Quality indicators for office-based medical oncology

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Background: The aim is to develop and utilize a set of quality indicators for office based medical oncology. The starter set of indicators will be used for an indicator-based quality measurement and peers to peer benchmarking by the WINHO department (collaboration of 210 medical oncology practices in Germany). The indicators should cover all areas of cancer care in office based oncology with a special emphasis on breast and colorectal cancer. The Quality Oncology Practice Initiative (USA) and its indicators are a paradigm for the WINHO indicator project.

Material & Methods: Relevant indicators were collected by internet and literature review. The indicator selection was done with a two-step expert rating procedure (modified RAND/UCLA). The indicators were rated concerning
1. importance/relevance,
2. benefit for patients,
3. whether they are within the responsibility of office based oncologists,
4. representation of high quality of care and
5. if the data is already present in patient records.

All indicators were rated on five stepped categorical rating scales. Based upon the results of the 1st rating session, the indicators were modified before the 2nd rating. The expert panel consisted of 25 experts from oncology associations, members of the open quality management group of the WINHO department and participants from patient support groups. We are currently doing a four-stepped feasibility test of all 46 WINHO indicators.

Results: A preliminary set of 272 quality indicators was collected by literature review. Due to redundancy and/or low specification level the set was reduced to 67 indicators. In the first rating session, 37 indicators were homogeneously rated as relevant and meaningful for high quality of care in office based oncology. The result of the second session was a set of 46 (32 documentation & therapy, 5 colon, 9 mama) quality indicators. First findings of our feasibility review indicate that the data for the calculation of 31 out of 46 indicators are already present in patient records.

Conclusions: QOP indicators are to some extent adoptable for German practices as well. First results of the feasibility test indicate that it will take considerable effort from all involved parties to embed the data collection for a starter set of quality indicators into a daily practices routine. A pilot study about the implementation of the routinely data collection for the indicators has to be done.

Disclosure: No conflict of interest disclosed.

Sexuality and fertility in male cancer patients

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Cryopreservation of human spermatozoa is the only chance for maintaining fertility in men with gonadotoxic disease and therapy. Male reproductive health can be severely impaired by chemo- and radiotherapy or surgical intervention. Thus, counselling at the time of diagnosis is highly important (Kliesch et al. 2003). Cryopreservation of spermatozoa before the start of therapy should generally be offered as a preventive measure to men at reproductive age who have not yet started or finished family planning. It can generally be offered to adolescents from the start of puberty as well as to adults. Among 851 consecutive patients of the CeRA, there are 111 adolescents between the age of 15 and 19. Semen quality does not differ significantly between (young) adults and adolescents before cryopreservation (Kliesch et al. 1996, Kamischke et al. 2004). The diagnoses of patients with oncological diseases among about 1,136 consecutive patients who visited the Centre of Reproductive Medicine and Andrology (CeRA) of the University Hospital of Münster for cryopreservation of their ejaculate comprises 30% with testicular tumors, 18% Hodgkin disease/non-Hodgkin disease, 9% leukemias and 7% bone tumors.

For cryopreservation the semen sample is frozen at -180°C in a sterile surrounding after cryoprotectants have been added. A period of at least 24 hours of abstinence is sufficient to avoid essential delays in starting therapy – especially in patients with advanced oncological disease. The collection of one to three semen samples does not cause any delay in the start of therapy. The analysis of the samples should be performed according to the WHO Guidelines (WHO Laboratory Manual 2010).

Oncological patients suffering from pre- or posttherapeutic azoospermia also profit from (microsurgical) testicular sperm extraction (TESE). The method is successful in almost 50% of the patients with testicular tumors (Schrader et al. 2003). In our own patient collective, we could detect spermatozoa in 55% and 47% of TESEs, resp., in pretherapeutic azoospermia as well as in azoospermia persisting after finishing therapy in 67 oncologic patients (testicular tumor, leukemia, lymphoma) (Werny et al. 2009). Analysis of questionnaires about two or three years post cryopreservation for oncological disease in adolescents and adults showed that cryopreservation can decrease concerns about fertility and facilitate coping with one’s disease and the discussion of fertility issues.

Disclosure: No conflict of interest disclosed.
Fortschritte in der Urologie: Systemische Therapie des Harnwegs-Carcinoms: Animalmodell- und Klinikstudie - Fortbildungsveranstaltung V338

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Introduction: The optimal treatment of bladder cancer remains a continuous subject of controversy. Radical cystectomy and pelvic lymphadenectomy is still the standard of care in the management of muscle-invasive bladder cancer. Surgery has resulted in effective local control and substantial survival rates. However, long-term mortality and reduced quality of life (QOL) due to urinary diversion and impairment of sexual function can be significant. Bladder-preservation trials have gained momentum because of comparable survival and recurrence rates in selected patients.

Methods: Overview of the literature to report long-term results of survival as well as treatment for inoperable situations and to summarise factors associated with bladder preservation and risk of relapse in patients treated with radiotherapy based multimodality therapy for invasive bladder cancer.

Results: During the past two decades, organ preservation by multimodality treatment has been investigated in prospective series with more than 1000 patients included. Current patient selection, maximum transurethral resection of bladder tumor, concomitant radiochemotherapy followed by cystoscopic evaluation of response with prompt salvage cystectomy for nonresponders constitute the basis of optimal bladder-preservation protocols. Bladder-preservation with such a multimodality therapy can achieve complete response rates of 60% to 80%. 5-Year survival rates of 50% to 60%, and survival rates with an intact bladder of 40% to 50%. Although no randomized comparisons between cystectomy and multimodality therapy exist, long-term data confirm that overall and disease-specific survival rates for patients in bladder-sparing protocols with salvage cystectomy, if necessary, are comparable to outcomes reported in series using primary cystectomy. A visibly and microscopically complete transurethral resection, younger age, lower T category, absence of multicentricity and no evidence of pelvic lymph node involvement are associated with an improvement in local control or survival. QOL studies demonstrate that the retained native bladder functions well and sexual function is often maintained.

Conclusion: In selected patients with muscle-invasive bladder cancer, trimodality therapy with bladder preservation represents a real alternative to radical cystectomy resulting in an acceptable rate of the long-term survivors retaining functional bladders. Quality of life and quality of bladder function is satisfactory for the majority of the patients.

Disclosure: No conflict of interest disclosed.

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Disclosure: No conflict of interest disclosed.
The full-day care of the patients in a rehabilitation facility allows for a profound assistance to optimize the nutrition within the scope of individual and group counseling, teaching kitchen and buffets. In this way initiated lifestyle modification can improve the quality of life and the outcome of our patients.

Disclosure: No conflict of interest disclosed.

V339
Coping and psychosocial interventions – what patients need psycho-oncological therapy?

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The diagnosis of cancer causes psychological distress in all affected patients. Symptoms of anxiety and depression often a consequence of a real threat of life and therefore not pathological. Maladaptive or anxiety disorders are considered for approximately 30 percent of cancer patients, all other patients develop adequate coping strategies.

The process of coping with a cancer disease is described by Verena KAST as a life crisis with different phases and the opportunity of personal development when coping is successful. Psycho-oncological therapies support cancer patients in this process of coping by offering emotional relief, strategies to expand their coping resources and developing a better quality of life. Psycho-oncological support is especially important for patients with additional conflicts in their families or distress in their working environment.

Special Psychotherapy is necessary for patients with pathological symptoms of anxiety or depression and patients with posttraumatic disorders. Several screening instruments have been implemented to select patients who benefit from psycho-oncological therapy.

Various techniques of psycho-oncological therapy applied in rehabilitation settings and outpatient care will be described in this report.

Disclosure: No conflict of interest disclosed.

Fortbildung
Adjuvante un neoadjuvante Behandlung beim Mammakarzinom
V340
Neoadjuvant treatment in breast cancer

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Neoadjuvant chemotherapy is standard of care in the treatment of locally advanced inoperable and inflammatory breast cancer. In patients with operable breast cancer several randomized trials showed similar disease free and overall survival when neoadjuvant and adjuvant treatment using identical regimens were compared. The higher rate of patients eligible for breast conserving surgery favored the neoadjuvant approach in these studies. Anthracyline-taxane based regimens given over 6 months achieve a pCR (pathological complete remission) rate of about 25%. Several studies showed that patients achieving a pCR had a significantly better outcome in terms of disease free and overall survival compared to those without a pCR. The pCR rate was 3-4 times higher when the hormone receptors were not expressed. Other factors predicting a higher chance of a pCR were poor grade, non lobular type, a triple negative status and young age below 40 years. In patients with HER2 positive disease the pCR rate between 40-50% could be observed when trastuzumab was added to neoadjuvant chemotherapy. Recent data suggest that these pCR rates may be further improved with dual blockade of the HER2 receptor by combining trastuzumab with lapatinib or pertuzumab in addition to chemotherapy. In patients with operable breast cancer neoadjuvant chemotherapy can be considered a valid treatment option when the same adjuvant treatment is indicated. Planned mastectomy but patient wishes breast conservation is the classical indication for neoadjuvant chemotherapy and may allow patients to undergo this less invasive surgical approach. Moreover the neoadjuvant setting provides a unique opportunity to investigate the impact of systemic therapy on breast cancer biology and may lead to a more rapid evaluation of new drugs or treatment modalities. Important open questions in the neoadjuvant treatment include the integration of sentinel node biopsy in the neoadjuvant setting and the approach when there is insufficient response of the tumor.

Disclosure: Jens Huober: Advisory Role: Sanofi-Aventis, BMS, GSK, Roche; Expert Testimony: GSK, Sanofi-Aventis, Roche

V334
Molecular subtypes – Relevant for therapy or for science?

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Breast cancer is no longer considered a single disease but represents a spectrum of malignant epithelial neoplasias of the breast with very diverse characteristics. For about three decades, breast cancer has been divided into estrogen receptor (ER) positive and negative subtypes (correlating with susceptibility to endocrine and cytotoxic therapies) of different histological grades (correlating with prognosis); more recently, a further subdivision was introduced by recognizing HER2 as an adverse prognostic factor and predictor of response to chemotherapy and treatment with drugs targeting the HER2 protein.

More recently, high throughput molecular methods led to the emergence of classifications based on differential gene expression (mRNA/DNA array) analysis. In addition to the prototypic classification into so-called intrinsic subtypes ‘luminal A’, ‘luminal B’, ‘HER2 positive’, and ‘basal-like’, other prognostic classification systems have been published such as the ‘Genomic Grade Index’. While the classification into ‘intrinsic subtypes’ proved to be reproducible with different sets of breast cancer samples and platforms, the individual allocation of patients to one group proved to be more elusive. The definitions and potential clinical use of molecular subtypes to guide the choice of therapy will be reviewed keeping in mind that to date, no prospective clinical trial has been reported to improve the outlook of patients suffering from breast cancer.

Disclosure: No conflict of interest disclosed.
New prognostic scoring systems in MDS

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The IPSS was the first comprehensive model predicting overall survival and leukemic transformation including cytogenetics as one of three major parameters (Greenberg et al., 1997). Shortcomings of the IPSS are the low number of patients with initial karyotypes, lack of prognostic data of rare abnormalities, the definition of intermediate karyotypes by exclusion only, the missing categorization of double and complex changes and the underscoring of bad risk cytogenetics in relation to blast counts. The WPSS was an advance since the prognostic weight of unfavorable cytogenetics was increased, however cytogenetic subtypes were adopted unchanged from the IPSS. In the MD Anderson Cancer Center (MDACC) Score only high risk abnormalities were considered. In comparison to blast counts their weight was higher as in the IPSS or WPSS, however their impact was limited by the inclusion of 7 other parameters. By performing multicentric international cooperative studies, our group aimed for a significant refinement of cytogenetic prognostic stratification. For this purpose univariate and multivariate analyses of OS and risk of transformation to AML were calculated. The prognostic impact of poor-risk cytogenetics (as defined by the IPSS) on OS was as univariate as an increased (>20%) blasts count. Remarkably, the predictive power of the IPSS cytogenetic subgroups was unaffected by the type of therapy given (Schanz et al., 2011). In the second study 19 cytogenetic subgroups were defined and condensed to 5 prognostic subgroups providing high predictive power for OS and AML transformation. The groups of King’s College in London and the Taussig Cancer Center in Cleveland provided a combined genetic prognostic scoring system considering additionally to cytogenetics the results of short nucleotide polymorphism analysis (Tu et al., 2011). Very recently, a revision of the IPSS (IPSS-R) based on an international data collection of 6388 pts. was presented containing cytogenetics as newly classified by our group, blast counts and cytopenias. As a result 5 distinct prognostic subgroups with a median OS ranging between 9.0 and 0.7 years and with a time to 25% development of AML between not reached and 0.7 years could be established.

Disclosure: No conflict of interest disclosed.

High-throughput methods for MDS – new clues to understand pathogenesis and to provide clinical information for diagnosis and prognosis

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Since nearly 30 years, the diagnosis of MDS is based on classification systems such as FAB and IPSS taken into account blast percentages and chromosomal aberrations. This is still true for today and the backbone of any diagnostic and prognostic approach for MDS patients. However, during the last 10 years, several findings detected by molecular genetic approaches became of importance also for MDS patients. This information is in some parts correlated to the biological understanding as already given by cytogenetic investigations. However, an increasing number of gene mutations such as deletions, duplications or insertions are now in the focus not only for research but also for clinical decision making and will also rise several ethical questions that are not solved so far.

It is a very important but also fascinating task to implement techniques such as next-generation sequencing and other high-throughput methods to diagnose MDS in the already available scenarios and to find better ways for understanding this very heterogeneous disease. For sure, patients will profit from molecular information in addition to standard techniques such as cytomorphology and cytogenetics in the near future.

Disclosure: No conflict of interest disclosed.
Medulloblastoma – molecular characterization and therapeutic implications

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Medulloblastoma comprises the most frequent malignant brain tumor in childhood and one of the most common causes of mortality in this age group. Modern multi-modal treatment approaches including surgery and adjuvant radio-chemotherapy have improved overall survival rates to around 70%, which is still comparatively low for childhood malignancies. Furthermore, many survivors experience severe long-term toxicity including neurological, cognitive, and endocrinological deficits as well as a highly increased risk of secondary malignancies. Thus, a better molecular understanding is urgently needed to tailor therapy to disease risk and tumor biology. Recent genome-wide studies using microarrays, and most recently next-generation sequencing techniques have consistently revealed at least four very distinct subtypes, which largely differ in terms of demographics and outcome. This extensive biological heterogeneity of tumors that look so similar under the microscope was further substantiated by the first sequencing studies that basically demonstrated that almost every tumor as a "private" repertoire of somatic mutations with only very few genes being recurrently mutated at a maximum frequency of still below 10%. These findings have immense clinical implications since they can on the one hand help to more accurately stratify patients into different risk groups, and on the other hand tell us that targeted therapy approaches will most likely only be effective in a small proportion of patients for a given drug requiring truly individualized approaches in the future.

Disclosure: No conflict of interest disclosed.

Chemotherapy for brain metastases

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Brain metastases are increasingly diagnosed in patients with solid tumors; although there is no prospective registry of brain metastases, the number of affected patients is estimated to make up to 20% of all cancer patients, which would translate in 6% of the general population. For patients with cancer, being diagnosed with brain metastases represents still the highest conceivable threat, even more than dying.¹ The mainstay of treatment of patients with brain metastases remains local treatment, either surgical resection and/or radiosurgery and/or whole brain radiotherapy. However, drug therapy in patients with brain metastases mostly is considered only when the previous options have failed. In the following, many survivors experience severe long-term toxicity including neurological, cognitive, and endocrinological deficits as well as a highly increased risk of secondary malignancies. Thus, a better molecular understanding is urgently needed to tailor therapy to disease risk and tumor biology. Recent genome-wide studies using microarrays, and most recently next-generation sequencing techniques have consistently revealed at least four very distinct subtypes, which largely differ in terms of demographics and outcome. This extensive biological heterogeneity of tumors that look so similar under the microscope was further substantiated by the first sequencing studies that basically demonstrated that almost every tumor as a "private" repertoire of somatic mutations with only very few genes being recurrently mutated at a maximum frequency of still below 10%. These findings have immense clinical implications since they can on the one hand help to more accurately stratify patients into different risk groups, and on the other hand tell us that targeted therapy approaches will most likely only be effective in a small proportion of patients for a given drug requiring truly individualized approaches in the future.

Disclosure: No conflict of interest disclosed.

Primary central nervous system lymphoma

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Primary NHL of the CNS (PCNSL) is a rare form of extranodal non-Hodgkin’s lymphoma (NHL). It accounts for 1 to 2% of all NHL and for 2 to 7% of all primary CNS tumours. Its incidence has increased over the past 30 years, particularly in immunocompetent individuals. Over 90% of PCNSLs are aggressive NHL of the B-cell type. Age > 60 years, performance status, elevated LDH, high CSP protein concentration, and involvement of deep regions of the brain were identified as prognostic factors. The outcome of patients with PCNSL is poor despite initially excellent responses to steroids and radiotherapy (RT). Adding methotrexate (MTX) to RT has improved the prognosis of PCNSL patients, although most eventually relapse. Recently, a clear superiority of combining high-dose cytarabine (HD-AraC) and HD-MTX compared to HD-MTX alone could be demonstrated. The impact of consolidating whole-brain radiotherapy has been controversially discussed and could not yet be determined conclusively. New radiotherapeutic techniques and lower doses of RT are under investigation and may reduce neurotoxicity. In younger patients with sequential MTX-based chemotherapy, intensified chemotherapy containing the alkylating agents Carmustine and thiopeta followed by peripheral stem-cell transplantation (ASCT) have shown high efficacy. Benefit and side effects of different consolidative strategies, that is conventional WBRT and high-dose chemotherapy supported by ASCT, is going to be compared in a randomized trial to draw definitive conclusions on the role of consolidation both on efficacy and neurotoxicity in patients with newly diagnosed PCNSL. A large proportion of PCNSL patients is older than 60 years and is still underrepresented in clinical trials. Treatment of elderly patients with PCNSL should consider the high risk of neurotoxicity; systemic chemotherapy without RT is recommended in those patients. The monoclonal antibody rituximab has produced promising results in small cohorts, but its value has to be determined.

In summary, diagnosis and treatment of primary central nervous system disease has changed dramatically within the last decade leading to long-term survival in a substantial proportion of patients. To optimize treatment strategies of this rare disease collaborative clinical trials are critical.

Disclosure: Gerald Illerhaus: Advisory Role: Riemser; Other Financial Relationships: Roche

Glioblastoma in the elderly

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Age is the major therapy-independent prognostic factor in gliomas across the World Health Organization (WHO) malignancy grades II-IV. Elderly patients with glioblastoma (GBM) are commonly treated less aggressively than younger patients. Thus, a differential attitude towards elderly compared with younger patients among neuro-oncologists may contribute to the overall poorer outcome in this patient population. Surrogate biological markers explaining this age effect have only recently been partly elucidated. For instance, the differential distribution of isocitrate dehydrogenase 1 (IDH1) mutations, which are almost absent in elderly glioblastoma patients and are prognostically favorable, may account for some of the prognostic impact of age. O6-methylguanine-DNA-methyltransferase (MGMT) promoter methylation identifies a subpopulation of glioblastoma patients with more favorable prognosis and predicts a benefit from alkylating agent chemotherapy. Unexpectedly, this molecular aberration appears to be more common in the elderly, but whether it has the same prognostic impact in the elderly, remains to be demonstrated.

Standards of care for elderly patients with glioblastoma are currently being defined by large randomized trials. The value of radiotherapy in this setting has been confirmed earlier in a small French trial comparing best supportive care versus radiotherapy alone. Two phase III trials have compared radiotherapy alone with temozolomide alone. Preliminary analyses of these trials arrived at different conclusions: the preliminary report from the Nordic trial...
Abstracts

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The last decade has seen some important changes in the management of meso-

dehlioma, driven by an increasing incidence and awareness among the public

and physicians and the extrapolation from treatment approaches and drugs

from other cancer types. Active chemotherapy is now available which, albeit palliative, will not only

moderate increase life expectancy but will also help in relieving symptoms. A small portion of patients might benefit from more aggressive interventions (EPP = extraperitoneal pneumectomy, PORT = postoperative radiotherapy, neo-

adjuvant chemotherapy) even with curative intent. Further improvement in advanced MPM is to be expected from drugs, concen-

trating on mesothelioma-specific targets (e.g. VEGFR, apoptotic pathways, folate receptors) or from selecting patients expressing one or more biomarkers. It is our duty as physicians to inform patients about all treatment options.

Disclosure: No conflict of interest disclosed.

Management of advanced mesothelioma

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Fortbildung

Keimzelltumore

V361

Treatment in stage I: does surveillance fit all, or is there a role for adjuvant therapy?

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Stage I disease is seen in 80-85% of pts with seminomas, and 60% of pts with nonseminomas. Management options in stage I are active surveillance, radio-

therapy, and adjuvant chemotherapy with carboplatin monotherapy in patients

(pts) with seminomas, and surveillance, adjuvant chemotherapy with 1-2

cycles of cisplatin, etoposide and bleomycin (PEB), and to a lesser extend

retroperitoneal lymph node dissection in pts with nonseminomas. Irrespective of cure rates of 99-100%, there is debate about the best strategy in stage I.

While an increase in cardiovascular toxicity and a higher incidence of second-

ary cancers is a concern in pts receiving adjuvant treatment, necessity of good

compliance, frequent CT scans at follow-up, and increased psychological

burden for pts are major drawbacks of surveillance. The European consensus

guidelines advocate an approach taking into account risk factors. In the absence of risk factors, which in the consensus were defined as invasion of the

rete testis and size of the primary tumor (< or ≥4cm) in seminomas, and pres-

ence of vascular invasion (VI) in nonseminomas, surveillance is favoured. Pts

presenting with risk factors are considered candidates for adjuvant therapy. In

seminomas, however, neither invasion of the rete testis, nor tumor size, postu-

lated as risk factors by Warde in 2002, could be confirmed in more recent

analyses, casting doubt on the clinical utility of this risk assessment. While

adjuvant radiotherapy or chemotherapy with 1-2 cycles of carboplatin

decreases relapse rates from 15-20% to 3-4%, both strategies have been criti-
cized for causing unnecessary toxicity. In nonseminoma, VI of both lymphatic

and blood vessels is a validated risk factor. Relapse rates are approximately

20% in pts without, and 50% in pts with VI. For the latter, the European con-
sensus advocates the use of 2 cycles of PEB. A recent Scandinavian case series

reported excellent results of a risk-adapted strategy in pts with nonsemino-
tous cancer, using surveillance for VI negative and 1 cycle of PEB for VI

positive pts. However, 1 cycle adjuvant PEB must be considered experimental, and therefore all pts with stage I nonseminoma and VI should be included in an ongoing randomized phase III trial by the German Testicular Cancer Study Group, comparing 1 versus 2 cycles PEB. Besides randomized trials, large

registries and meticulous follow-up of pts with stage I disease could help to
document toxicity and outcome and determine best management.

Disclosure: No conflict of interest disclosed.

Freie Vorträge

Indolente B-Zell-Lymphome

V365

Quality of life of long term survivors with follicular lymphoma after high-dose chemotherapy with autologous stem cell transplantation and conventional chemotherapy

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Introduction: High-dose chemotherapy (HDCT) followed by peripheral blood stem cell transplantation (PBSCT) is frequently applied in eligible patients (pts) with relapsed or refractory follicular lymphoma (FL). The toxicity of HDCT, however, might manifest itself in reduced quality of life (QoL). In this study we investigated QoL of long term survivors after HDCT in comparison with patients after conventional chemotherapy (CT) and the healthy German population.

Patients and Methods: QoL was evaluated with the standardized questionnaires EORTC QLQ-C30 and EQ-SD. A total of 124 pts with FL were included in the study. 63 pts received HDCT with PBSCT. This group was compared with 61 pts who were treated with Rituximab and CHOP chemotherapy and supplementary radiation. Median follow-up was 9 years for the HDCT group and 4.4 years for the CT group. At the time of the study, 88% of

No conflict of interest disclosed.

Disclosure: No conflict of interest disclosed.
pts of the HDCT group and 41% of patients of the CT group were in continuous complete remission (CR). In addition, QoL of the pts was compared to QoL of healthy people of the German population.

Results: In the HDCT group, the results of the questionnaires show a reduced QoL compared to the healthy population. The decrease in QoL was significant with p<0.05 in four of five of the subcategories of the functional state and six of the nine subcategories of the symptomatic state. Pts who received CT also showed a reduced QoL compared to the healthy population in all of the three main categories of the EORTC-QLQ-C30, in particular in the category of functional and symptomatic state. The differences of the functional state were statistically significant in all subcategories (p=0.001) and the differences of the symptomatic state were statistically significant in seven of nine subcategories.

When QoL of the HDCT group and the CT group were compared, there were significant differences in favor of the HDCT group in the functional subcategory social functioning (p=0.04) and the symptomatic subcategory pain (p=0.01). In the EQ-5D questionnaire, we also found a reduced QoL in patients after CT compared to HDCT pts (p=0.05) and healthy people (p=0.02).

Conclusions: In this long term follow up study, we found a reduced QoL in FL pts after CT as well as after HDCT compared to the healthy population. There was a tendency of a better QoL in pts of the HDCT group compared to pts of the CT group, maybe due to a higher proportion of pts in CR or a longer follow up period in the HDCT group.

Disclosure: No conflict of interest disclosed.

V366
High-dose chemotherapy with autologous stem cell transplantation compared to chemotherapy or immunotherapy in patients with follicular lymphoma: a systematic review with meta-analysis

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Background: Follicular lymphoma (FL) is the most common indolent and second most common Non-Hodgkin’s lymphoma in the Western World. Standard treatment usually includes rituximab and chemotherapy. High-dose chemotherapy and autologous stem cell transplantation (ASCT) is an option for patients in advanced stages or for second line therapy, leading to improved progression-free survival rates. However, the impact of ASCT remains unclear, as there are hints for increased risk for second cancers.

Objectives: We performed a systematic review with meta-analysis of randomised controlled trials (RCTs) comparing ASCT with chemotherapy or immuno-chemotherapy in patients with follicular lymphoma with respect to overall survival (OS), progression-free survival (PFS), treatment related mortality (TRM), adverse events and secondary malignancies. Methods: We searched MEDLINE, EMBASE and CENTRAL as well as conference proceedings from January 1985 to November 2010 for RCTs. Two review authors independently screened search results, extracted data and assessed quality of trials.

Results: Our search strategies led to 2971 potentially relevant references. Of these, five RCTs involving 1093 patients were included. In none of these trials rituximab was administered to all patients or only those in the comparator arm. Although the increase of PFS is statistically significant for patients in the ASCT arm (hazard ratio (HR) = 0.40 (95% confidence interval (CI) 0.32 to 0.52), this does not transfer in a statistically significant OS advantage (HR = 0.85; 95% 0.60 to 1.20). However, there are no statistically significant differences in terms of treatment related mortality (RR = 1.28; 95% CI 0.25 to 6.61) or secondary AML/MDS (RR = 2.87; 95% CI 0.25 to 6.61) or solid cancers (RR = 1.20; 95% CI 0.25 to 5.77).

Conclusions: In summary, the currently available evidence suggests a PFS benefit and no statistically significant differences in terms of TRM and secondary cancers for ASCT compared to chemotherapy or immuno-chemotherapy for patients with follicular lymphoma. Further trials with additional rituximab and longer follow-up are needed to determine whether one of these therapies leads to a survival advantage.

Disclosure: No conflict of interest disclosed.
Maintenance Rituximab every 2 months for 2 years is effective and well tolerated in patients with follicular lymphoma with both standard or rapid infusion: Updated results from the phase IIIb MAXIMA study


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Background: MAXIMA is a Phase IIIb study evaluating the safety of rituximab (Rituxan®, MabThera®) maintenance therapy given at either a standard infusion rate or as a rapid infusion in patients with treatment-naïve or previously treated follicular lymphoma (FL) responding to induction treatment. Data are described here at a median follow-up of 28.8 months.

Methods: Patients (pts) from 211 centers in 24 countries who achieved a CR/CRu or PR following induction therapy with 8 cycles of a rituximab-containing regimen, received maintenance treatment with rituximab (375 mg/m²) every 2 months for a maximum of 2 years. Rituximab could be administered at a standard infusion rate (>90 minutes) or as a rapid infusion (≤90 minutes).

Results: A total of 545 pts responding to induction treatment were enrolled. Median age was 57 years (range 29-86), with 11.7% over 70 years. 395 (72.5%) pts were previously untreated. 381 (69.9%) pts entered the study in post-induction CR/CRu, 353 (92.7%) remained in CR/CRu during maintenance (progressive disease 7.1%; missing 0.3%). Of the 164 pts achieving PR following induction therapy with 8 cycles of a rituximab-containing regimen, received maintenance treatment with rituximab (375 mg/m²) every 2 months for a maximum of 2 years. Rituximab could be administered at a standard infusion rate (>90 minutes) or as a rapid infusion (≤90 minutes).

Conclusions: Maintenance rituximab every 2 months for 2 years was well tolerated with little rituximab-related toxicity with no apparent difference in tolerability when rituximab was administered as standard or rapid infusion.

Disclosure: No conflict of interest disclosed.
Interferon maintenance prolongs remission duration after retrospective analysis of the German Low-Grade Lymphoma Study Group (GLSG)

Hoster, E.¹,², Unterhalt, M.¹, Forstpointner, R.¹, Pfeundschuh, M.³, Hallek, M.¹, Kneba, M.³, Lengfelder, E.³, Metzner, B.³, Wandt, H.², Dreyling, M.¹, Hiddemann, W.⁴, On behalf of the German Low-Grade Lymphoma Study Group (GLSG)

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Introduction: Rituximab (R)-maintenance in first remission of follicular lymphoma (FL) has been shown to improve remission duration (RD) compared to observation (Salles et al., ASCO 2010). Previously, maintenance using interferon-α (IFN) had been shown to be effective in indolent lymphoma (Solal-Céligny et al., NEJM 1993, Hagenbeek et al., JCO 1998). However, data on the impact of IFN-maintenance after immunochemotherapy are rare. In two GLSG first-line trials IFN-maintenance was intended in all patients responding to induction with MCP, CHOP, or R-CHOP, and not assigned to high-dose therapy. We performed a retrospective analysis to compare the outcome of FL patients who received IFN-maintenance or no consolidation or maintenance.

Methods: We compared patient characteristics to detect reasons why IFN-maintenance was not started. Analysis of RD was adjusted for the potential confounders FLIPI, performance status, R-containing induction and remission status.

Results: IFN-maintenance was started in 716 (76%) of 939 responding FL patients not treated with high-dose therapy. Patients with IFN-maintenance were younger (57 vs. 59 years, p=0.002), had less frequently a high risk FLIPI (46% vs. 55%, p=0.038), but not more frequently a better performance status (ECOG 0-1: 93% vs. 90%, p=0.18). IFN patients had been less frequently treated with R during induction (43% vs. 64%, p<0.001). Patients with IFN-maintenance had significantly longer RD (hazard ratio, HR, 0.70, 95% CI 0.56 to 0.87, p=0.002) which was even more pronounced after adjustment for potential confounders (adjusted HR 0.55, 95% CI 0.44 to 0.70, p<0.001). Of 452 patients responding to R-CHOP, IFN-maintenance was started in 309 (68%). RD at 3 years was 78% vs. 63% (p<0.001) and the adjusted HR for IFN was 0.55 (95% CI 0.37 to 0.81, p=0.003).

Conclusions: Although this is not a randomized comparison, our results suggest that the impact of IFN might be still valid in the era of immunochemotherapy. Since R-maintenance has fewer side effects and appears to be more effective than IFN-maintenance, we recommend its use in selected cases.

Disclosure: Andreas Hochhaus: Advisory Role: Novartis, BMS, Pfizer, Ariad; Financing of Scientific Research: Novartis, BMS, Pfizer, Ariad; Expert Testimony: Novartis, BMS, Pfizer, Ariad

How to proceed in case of imatinib failure?

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Based on the impressive results of the IRIS study (O’Brien S et al, NEJM. 2003;348(11):994-1004) and the meanwhile long-term experience with imatinib (~10 years) imatinib 400mg QD is currently the standard of care for patients with chronic myeloid leukemia (CML) in chronic phase regardless of age and Sokal risk score. Nevertheless, around 20 – 30% of the patients do not respond adequate to imatinib therapy in a way that they either do not achieve an appropriate cytogenetic or molecular remission according to established guidelines from the European Leukemia Net (ELN; Baccarani et al, JCO. 2009;27:6041-6051) or that they lose their imatinib-response. In case of an imatinib failure, a complete re-evaluation must be performed. This includes a bone marrow (BM) biopsy with subsequent histologic and morphologic analysis. Furthermore, the BM specimen must be analysed cytogenetically (optionally by FISH) and molecularly to measure levels of BCR/ABL and search for potentially present mutations in the BCR/ABL kinase. At this time it is also important to consider a poor compliance or adherence to the imatinib therapy. This option should be seriously discussed with the patient. Additionally performed measurements of imatinib trough levels are recommended. After having performed all these diagnostic procedures a subsequent change in therapy must be undertaken. Therapeutic options include an increase of the imatinib dose, change to a second generation tyrosine kinase inhibitor (TKI; e. g. dasatinib, nilotinib), consideration of an allogeneic transplantation, involvement into a clinical trial with a new TKI or aurora kinase inhibitor or treatment with drugs from the pre-imatinib era like interferon-alpha or hydroxyurea. Parameters that impact on the type of second line therapy after imatinib failure are the presence (and type) or absence of a BCR/ABL mutation, presence of side effects under the therapy with 400mg imatinib, patient’s age and performance status, donor availability and co-morbidities. Updated

Onkologie 2011;34(suppl 6):1–305
Abstracts

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pulmonale Rundherde annähernd verdoppeln. Die höchste Sensitivität erreicht
lagerungsfrei dargestellt werden. Hierdurch lassen sich die Detektionsraten für
anschließend 25-60 Schichten berechnen, in denen solide Befunde über-
verschwenkt wird. Aus den digitalisierten Projektionsdaten lassen sich
werden. Systeme zur computerunterstützten Detektion (CAD) verbessern die
erne Strukturen oder Weichteile können auch relativ große Befunde über-
große Rundherde liegt sie bei nur ca. 20-30%. Bei Überlagerung durch knöch-
in Allgemeinanästhesie, ist ein häufiges Problem des klinischen Alltags. Die
Die Entdeckung eines pulmonalen Nodulus z.B. vor einem operativen Eingriff
1

V375

Was is the appropriate role of “clinical equipoise” in risk-
benefit evaluations of clinical research?

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An aceptable risk-benefit ratio is a central ethical requirement for clinical
research studies. The concept of „clinical equipoise“ has traditionally played
an important role in evaluating the risk-benefit profile of investigational drugs.
„Clinical equipoise“ is commonly defined as a state of honest professional
disagreement among expert clinicians about which treatment – routine clinical
care or the given investigational drug – is to be preferred from the patient’s
perspective. Thus, a state of „clinical equipoise“ indicates that an investiga-
tional drug has a risk-benefit profile that is at least comparable to the risk-
benefit profile of standard care.

According to the standard approach to risk-benefit evaluations in clinical
research, „clinical equipoise“ is a necessary requirement for so-called thera-
petic research interventions. If follows from this approach that an investiga-
tional drug or intervention can be administered only if it has a comparable
risk-benefit profile to routine clinical care. The standard approach has recently
been criticized as being fundamentally flawed.

The presentation will reject this radical critique of „clinical equipoise“. It will
argue that „clinical equipoise“ has two important functions in evaluating the risk-
benefit profile of clinical research studies. First, „clinical equipoise“ is a good
indication of the social value of research studies. Second, „clinical equipoise“ is a sufficient – but not a necessary – requirement for an acceptable
risk-benefit profile of investigational drugs and other research interventions.

The proposed partial rehabilitation of „clinical equipoise“ potentially offers a
way out of the controversy about this concept and the appropriate role of
„clinical equipoise“ in risk-benefit evaluations. It also has important implica-
tions for evaluating the risks and potential benefits of clinical research stud-
ies.

Disclosure: No conflict of interest disclosed.

Fortbildung

Abklärung eines Lungenrundherdes und lokaleTherapie

V377

Current status and recent developments in lung nodule imaging

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Die Entdeckung eines pulmonalen Nodulus z.B. vor einem operativen Eingriff
in Allgemeinanästhesie, ist ein häufiges Problem des klinischen Alltags. Die
Sensitivität der Röntgenuntersuchung ist jedoch begrenzt: für 0,5-1,5 cm
große Rundherde liegt sie bei nur ca. 20-30%. Bei Überlagerung durch knoch-
erne Strukturen oder Weichteile können auch relativ große Befunde über-
sehen werden. Systeme zur computunterstützten Detektion (CAD) verbessern die
Detektionsrate mit digitalen Standard-Röntgenaufnahmen auf ca. 80%. Ein
relativ neues Verfahren ist die Tomosynthese, bei dem die Röhrre eines Röntgensystems während der Aufnahme gegenüber dem digitalen Detektor
verschwenkt wird. Aus den digitalisierten Projektionsdaten lassen sich
anschließend 25-60 Schichten berechnen, in denen solide Befunde über-
lagerungsfrei dargestellt werden. Hierdurch lassen sich die Detektionsraten
für pulmonale Rundherde annähernd verdoppeln. Die höchste Sensitivität erreicht
das Thorax-CT, mit dem ca. 65-85% aller 0,5-1 cm großen Rundherde bei der
Sichtbefundung detektiert werden. Mit computerunterstützten Verfahren steigt
die Detektionsrate auf bis zu 90%. Die Strahlenexposition erreicht bei einer
Röntgenübersicht in zwei Ebenen max. ca. 0,05 mSv, bei einer Tomosynthese
0,1-0,2 mSv und bei einer CT 1 mSv (low dose Technik) bis zu 10 mSv
(hochauflösende Mehrzeilentechnik). Derart hinaus ist die Positronen-
Emissionstomographie (¹⁸F-FDG-PET) mit Korrektur der Atembewegungen
(Gating) zur Identifikation kleiner, stoffwechselaktiver Rundherde auch unter
1 cm Größe geeignet. Als alternativeren Verfahren ohne Strahlenexposition steht
die MRT Verfügung. Mit standardisierten Protokollen erreicht sie bei guten
Untersuchungsbedingungen eine Sensitivität von ca. 80% bei 3-5 mm großen
und 90% bei 6-10 mm großen Rundherden. Offen bleibt die Frage, inwieweit
das CT (technisch geeignet wären auch Tomosynthese und MRT) sinnvoll für
ein Lungenkrebs-Früherkennungsprogramm bei Hochrisikogruppen (langjähr-
rigen Rauchern) empfohlen werden kann. Erste Daten aus dem National Lung
Screening Trial in den USA weisen auf eine mit dem CT-Screening erreich-
bare Reduktion der Lungenkrebsmortalität um 20% hin. Endgültige Daten
stehen bisher jedoch aus, so dass bei Empfehlungen zur Umsetzung ähnlicher
Programme noch Zurückhaltung geübt wird. Als Beispiel soll die aktuelle
Stellungnahme der DGP und DRG im Rahmen des Vortrags vorgestellt und
diskutiert werden.

Disclosure: No conflict of interest disclosed.

Fortbildung

Prävention / Screening

V381

Nicotine abstinence of cancer patients before, during and after treatment

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Tobacco use accounts for at least 25% of all cancer deaths including carci-
noma of the lung, lip, oral cavity, pharynx, larynx, esophagus, pancreas, uterine cervix, kidney, bladder and stomach.

Smoking cessation is an important opportunity to enhance effectiveness of
cancer treatment, like surgery, radiotherapy, and systemic therapy. It is impor-
tant not only for cancer patients but also for all patients who need surgical therapy.
Smokers have an increased mortality risk, more pulmonary and respi-
atory complications attributable to limited lung function, wound infections
and delayed surgical wound healing. For example, in colorectal surgery, smok-
ing patients have a major possibility for anastomatic leakage compared to non-
smokers. In breast reconstruction smoking impairs the free TRAM flap microcirculation. In Liver transplant, smoking is associated with more cardio-
vascular-related and sepsis-specific mortality and vascular complications and
higher incidence of ascites and encephalopathy. Smoking cessation shortly
before thoracic surgery is associated with improved long-term survival.
Tobacco smoking during radiotherapy in patients with head and neck cancer is
associated with unfavourable outcome. Current smoker with lung cancer dur-
ing radiotherapy have a 20% greater probability of radiation pneumonitis.
During chemotherapy, smoking exacerbates oral mucosites, loss of taste, xero-
tomia, weight loss and fatigue.

Tobacco smoking after cancer diagnosis has also a negative impact on prognosis
(e.g. small cell lung cancer, renal clear cell carcinoma, Non-Hodgkin’s
lymphoma, breast cancer) as well as on quality of life.

Therefore, physicians should motivate and help their cancer patients to quit
smoking as soon as possible before cancer treatment. Non-smoking is not only
cool, it also enhance effectiveness of cancer treatment and had a positive
impact on survival.
Every comprehensive cancer center should offer cancer patients and also the
community as a whole a service of smoking cessation and also promote
tobacco control in adolescents and adults.

Disclosure: No conflict of interest disclosed.

Oberfluoreszenz und Bildgebung mit PET/CT in der Lungenchirurgie

V380

Zentrale diagnostische und therapeutische Rolle der PET/CT in der Lungenchirurgie

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Onkologie 2011;34(suppl 6):1–305

Abstracts
Lifestyle and environmental factors play an important role in the etiology of cancer. Besides alcohol, smoking and obesity, inactivity is a modifiable established risk factor for different types of cancer. In the past decades increasing number of studies support the protective effect of regular physical activity, however the effect varies by cancer site. There is convincing evidence that regular physical activity protects against colon cancer. Epidemiologic observational studies suggest a strong inverse relation between physical activity and the risk of colon cancer with an average risk reduction of approximately 25% for the most active compared to the least active individuals. A similar protective effect has been established for the development of breast cancer. An average risk decrease of 25% has been observed for the most active compared to the least active women. A protective role of regular physical activity has also been suggested for endometrial cancer. The beneficial effect of physical activity for other cancer sites is not consistent. For prostate cancer only a weak association has been shown, while the effect of physical activity for developing lung cancer remains unclear. Little evidence exists for other cancer sites like gastric or rectal cancer. For colon and breast cancer there is growing evidence for a dose response effect of physical activity. The combination of intensity, frequency and duration determines the total level of physical activity. People who are physically active over their lifetime seem to have the greatest benefit. This positive effect has been observed for both recreational and occupational activity. Public health recommendations include regular moderate physical activity, equivalent to brisk walking, for 30 min. per day and at least 5 times a week to reduce cancer risk. Data suggest that physical activity exerts its cancer-protective effects through many mechanisms, however a lot of pathways are currently not fully understood. Besides its impact on energy balance and weight, an independent effect of physical activity has also been suggested for endometrial cancer. The beneficial effect of physical activity for other cancer sites is not consistent. For prostate cancer only a weak association has been shown, while the effect of physical activity for developing lung cancer remains unclear. Little evidence exists for other cancer sites like gastric or rectal cancer. For colon and breast cancer there is growing evidence for a dose response effect of physical activity. The combination of intensity, frequency and duration determines the total level of physical activity. People who are physically active over their lifetime seem to have the greatest benefit. This positive effect has been observed for both recreational and occupational activity. Public health recommendations include regular moderate physical activity, equivalent to brisk walking, for 30 min. per day and at least 5 times a week to reduce cancer risk. Data suggest that physical activity exerts its cancer-protective effects through many mechanisms, however a lot of pathways are currently not fully understood. Besides its impact on energy balance and weight, an independent effect of physical activity is assumed. Physical activity influences sex hormones, metabolic hormones, the immune function and inflammation and may mediate its beneficial effect through these mechanisms. Future research is needed to understand the underlying mechanisms and to be able to provide more detailed recommendations regarding the duration, intensity and frequency of physical activity to reduce the cancer risk. Disclosure: No conflict of interest disclosed.
V385

Docetaxel, cisplatin and capecitabine (DCX) as perioperative chemotherapy in gastro-esophageal adenocarcinoma. (Mature results of a phase II study of the Arbeitsgemeinschaft Internistische Onkologie AIO)

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Background: Perioperative chemotherapy with ECF or ECX is a standard approach in Europe for resectable gastro-esophageal cancer. Docetaxel significantly improves efficacy if added to cisplatin and 5-FU as palliative treatment. To translate this efficacy into the curative approach this phase II study investigates docetaxel (D), cisplatin (C) and capecitabine (X) in the perioperative setting.

Methods: Pts with curatively resectable adenocarcinoma of the distal esophagus, gastro-esophageal junction (GEJ) or stomach UICC stage > 2 were treated with 3 preop and 3 postop cycles of chemotherapy: D 75 mg/m² d1, C 60 mg/m² d1 and X 1875 mg/m²/d d1-14, q3w. Primary endpoint: R0 resection rate. G-CSF was not routinely administered.

Results: From Nov 2008 to Jan 2010, a total of 51 pts were included. Pts' characteristics: Male/female: 48/3: Med. age: 65 yrs (37-74). Karnofsky PS: 100%/90%/80%/70%: 22/24/4/pts. Localisation: distal esophagus 9.8%, GEJ 60.7%, gastric body 29.4%. Histology: intestinal 39.2%, diffuse 21.6%, not specified 39.2%. Cycles delivered preop: 0/1/2/3: 1/5/9/41.4% of pts, postop: 0/1/2/3: 27/5/3/75.9/5/92.9% of pts. 51.4% of pts starting postop chemo had at least one drug reduction. Main toxicities CTC grade 3 + 4 (>5%): preop/postop: nausea 6.1%/ 5.6%, loss of appetite 6.1%/ 2.8%, diarrhea 12.2%/ 5.6%, neutropenia 79.5%/ 61.1%. Efficacy: Surgical resection rate: R0: 90.2%, R1: 5.9%, no resection: 3.9%, reported D2 LN dissection: 82.4%, post-OP complications: 29.4%, 30 day mortality: 0%, Histopathologic remission rate (Becker et al. 2003): 1a: pCR 13.7%, b (<10% viable tumor cells): 7.8%. After a median follow up of 11.8 months (range 3.7-25.8) 9 pts had tumor recurrence so far.

Conclusions: DCX can be safely administered as perioperative treatment for locally advanced esophageal-gastric adenocarcinoma with 94.1% of pts receiving all scheduled preop cycles and 52.9% receiving all postop cycles. Routine use of G-CSF may be considered. The pCR rate of 13.7% is among the highest reported thus far in comparable trials using three-drug regimens.


V386

Defining two prognostic groups of metastatic gastric cancer: FLOT3 trial of the Arbeitsgemeinschaft Internistische Onkologie (AIO)


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Introduction: A prognostic model for metastatic gastric cancer has not been established yet.

Methods: Pts with untreated gastric cancer were prospectively stratified into 3 groups: operable (OD), limited metastatic (LD), or extensive metastatic (ED) disease using a predefined algorithm and treated with 5-FU, oxaliplatin, leucovorin and docetaxel (FLOT). LD was defined as having all of the following: distant intra-abdominal lymph node metastases and/or a maximum of 1 organ involved in metastatic disease (distant extra-abdominal lymph node metastases were considered as 1 organ), normal serum alkaline phosphatase, < 5 liver lesions, no clinically apparent peritoneal carcinomatosis, no lymphangiosis carcinomatosa of the lung or malignant pleural effusion, and ECOG ≤ 1. All metastatic pts were ED. Pts with OD received 4 preoperative cycles of FLOT and were operated (D2-resection) followed by 4 postoperative cycles. Pts with LD received 8 cycles with surgery allowed for complete macroscopic resection. Pts with ED received 8 cycles with surgery allowed for palliation only. The study had 90% power to detect a HR of 0.55 for overall survival in favor of the LD group (vs. ED group, 2-sided log-rank p<0.05).

Results: A total of 252 pts (OD 52/80/132) were included between Feb 2009 and Jan 2010 in 47 centers, of whom 239 pts were eligible. All 3 groups received a median of 8 cycles of FLOT. The primary endpoint of the study was met: median OS was 18.6 vs. 10.9 months in pts with LD vs. ED, respectively (HR 0.4, 95% CI 0.28 – 0.63, p<0.001). Median OS in the OD group (not reached) was superior to LD and ED groups. There were also significant differences in the distribution of PFS according to group (p<0.001 for all comparisons). Surgical or local ablative treatment was conducted in 95.7%, 42.4%, and 5.4% in the OD, LD, and ED groups, respectively (p<0.001 for all comparisons). Grade 3/4 toxicities were similar among groups (70.6%, 72.1%, and 80.9%, respectively), but anemia, pain, and infection were significantly more frequent in ED pts.

Conclusions: This convenient clinical score successfully stratified metastatic gastric cancer patients into meaningful prognostic groups, aiming to further optimize the treatment options for these patients.

V387

An open-label, multicenter biomarker-oriented AIO phase II trial of sunitinib for patients with chemo-refractory advanced gastric cancer

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Background: Sunitinib monotherapy in pretreated patients with advanced gastric cancer (AGC) was investigated. Preplanned analyses of tumour biomarkers on treatment outcome were performed.

Patients and Methods: Patients received sunitinib 50 mg/day for 4 weeks with 2 weeks rest until disease progression or unacceptable toxicity. The primary endpoint was objective response rate (ORR). Secondary endpoints included progression-free survival (PFS), overall survival (OS) and safety.

Results: Fifty two patients were enrolled and treated (safety population, SP). In the intention to treat population (n=51); the ORR was 3.9%, median PFS was 1.28 months [95% CI, 1.18-1.90], median OS was 5.81 months [95% CI, 3.48-12.32], the estimated one-year survival rate was 23.7% [95%CI: 12.8-36.5]. In subgroup analyses, tumour VEGF-C expression compared with no expression was associated with shorter median PFS (1.23 vs. 2.86 months) but there was no difference in tumour control rate (p=0.142). In the SP, serious adverse events occurred in 26 patients, leading to 13 deaths, all sunitinib unrele.

Discussion: Marko Moehler: Advisory Role; Pfizer; Expert Testimony: Pfizer

Peter Galle: No conflict of interest disclosed.

V388

Prognostic significance of human epidermal growth factor-2 (HER2) in advanced gastric cancer: A U.S. and European international collaborative analysis


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Introduction: HER2 is a valid therapeutic target in advanced gastric cancer, however the prognostic significance of HER2 in this disease remains unknown. This analysis was performed to evaluate the prognostic significance of HER2 gene amplification or protein overexpression (HER2+) in advanced gastric cancer.

Methods: Paraffin-embedded tumor samples of gastric cancer pts from six prospective first-line therapeutic trials of chemotherapy without trastuzumab performed in the U.S. and Europe were examined for HER2 by immunohistochemistry (IHC, 4B5) and in situ hybridization ISH (FISH or HER2 Dual ISH). HER2 positive disease (HER2+ pts) was defined as IHC 3+ orISH+ (ISH

>2.0 HER2/CEP17 FISH or HER2 Dual ISH). The impact of HER2 status was correlated with outcome using univariate and multivariate analysis.

Results: 381 pts were evaluated: age 63 (28-88), M/F 256/125, GEI gastric/140/191, PS 0-1 338, Intestinal/Diffuse 184/177. 20.5% of all pts are Her2+. There were significant differences in HER2+ rates according to histological type (intestinal, 33%; diffuse/mixed, 8%; p<0.0001), sex (M, 26%; F, 10%; p=0.002), liver metastasis (yes, 31%; no, 11%; p<0.001) and peritoneum metastasis (yes, 11%; no, 26.2%; p=0.004). However, in the multivariate setting, only intestinal type and liver involvement were linked to higher rates of HER2 expression. Similar rates of HER2+ tumors were found in biopsies vs. resections (21% vs. 19%) and in primary tumors vs. metastases (21% vs. 16%). Median overall survival was significantly longer in HER2+ pts (13.9 vs. 11.4 mos; p=0.047) on univariate analysis. This prognostic value disappeared in multivariate analysis (p=0.3). Factors associated with favorable survival in the multivariate analysis were 3-drug combination, female sex, recurrent disease, no peritoneal metastasis and ECOG PS 0-1.

Conclusion: Approximately 20% of Western patients with advanced gastric cancer are HER2+. Although on univariate analysis, HER2+ was associated with improved overall survival, this was not independent of other known prognostic variables. Unlike breast cancer where HER2+ disease carries an adverse prognostic value, HER2+ disease is not an independent prognostic factor for pt outcome in advanced gastric cancer.

Key words: Gastric cancer, esophagogastrectomy, human epidermal growth factor-2, survival.

Disclosure: Dominique Werner: No conflict of interest disclosed.

Salab-Eddin Al-Batran: Advisory Role; Roche; Financing of Scientific Research: Roche; Expert Testimony: Roche.

V389

1-year follow-up findings from a phase II study of catumaxomab as part of a multimodal approach in patients with primarily resectable gastric cancer


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Introduction: Perioperative chemotherapy (CT) has been shown to be beneficial in gastric cancer (GC), although a significant number of patients (pts) relapse after surgery and overall prognosis is poor. The trifunctional antibody catumaxomab (anti-EpCAM x anti-CD3) is approved in Europe for the treatment of malignant ascites based on a pivotal trial involving GC pts. We evaluated the combination of neoadjuvant CT, potentially curative gastrectomy and intraperitoneal (i.p) immunotherapy with catumaxomab in a single-arm phase II study.

Methods: Pts with GC (TNM status T2/T3/T4, N+, M0) received neoadjuvant platinum-based CT followed by R0-gastrectomy (‘en-bloc’). Pts received catumaxomab i.p. as one intraoperative bolus (10 µg) and four successive 3-hour infusions of 10, 20, 50 and 150 µg. The primary safety endpoint was the rate of predefined postoperative complications observed during 30 days after surgery. Key efficacy endpoints included disease-free survival (DFS) and overall survival.

Results: 54 pts received ≥1 cycle of CT, surgery and ≥1 doses of catumaxomab. Of these, 30 (56%) received all five catumaxomab infusions. No deaths were observed within 30 days after surgery. Predefined postoperative complications were reported for 18 pts (33%; 95% CI: 21-48%). As this was less than the predefined maximum tolerable rate of 62%, the primary study endpoint was met. The most frequent complications were pulmonary infection (17%), anastomosis insufficiency (11%) and abscess (7%). Most adverse events occurred after the first intraoperative dose. Postoperative infusions were better tolerated. Transient changes in white blood cell count and peripheral lymphocytes without clinical manifestations were commonly seen after each infusion. Mean ALT, AST, bilirubin, amylase and AP values remained normal. During a

Onkologie 2011;34(suppl 6):1–305
V380

Downstaging capacity of neoadjuvant chemotherapy in distal vs. proximal gastric carcinoma: Preliminary data from an epidemiological tumor registry

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Introduction: Following the results of 3 randomized controlled trials, neoadjuvant chemotherapy has been adopted as standard of care for locally advanced gastric cancer by many European centers. All of these trials included patients with cancers located in the gastric corpus, antrum, and pylorus (distal gastric cancers, DGC) as well as cancers of the esophagogastic junction (proximal gastric cancers, PGC). However, epidemiological as well as gene expression data suggest that DGC and PGC may differ in their response to neoadjuvant chemotherapy to downstage gastric cancers is different for DGC and PGC. However, epidemiological as well as gene expression studies. The 1-year follow-up efficacy data suggest a beneficial effect on DFS. Follow-up investigations are ongoing over a total observation period of 4 years.


V391

Novel concepts in allogeneic stem cell transplantation

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Allogeneic hematopoietic stem cell transplantation (alloHSCT) is the therapeutical modality with the highest antileukemic activity in acute leukemia and myelodysplastic syndrome. Although major improvements in HLA typing and supportive care have lead to decreased transplant-related mortality, the anti-leukemic activity of alloHSCT might be offset by an increased risk for transplant-related death, especially in cases with low risk disease. Currently, alloHSCT is therefore recommended in patients with intermediate or high-risk disease as well as for patients with primary refractory or relapsed disease. A major step-forward had been the development of reduced-intensity and non-myeloblastic conditioning regimens which allow referring older patients and those with comorbidities to alloHSCT. Allogeneic graft-versus-leukemia effects seem to be so prominent, that even less intensive conditioning protocols may lead to cure of the disease. Besides modified and personalized conditioning therapy the availability of alternative graft sources like matched unrelated donors, cord blood and even haploidentical stem cell grafts has increased the number of patients with AML who could potentially undergo alloHSCT whenever the risk of therapeutic failure with conventional therapy is considerable. The role of HLA-matching and comorbidities has recently been defined enabling clinicians to counterbalance transplant- and disease-related risk factors. A major effort of translational research has been the investigation of AML-specific targets amenable for specific immunotherapy. Additionally, novel compounds like targeted tyrosine-kinase inhibitors, immunomodulatory drugs and demethylating agents are increasingly employed to treat the significant number of patients relapsing after alloHSCT. The availability of more sensitive and specific diagnostic tools for the quantification of minimal residual disease allows for preemptive intervention strategies combining novel drugs and adoptive cell therapy. The major challenge of the next era of clinical trials will be to integrate the aforementioned strategies with the aim of improving the probability of overall survival for the increasing number of patients undergoing alloHSCT.

Disclosure: Martin Bornhäuser: Advisory Role: Riemser; Financing of Scientific Research: Celgene, Novartis, Medac, Roche, Wyeth; Expert Testimony: Roche, Novartis

Freie Vorträge

Akute myeloische Leukämie I (experimentell)

V394

Genome-wide analysis of transcriptional reprogramming in mouse models of acute myeloid leukaemia

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Introduction: Acute myeloid leukaemia (AML) is caused by heterogeneous genetic/epigenetic alterations that interfere with transcriptional programs of haematopoietic stem/progenitor cells (HSPCs). Despite genetic/epigenetic heterogeneity, critical oncogenic pathways and their corresponding leukaemic transcriptional programs are thought to be shared. High-throughput molecular technologies and integration of global profiles are supposed to enhance the development of personalised treatment by identification of leukaemic transcriptional programs acting on individualised level.

Methods: In this study, an integrated genomic approach was used in established AML transplant mouse models, induced by MLL-ENL and MOZ-TIF2 fusion proteins. We combined gene-expression and epigenetic profiles (H3K9ac ChIP-Seq) to generate whole-genome data-sets at different stages of leukaemic progression. Candidate HSPC transcription factors were identified by bioinformatic approaches and validated by retroviral gene-targeting experiments in leukaemic cells in competitive proliferation assays.

Results: H3K9ac ChIP-Seq allowed us to identify several thousands candidate regulatory regions and an array of differentially accessible consensus motifs for key HSPC transcription factors, such as Gata2, Gfi1 and Sfpi1/Pa-1. Bioinformatic integration revealed that repression of Gata2 was mirrored by loss of accessible GATA motifs (loss of H3K9ac) within candidate regulatory elements. Our validation experiments showed that forced re-expression of Gata2 in leukaemic cells was not compatible with sustained proliferation, which is in line with a previously reported role of Gata2 in hematopoietic control.

Disclosure: No conflict of interest disclosed.
of expansion/differentiation of normal HSPCs. Moreover, large scale human AML datasets confirmed low GATA2 expression in AML blast cells compared to HSPCs from healthy controls.

**Conclusions:** We conclude that oncogenic pathways leading to Gata2 repression represent a recurrent theme in AML transcriptional reprogramming. We propose that Gata2 repression contributes to leukaemogenesis by deprivation of homeostatic control of expansion/differentiation in normal HSPCs. Finally, we suggest that our integrated genomic approach can be applied in human AML to identify critical factors required for leukaemic reprogramming that can be missed by means of gene-expression studies alone.

**Disclosure:** No conflict of interest disclosed.

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**V395**

The leukemogenic potential of DEK/CAN is abolished by histone deacetylase inhibitors

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²Acute myeloid leukemia (AML) is characterized by an abnormal accumulation of hematopoietic progenitors in the bone marrow (BM). The AML phenotype is maintained by an accelerated proliferation due to a differentiation block that prevents progenitors from reaching the post-proliferative stage of blast cells. This is supported by the aberrant stem cell capacity of poorly defined leukemic stem cells (LSC). Specific chromosomal translocations, such as (t(8;21), (t(15;17), (t(6;9) represent the leukemia initiating event. The related AML associated fusion proteins (AAFPs) such as PML/RARα, AML-1/ETO or DEK/CAN recapitulate the leukemic phenotype in vitro and in vivo. Most of the AAFP interfere with the epigenetic regulation of transcription by modifying key processes of chromatin remodeling such as histone acetylation and methylation as well as DNA methylation. In the DEK/CAN fusion protein all the chromatin binding domains of DEK are conserved and we recently showed that DEK/CAN is associated to chromatin and strongly interferes with chromatin modeling by inhibiting the decondensation of chromatin and accessibility to transcription. Here we investigated, whether it is possible to revert the leukemogenic potential of DEK/CAN by histone-deacetylation (HDAC) inhibitors (HDACi). We employed Valproic acid (VPA), and the two hydroxamic acid-derived HDACi Dacinostat Daciniostat and Vorinostat and studied their effects on a mouse model of DEK/CAN-positive leukemia. Here we report that Daciniostat and Vorinostat reduced the replating efficiency of DEK/CAN-positive LSC to a larger extent than VPA. Furthermore all HDACi abolished the stem cell capacity of DEK/CAN-positive LSC as revealed by a strongly reduced number of colonies in a colony forming unit spleen day 12 assay (CFU-S12), in which DEK/CAN was not anymore detectable even by RT-PCR upon exposure to HDACi. These results suggest that the leukemogenic potential of DEK/CAN is mainly mediated by an aberrant chromatin remodeling, which can be reverted by potent HDACi.

**Disclosure:** No conflict of interest disclosed.

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**V396**

The Clathrin binding and the OM-LZ domains of the CALM-AF10 fusion are sufficient to induce acute myeloid leukemia in mice

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**Introduction:** The t(10;11)(p13-14;q14-21) translocation, giving rise to the CALM-AF10 fusion gene, is a recurrent chromosomal rearrangement observed in patients with poor prognosis acute myeloid leukemia (AML). Although splitting of the CALM-AF10 fusion transcripts has been described in AML patients, the contribution of different CALM and AF10 domains to leukemogenesis is undefined. To understand the mechanism of CALM-AF10-induced AML, we defined the minimal portion of CALM-AF10 sufficient for myeloid leukemogenesis.

**Methods:** Methylcellulose-based colony forming cell (CFC) and day-12 colony forming unit spleen (CFU-S) assays were performed to assess the impact of the expression of the various CALM-AF10 mutants on transduced bone marrow cells in vitro and in vivo respectively. Long-term bone marrow transplantation assays were performed in lethally irradiated mice to ascertain the potential of the CALM-AF10 mutants in leukemia generation. Global gene expression analysis was performed for the various CALM-AF10 mutants via microarrays.

**Results:** Fusion of the clathrin-binding region of CALM (amino acids 400-648) to the OM-LZ domain of AF10 (amino acids 677-758) generated a 37 kD fusion protein (CALM-AF10 minimal fusion (MF)) with strikingly enhanced transformation capabilities in short-term in vitro and in vivo colony assays. Interestingly, in long-term bone marrow transplantation experiments, murine leukemias induced by the CALM-AF10(MF) faithfully recapitulated multiple aspects of full-length CALM-AF10 induced leukemias such as disease latency. In contrast, the CALM portion of the fusion protein including the clathrin binding domain was not sufficient on its own to induce increased activity in the CFU-S assay, indicating that the clathrin binding domain has to collaborate with the OM-LZ domain for full transforming activity. Microarray analyses demonstrated that the enhanced transforming activity of CALM-AF10(MF) is paralleled by further upregulation of the Mox-a cluster and miR-196b when compared to the CALM-AF10 full length fusion.

**Conclusion:** Our study indicates that collaboration of the OM-LZ and the clathrin binding domain of CALM-AF10 is sufficient to induce AML and suggests that future approaches to antagonize CALM-AF10-induced AML should incorporate strategies which aim at blocking these key domains.

**Disclosure:** No conflict of interest disclosed.

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**V397**

Comprehensive high density SNP array analysis of acute promyelocytic leukemia (APL) identifies multiple collateral genomic lesions and indicates recurrent micro-deletions of chromosome 1q31.3 as a new marker of unfavorable outcome

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**Introduction:** In acute promyelocytic leukemia (APL) the PML-RARA fusion product is necessary but not sufficient for the generation of leukemia. This circumstance motivates the search for additional leukemogenic and cooperating molecular lesions, which are of pathomechanistic relevance and may serve as molecular markers for improved risk stratification in APL.

**Methods:** We have analyzed 101 APL patients’ DNA samples from bone marrow blasts with high-density Genome-Wide Human SNP 6.0 arrays in search for new molecular lesions, which are of pathomechanistic relevance and may serve as molecular markers for improved risk stratification in APL.

**Results:** With our SNP array analyses a total of 279 acquired CNAs were identified consisting of 185 heterozygous deletions, 87 amplifications and 7 regions of copy number neutral loss of heterozygosity (CNLOH). Numerous
new and unknown recurrent micro-deletions were discovered. The most common of these were somatically acquired ~100 kilobase deletions—chromosome 1q31.3 in 13 of 101 (13%) samples. These deletions contained the microRNA-181a1 and microRNA-181b1 and the transcript EF413001. Patients with these deletions displayed significantly higher white blood cell counts (p=0.012), higher serum LDH levels (p=0.02), higher peripheral blood blast counts (p=0.047), and higher numbers of additional genomic alterations at initial diagnosis (p<0.0001) than patients without the deletion. Furthermore, patients with this recurrent deletion had a significantly higher cumulative incidence of relapse as compared to those without deletion (44% vs. 13%, p=0.007).

Conclusion: This study identified a plethora of novel, biologically interesting genomic lesions, which could shed more light on molecular defects cooperating with PML-RARA in the pathogenesis of APL. Furthermore, it also demonstrated for the first time that the profiles of genomic alterations in addition to PML-RARA in APL patients were heterogeneous and have an influence on disease risk definition. The further pursuit of the new genomic lesions discovered in this study is highly warranted as it could improve the current risk stratification of APL.

Disclosure: No conflict of interest disclosed.

V398
Autologous T cells from AML patients can be effectively recruited for in-vitro lysis of blasts by a novel CD33/CD3-bispecific BiTE antibody
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The CD19/CD3-bispecific T-cell engaging (BiTE®) antibody blinatumomab has shown in phase 1 and 2 clinical trials very high response rates in patients with non-Hodgkin’s lymphoma and acute lymphoblastic leukemia. Here, we report on the potential of a novel BiTE antibody targeting CD33, an antigen broadly expressed by myeloid cells including acute myelogenous leukemia (AML) blasts, in redirecting autologous T cells for in vitro lysis of blasts from AML patients.

In a first step, the cytolytic potential of the CD33-specific BiTE was investigated in co-cultures of enriched resting CD8⁺ T cells from healthy donors and CD33⁺ leukemic cell lines. CD33 BiTE concentrations as low as 0.1 nmol (1.8 µM) mediated effective lysis of leukemic cell lines at effector to target (E:T) ratios of 1:1, whereas no lysis was observed with a solely CD3-binding (1.8 pM) mediated effective lysis of leukemic cell lines. Redirected T cells from AML-induced upregulation of activation markers on the majority of T cells. We also studied the impact of TET2 mutations, global gene expression profiles from 28 patients. Results: In 23.3% of the samples we found TET2 mutations; three patients had mutations at amino acid R140 in IDH2. The analysis of the 5mC levels of the patients’ DNA revealed a 5mC content of the DNA ranging from 0.006% to 0.054%. Patients with TET2 mutations show significantly lower 5mC levels. GEP of 28 of these 30 patients demonstrated that patients with low 5mC levels had a distinct gene expression profile, whereas there was only a weak association between global GEPs and TET2 mutational status.

Conclusions: These results indicate that 5mC levels are most likely a more relevant measurement to define biologically distinct secondary leukemia subtypes than the TET2 (or IDH1/2) mutational status. To further elucidate the regulation of 5mC levels and their role in leukemogenesis larger groups of sAML as well as de novo AML patients need to be studied.

Disclosure: No conflict of interest disclosed.
Methods: Patients receiving a new line of palliative cancer treatment were eligible and completed a multi-item questionnaire after at least one cycle of a new treatment. Various demographic and patient related data were assessed: Patients’ main treatment goals and preferred behaviors, core domains of quality-of-life, locus of control (LoC), which defines the extent to which individuals believe that they can control events that affect them, and satisfaction with treatment decision. Oncology professionals provided data on treatment line, type of tumor, estimation of prognosis, localization of metastasis, Karnofsky index and consultation time. Factors influencing satisfaction with decision will be analyzed using a multiple regression model. For a significance level of 5% and a power of 80%, 415 evaluable patients are required.

Results: The survey was performed in 8 oncology hospitals and 2 private oncology practices between February 2009 and April 2011. Of 564 distributed questionnaires, 479 were collected of which 16 were incomplete and 73 patients refused participation with the following reasons: no specific reason (11), language problems (26), and other specific reasons (36) like too difficult questions. So far, more than 420 questionnaires are fully evaluable for the primary endpoint, resulting in 80% power.

Conclusion: A complex survey is feasible with a high completion rate in a multi-institutional setting. Multi-factorial testing of factors influencing a well-perceived decision for a new palliative treatment is ongoing. Full results will be available at the annual meeting in Basel.

Disclosure: No conflict of interest disclosed.

V401
Evaluation of psychosocial distress in main care-givers of patients with a metastatic solid tumor who receive treatment in a community based oncology group practice


Introduction: It is well known that people who care for patients with a metastatic solid tumor are exposed to an above-average level of distress. No data are available concerning the psychosocial distress of main care-givers of patients with metastatic solid tumors, who are treated in a community based oncology group practice.

Methods: Standardized cross-sectional survey of main care-givers and patients with a metastatic solid tumor who were treated in a community based oncology group practice in Germany between 04/2010-03/2011. Psychosocial distress of the patients and their main care-givers were evaluated using the German versions of the Distress Thermometer (DT) and the Problem List (PL). In addition anxiety and depression of the main care-givers were assessed using the Hospital Anxiety and Depression Scale (HADS-D).

Results: 200 patients (35% male, 66% female) with a median age of 68 (38-93) were interviewed. 5% did not have a main care-giver, 5% indicated that they needed no support, 52% reported one main care-giver and 38% several. Partners (68%), children (43%) and siblings (11%) were the most important care-givers. 49% of patients preferred visiting the practice in companion with their care-givers. The patients’ median score on the DT was 5 (0-10), with 35% scoring above cut-off (> 5) for psychosocial distress.

137 main care-givers (41% male, 59% female) with a median age of 61.5 (25-86) were interviewed. The relationships to the patients were as follows: partners 66%, children 23%, mothers 4%, siblings 3%, friends 3%, others 1%. The main care-givers themselves were supported by partners (41%), children (31%), friends (26%) and siblings (14%), 15% did not receive any support. The median score on the DT was 5 (0-10), with 46% scoring above cut-off (> 5) for psychosocial distress. According to the HADS-D 44% (cut-off ≥ 8) of the care-givers reported anxiety, with a mean score of 7.2 (0-21). 22% could be regarded as depressed (cut-off ≥ 8), with a mean score of 4.9 (0-17).

Conclusions: The main care-givers are highly distressed, even more than the patients themselves. Anxiety and depression are widely spread among care-givers and should be addressed by healthcare professionals.

Disclosure: No conflict of interest disclosed.

V402
The impact of the unknown and the unknowable: Development and validation of a questionnaire assessing patients’ decisional uncertainties

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Introduction: Patients making far-reaching medical decisions with their doctors have to process comprehensive information. Even more they deal with various uncertainties. A patient with prostate cancer might perceive uncertainty regarding the chance of a benefit from radical surgery and about how to weigh this up against possible side-effects, such as impotence or incontinence. Moreover, he might deal with resulting questions regarding his social life or trustability of his doctor. Some uncertainties are resolvable, others have to be accepted. However, these uncertainties challenge patients’ decision making process in particular and their well-being in general.

The QUaCC questionnaire reliably assesses 8 distinct categories of decision related uncertainty referring to a) disease related issues (prognosis/diagnosis, treatment), b) risk communication issues (deciphering information, role in the doctor-patient dyad, physician’s trustability) and c) aspects of coping with life considering the disease (mastering requirements, social integration, causal attribution).

This study aimed to develop and validate a short version of the QUaCC (QUaCC24).

Methods: The validation sample of the long version (N=708) was halved. The first half was used to identify the best 3 out of 5-6 items per category with regard to reliability (corrected item-total-correlation), item difficulty and representativeness. The second half was used for cross-validation of this selection. The resulting questionnaire was validated in two versions with slightly different item format and wording (each N=100) among cancer patients undergoing radiotherapy. A subsample from the original data base with the original wording and design (N=100) served as reference sample.

Results: Median reliability (Cronbach’s alpha) for the two modified versions was .74 and .75 respectively. The short version with the original wording and design achieved a median reliability of .81. Item difficulty was comparably satisfying for all versions.

Conclusions: The QUaCC24 combines sufficient accuracy with high practicability (administration time 10min.). It can inform physicians and psychosocial oncologists about the uncertainties patients are concerned with. This can help to tailor support to each patient’s individual needs in three steps: 1) Mapping a patient’s uncertainties, 2) Identify the changeable ones to resolve them, e.g. by providing suitable information, 3) Develop individual coping strategies to manage the unchangeable ones.

Disclosure: No conflict of interest disclosed.

Abstracts

Oncologie 2011;34(suppl 6):1–305
Abstracts

V403
Comparison of emotional well-being, perceived social support and quality of life of patients undergoing autologous peripheral blood stem cell transplantation (PBSCT) and their caregivers: a one year longitudinal investigation

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Introduction: Patients (pts) rely on their partners/caregivers (cs) as their main source of social support. In oncological daily practice the focus often laid on the pts, whereas the cs is not perceived as an individual with his/her own specific needs. The aim of this study is to determine the differences of patients (pts) and caregivers (cs) in emotional well-being, perceived social support and Quality of Life (Qol) during the course of one year following PBSCT.

Methods: 99 pts suffering from MM (69%), relapsed NHL (21%) and other malignant diseases (10%) and their cs completed the Profile of Mood States (POMS), the Illness-Specific Social Support Scale (ISSS) and the EORTC Quality of Life (Qol) during the course of one year following PBSCT.

Results: Pts as well as cs showed significantly decreasing levels of depression (pts t1: 11.7 , t2: 7.1; t3: 7.9 (p< 0.01) vs. cs t1: 11.7 ; t2: 7.1; t3: 8.5 (p< 0.01) and fatigue (pts 9.3; 7.8; 7.7 (p< 0.01) vs. cs 8.3; 6.7; 7.1 (p< 0.01)) and increasing vigor (pts 7.1; 9.6; 10.4 (p< 0.01) vs cs 9.1; 10.4; 10.8 (p< 0.05)) over the period of one year. Compared to pts cs showed more vigor prior PBSCT (pc 0.01), depression, fatigue and anger did not differ. The perception of social support remained stable over time for couples. Pts experienced more positive support (pts 3.1; 3.0; 2.98 vs. cs 2.7; 2.6; 2.6 (p< 0.01) as well as more problematic support at all time points than cs (pts 0.9; 0.8; 0.9 vs. cs 0.8; 0.8; 0.6) (p< 0.01).

Conclusion: The psycho-social situation of pts as well as their cs is improving during the first year post PBSCT. The situation of the cs is described by a significant reduction in social function and less perceived positive support than the pts. Whereas pts are in the focus of medical care, the focus of psycho-oncological attention should be at least as much on cs as on pts.

Disclosure: No conflict of interest disclosed.

V404
Generic health-related quality of life assessment in patients with autologous hematopoietic stem cell transplantation following high-dose chemotherapy

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Introduction: Longitudinal studies with health-related quality of life (HRQoL) as outcome variable are still underrepresented for patients with hematological malignancies and autologous hematopoietic stem cell transplantation (HSCT) following high-dose chemotherapy (HDC), in particular the assessment at treatment-relevant time points from patients’ and physicians’ perspective.

Methods: 63 patients undergoing six different HDC-regimens (e.g., BEAM, carmustin, etoposid, cytarabine, melphalan) completed prospectively the SF-36 (medical outcomes study-36 Short Form Health Survey) at 3 relevant time points during treatment (Course: T1) at beginning of stem cell separation, (T2) at beginning and (T3) at discharge after HDC. Data were compared to T1 (baseline) as longitudinal examination and in addition to the representative norm data using the SF-36 in the German National Health Survey.

Results: The longitudinal examination shows a significant decrease in the physical component summary score (p< 0.05) and most of the physical subscales (physical functioning, role-physical, pain), but no change of the mental component summary score and most of the mental subscales (role-emotional, social functioning, mental health). In comparison with the German norm data the four physical subscales and three mental subscales indicate a significant decrease at T3 (p< 0.0167). However, patients’ mental health is at no treatment-relevant time point different from the German norm data.

Conclusion: Patients’ mental health is more stable than the physical health. Objectively negative factors in patients’ situation have probably relatively little effect on their subjective mental well-being. Clinicians have to scrutinise patients’ answers to the question “How are you doing?”. The comparison with German norm data implicates additional results for clinical interpretation. We recommend the use of a routine HRQoL assessment in the primary cancer care and a multidisciplinary transplantation team.

Disclosure: No conflict of interest disclosed.

V405
Communication preferences and quality of life in ambulant oncology patients

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Background: Successful communication with their care givers is a key factor in quality of care in cancer patients. Coping with the disease-related stress, adherence to therapy and other outcome variables are considerably influenced by the personal communication during consultation. Different communication styles and needs in relation to different disease situations thus are a challenge for doctors to ensure appropriate patient conversations. The study is aimed at a better understanding of the informational, emotional and supportive aspects of patient needs in the doctor-patient conversation.

Methods: In fall 2010, 31 office-based oncology practices in Germany received 1860 questionnaires (60 per each practice) concerning patient related communication preferences (KOPRA, by Farin-Glattacker et al. 2009). The KOPRA-sheet contains 32 items that can be grouped into four scales. It has been psychometrically tested. This questionnaire for chronically ill patients has so far been used in orthopedic and cardiac patients. The in-house survey was based on the patient surveys of WINHO wherein more than 200 oncology practices are able to participate. To display the patient situation more closely, the common Euro-Qol-questionnaire (EQ-5D) was used next to the KOPRA instrument. The questionnaires were scanned electronically. Statistical evaluation was done with SPSS (version 17).

Results: 1731 questionnaires were returned. 82% of respondents reported an oncology diagnosis. 57% are female. Just as the mean age (63.2 women / 63.4 men), patient characteristics are fully consistent with patient patterns in oncology practices. Cancer patients showed in many dimensions of KOPRA stronger communication-preferences at the treatment-relevant time point different from the German norm data.

Conclusions: Overall, the results show a wide range of communication needs in cancer patients, each of which is activated and boosted under specific conditions. The results can support physicians to detect constellations for specific communication requirements. Further evaluation is to be done to determine the challenges for communication skills and backing of treating physicians.

Disclosure: No conflict of interest disclosed.
A phase I study of doxorubicin-loaded anti-EGFR immunoliposomes in patients with advanced solid tumors

Methods: ILs were modularly fabricated under GMP conditions with Fab' fragments from Mab C225 (cetuximab), covalently linked to pegylated lipo- somes containing doxorubicin (PLD). This first in man single-center phase I clinical trial of anti-EGFR ILs-dox was designed for patients (pts) with various solid tumors, overexpressing EGFR (DAKO EGFR pharmDx-test). ILs- dox was administered i.v. q 4 weeks at a doxorubicin (dox) dose of 5, 10, 20, 30, 40, 50 and 60 mg/m², 3 pts per dose level, for a maximum of 6 cycles. In addition to weekly safety monitoring, echocardiography was performed q 2 cycles, and pharmacokinetic assessments during cycle 1. The primary objec- tive of this study was the establishment of MTD; secondary objectives included PK, tumor response, and time-to-progression.

Results: After failure of standard treatments 26 pts were included between January 2007 and May 2010. Median age was 62 years, WHO PS-0 in 3, PS-1 in 19 and PS-2 in 4 pts. Most common histologies included pancreatic, H&N, colorectal and urothelial cancer. Two cases of neutropenia, defined as a dose limiting toxicity, occurred on dose level 7 (= 60 mg dox/m²). On all lower doses the compound was very well tolerated, e.g. skin toxicity grade 1 in 2 pts, no hand-foot-syndrome, no alopecia, no cardio-toxicity, no cumulative toxic- ity. Therefore, 50 mg dox/m² was defined as the maximum recommended dose for further phase II development. Best response to treatment included 1 CR, 1 PR and 8 SD lasting 2-12 mo (median 5.75 mo). Mean total dox half-life was calculated to be 31.0 hrs (± 7.6 hrs) and for the attached monoclonal antibody fragment of C225 17.7 hrs (± 4.3 hrs), respectively.

Conclusions: Anti-EGFR DOX loaded ILs are safe and well tolerated up to 50 mg dox/m². Clear evidence of clinical activity was observed warranting further evaluation in phase II trials.

Disclosure: Christoph Rochlitz: Stock Ownership: Lipotarg GmbH (Patenthalter)
Christoph Mamot: Stock Ownership: Lipotarg GmbH (Patenthalter)
of 21 evaluable pts stable disease was observed for four months. Both study arms will continue recruitment of pts to determine the optimal dose and regimen for phase II trials.

Disclosure: No conflict of interest disclosed.

V409
Phase I study with paclitaxel in combination with sorafenib and bevacizumab in patients with locally advanced or metastatic solid tumors

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Background: High levels of VEGF protein correlate with a poor prognosis including poor overall survival of cancer patients. Bevacizumab in addition to paclitaxel significantly prolonged PFS and increased the objective RR in patients with breast cancer. Levels of VEGF increased in a dose-dependent manner with all oral TKIs inhibiting VEGFR-2, which reflects a potential escape mechanism of the tumor to overcome antiangiogenic TKI activity.

Methods: To determine the minimal effective (MED) and maximum tolerable doses (MTD) of bevacizumab in combination with standard dose weekly paclitaxel (90mg/m2 d1, 8, 15 q4wks) and continuous oral sorafenib (400mg BID). A total of 3-6 pts will be treated per dose level with 0mg, 1mg, 2mg, 4mg, and 10mg/kg bevacizumab, respectively. Blood levels of VEGF are assessed.

Results: Currently 18 pts with various solid tumors were enrolled with bevaciazumab doses of 0mg (n=6), 1mg (n=6), 2mg (n=3), and 4mg (n=3). Most frequent adverse events were leucopenia, anemia, hand-foot-skin reaction, rash, fatigue, voice changes, and stomatitis. Five dose limiting toxicities (DLTs) occurred: rash and stomatitis (cohort I) and 3 patients with hand-foot-skin reaction (cohort II). After dose reduction of sorafenib (200mg BID), no further DLT occurred in cohort III & IV. Therefore, the maximum tolerated dose (MTD) of sorafenib in combination with standard dose weekly paclitaxel is 200mg BID continuous dosing. An additional effect of bevacizumab on plasma VEGF was not observed at 4mg/kg. Further dose escalation is ongoing. So far, twelve patients had stable disease (clinical benefit 66%).

Conclusion: Standard dose weekly paclitaxel combined with continuously dosed sorafenib 200mg bid shows antitumor activity and an acceptable toxicity profile. Additional bevacizumab up to 4mg/kg does not enhance toxicity. Final data will be presented at the meeting.

Disclosure: No conflict of interest disclosed.

V410
Tropism-modified, microRNA-1d-regulated AAV vectors for breast cancer-targeted therapeutic gene transfer

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Introduction: Vectors derived from adeno-associated virus serotype 9 (AAV9) are particularly attractive for in vivo gene transfer due to their high transduction efficiency and their excellent safety profile. Here we report the engineering of breast cancer-targeted AAV9, using a combined approach of vector targeting by incorporation of tumor specific peptides into the three-fold spike of the viral capsid and microRNA (mir)-regulated gene expression.

Methods: AAV9 vectors displaying a breast cancer-targeted peptide were established, either harbouring luciferase, or an HSV-tk gene and a mir-1d binding domain in the 3’UTR region of the expression cassette. Enzymatic removal of steric was performed by treatment of cells with neumarinidase. For biodistribution analysis, the amount of vector DNA was determined in various tissues after injection into transgenic PymT breast cancer-bearing mice by real time PCR. In vivo transduction of rAAV-luciferase was determined by bioluminescence imaging and single organ transduction was analyzed by haminometry. Cytotoxicity of TK vectors was assessed by MTT assay.

Results: Neuraminidase treatment enhanced transduction of AAV9, while AAV9 vectors displaying tumor targeted peptide ligands (AAV9-ESG) remained unaffected. After systemic vector injection, biodistribution analysis indicated that AA9-ESG de-targeted homing to heart and liver while increasing tumor homing. Compared to wild type vectors, AAV-ESG mediated strong gene expression in tumor tissue while expression decreased in almost all control tissues including heart and liver. Gene expression was not entirely specific to breast tumor tissue but was also detected in the heart. Insertions of the heart-specific microRNA sequence mir-1-d into the expression cassette strongly reduced unintended cardiac gene expression while tumor expression was retained. Finally, AAV capsid harboring SR39 mir-1d mediated a strong dose-dependent killing of HEK-293T cells after gancyclovir treatment.

Conclusion: Insertion of targeting peptides into putative receptor-binding capsid regions of AAV9 retargets homing and gene expression of AAV9 to breast cancer via an alternative cellular receptor. Tumor-specific gene expression was further improved by microRNA-regulated transgene expression. HSV-tk suicide gene transfer efficiently kill tumor cells in vitro. This emphasizes the potential of targeted AAV as promising candidate for pre-clinical therapeutic applications in breast cancer.

Disclosure: No conflict of interest disclosed.

V411
Moguntinones – new selective inhibitors for treating human gastrointestinal tumours

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Moguntinones are new synthetic designed small molecules with structural features of 3 natural products. They have been invented and patent-protected as tyrosine kinase inhibitors by Institute of Pharmacy in cooperation with I. and III. Dept. of Medicine, Mainz. Moguntinones display a new generation of inhibitors for tumor progression, angiogenesis and tumor cell resistance. Our aim was to analyse their antineoplastic effects in vitro and in vivo in human gastrointestinal cancers.

Methods: To establish their mode of action, Moguntinones were analysed in the HET-CAM assay and characterized using IC50 values of kinase assays. Secondly, the human colon cancer HT-29, DLD-1, SW480 and gastric cancer MKN-45, AGS cells were analysed in vitro and in vivo after incubation with Moguntinones, for their interference with signalling pathways by RNA and protein levels (RT-PCR, Western, ELISA, FACS). Additionally, different viability and apoptosis assays were analysed after Moguntinones were combined with or without cytostatic drugs. The in vitro data were then verified in a human xenograft NOD/SCID mouse models.

Results: The first generation of Moguntinones showed clear antiangiogenic effects in HET-CAM assays and different spectra of activity in the kinase kinome, most commonly acting on VEGFR 1-3, PDGFR and FLT-3 receptor. Secondarily, the human colon cancer HT-29, DLD-1, SW480 and gastric cancer MKN-45, AGS cells were analysed in vitro and in vivo after incubation with Moguntinones, for their interference with signalling pathways by RNA and protein levels (RT-PCR, Western, ELISA, FACS). Additionally, different viability and apoptosis assays were analysed after Moguntinones were combined with or without cytostatic drugs. The in vitro data were then verified in a human xenograft NOD/SCID mouse models.

Conclusion: Moguntinones retained their affinity to growth inhibition and cell death of human gastrointestinal cancer cell lines in vitro, which was then confirmed in vivo in the xenograft NOD/SCID mouse models.

Disclosure: No conflict of interest disclosed.
The dose of the taxanes, gemcitabine, irinotecan, vinorelbine and the anthracyclines has to be reduced in patients with elevated bilirubin, and withheld in patients with bilirubin levels higher than approximately 30 μmol/L. As a note of caution, there is already a large interindividual variability of the elimination of TKI in patients with normal liver biochemical tests. Surrogate markers for impaired liver function: Total bilirubin is the most reliable surrogate parameter for liver function, although aspartate aminotransferase (AST) has been correlated with the elimination capacity of paclitaxel, gemcitabine and epirubicin. Scoring systems such as the Child-Pugh or Model for End-stage Liver Disease (MELD) index are inferior to bilirubin for assessing liver metabolic capacity. Phenotyping assays such as midazolam clearance and pharmacogenetic tests for selected drugs (e.g. UGT1A1 for irinotecan) will become more important in the future.

Conclusions: Treatment individualization in patients with impaired liver function in oncology is feasible, but has to be adapted to the specific anticancer drug administered, and to the (presumed) etiology of liver impairment.

Disclosure: No conflict of interest disclosed.

V416
Systemic chemotherapy in patients with renal insufficiency

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Renal insufficiency is common in cancer patients and can hamper the administration of chemotherapy in appropriate dosages. In addition, several chemotherapeutic drugs can directly affect renal function. Furthermore, several clinical factors, including intravascular volume depletion, the concomitant use of non-chemotherapeutic nephrotoxic drugs (e.g. antibiotics) or radiographic contrast media and tumor-related urinary tract obstruction, can enhance renal dysfunction. The Renal Insufficiency and Anticancer Medications (IRMA) study has shown that about 7% of cancer patients have a pre-existing elevated serum creatinine level. Notably, the results of this study also demonstrated that more than 50% of these patients had an abnormal renal function based on Cockcroft-Gault or abbreviated Modification of Diet in Renal Disease (aMDRD) calculation. Therefore, management of patients with renal insufficiency is a frequent problem in daily clinical practice. For many drugs dose modification is required when administered to patients with reduction in renal function. In this context, haemodialysis represents a particular clinical setting, which requires special considerations for medical oncologists. In this part of the educational session we will discuss advantages and disadvantages of different methods for the calculation of the glomerular filtration rate as the best index for renal function and mechanisms of nephrotoxicity for frequently used novel and well-established anticancer drugs. Moreover, we will present suggestions for chemotherapy dose modification in patients with impaired renal function, including patients on haemodialysis.

Disclosure: No conflict of interest disclosed.

V418
Systemic tumor therapy with organ insufficiency: Pre-existing neuropathy

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Toxicity to the peripheral nervous system due to various chemotherapeutic agents is a common phenomenon of cancer therapy. Platinum compounds, vincristine, microtubule-stabilizing agents (i.e. taxanes), antiangiogenic agents (e.g. thalidomide) and protease inhibitors (e.g. bortezomib) are the most neurotoxic drugs. As a dose-limiting toxicity chemotherapeutic induced peripheral neuropathy (CIPN) is often associated with severe neuro-

Disclosure: No conflict of interest disclosed.

V415
Systemic anticancer treatment in patients with impaired liver function

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Introduction: Treating cancer patients with impaired liver function is an important clinical problem, and data are limited for most anticancer drugs. Still, treating oncologists must understand the meaning and limitations of liver biochemical tests, and be aware how anticancer drugs can be used in patients with impaired liver function.

Chemotherapeutic drugs: The dose of the taxanes, gemcitabine, irinotecan, vinorelbine and the anthracyclines has to be reduced in patients with elevated bilirubin, and withheld in patients with bilirubin levels higher than approximately 80 μmol/L. There is a substantial risk for gemcitabine-related deterioration of liver function in patients that have already some degree of liver impairment at the start of treatment. Clinicians should differentiate between increased bilirubin levels due to extrahepatic cholestasis that is successfully treated by local stenting, and intrahepatic cholestasis due to widespread metastases.

Molecularly-targeted drugs: The oral tyrosine kinase inhibitors (TKI) are predominantly metabolized by hepatic CYP3A4, and the elimination of these drugs is sensitive to impaired liver function. Accordingly, the dose of erlotinib, sorafenib and sunitinib (among others) has to be reduced by roughly 50% in patients with a serum bilirubin of >30 μmol/L. As a note of caution, there is already a large interindividual variability of the elimination of TKI in patients with normal liver biochemical tests. Surrogate markers for impaired liver function: Total bilirubin is the most reliable surrogate parameter for liver function, although aspartate aminotransferase (AST) has been correlated with the elimination capacity of paclitaxel, gemcitabine and epirubicin. Scoring systems such as the Child-Pugh or Model for End-stage Liver Disease (MELD) index are inferior to bilirubin for assessing liver metabolic capacity. Phenotyping assays such as midazolam clearance and pharmacogenetic tests for selected drugs (e.g. UGT1A1 for irinotecan) will become more important in the future.

Conclusions: Treatment individualization in patients with impaired liver function in oncology is feasible, but has to be adapted to the specific anticancer drug administered, and to the (presumed) etiology of liver impairment.

Disclosure: No conflict of interest disclosed.

V414
Dose adjustment in patients with organ insufficiency

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Anticancer drugs have to be used novel and well-established anticancer drugs. Moreover, we will present advantages and disadvantages of different methods for the calculation of the glomerular filtration rate as the best index for renal function and mechanisms of nephrotoxicity for frequently used novel and well-established anticancer drugs. Moreover, we will present suggestions for chemotherapy dose modification in patients with impaired renal function, including patients on haemodialysis.

Disclosure: No conflict of interest disclosed.

V412
Mummies, medicine and molecules

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The pathopathological investigation of the human remains from various necropoles during the last decades has enabled us to obtain a clearer image of the life and disease conditions in ancient Egyptian populations. A methodical approach including modern methods of CT scans and molecular investigations offers considerable insight into the disease spectrum and the consequences of various disorders on the affected populations.

Our studies cover a time period from the early/predynastic period (c. 3,200 BC), the Old Kingdom (c. 2,600 – 2,400 BC), the Middle Kingdom (MK; c. 2050 – 1650 BC), the New Kingdom (NK; c. 1550 – 1070 BC) and the Late Period (LP, c. 700 – 500 BC). Besides on-site pathopathological examination selected specimens were investigated by radiology, including CT scans. Furthermore, we applied molecular biological techniques to search for ancient pathogenies with a main focus on ancient tuberculosis.

The age at death and the sex ratio were constant between the periods with the highest death rate between the 2nd and 3rd decade of life. The infant/adolescent mortality was comparably low in the different tombs. A whole variety of diseases was detected, such as infectious diseases, including tuberculosis which was molecularly proven in up to c. 25% of certain populations. Metabolic disorders, such as scurvy, osteomalacia and chronic anemia (crbeha orbita1is, porotic hyperostosis) were seen in high frequency in the MK populations, but significantly less in the NK-LP people. Trauma rates were comparatively high, and lesions due to degenerative joint and vertebral diseases were significantly higher in LP than in MK or NK individuals suggesting higher mechanical load in the later population. Isolated cases of benign and malignant (secondary) bone tumors and various soft tissue/organ diseases indicate that „civilisation“ disorders were present when the living conditions assured survival into advanced age. Finally, we obtained circumstantial evidence for social care of deceased individuals and have detected signs for medical practise, in particular surgical interventions.

In summary, we provide first data on various disease in ancient Egypt including initial epidemiological aspects. The molecular analysis helps us to identify ancient pathogenies and will hopefully enable us to track evolutionary pathways of tuberculosis, malaria and leishmaniasis.

Thereby, we unravel a more and more clearer picture of live and living conditions in ancient Egypt.

Disclosure: No conflict of interest disclosed.

V413
Systemic tumor therapy with organ insufficiency: Pre-existing neuropathy

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Toxicity to the peripheral nervous system due to various chemotherapeutic agents is a common phenomenon of cancer therapy. Platinum compounds, vincristine, microtubule-stabilizing agents (i.e. taxanes), antiangiogenic agents (i.e. thalidomide) and protease inhibitors (i.e. bortezomib) are the most neurotoxic drugs. As a dose-limiting toxicity chemotherapeutic induced peripheral neuropathy (CIPN) is often associated with severe neuro-

Disclosure: No conflict of interest disclosed.
logical disability and neuropathic pain leading to reduced quality of life. Depending on the cancer drug used length-dependent sensory, sensorimotor and autonomic dysfunctions arise. Acute (i.e. oxaliplatin cold-induced hyperpathy) and chronic neurotoxic effects can be differentiated. Platinum compounds are unique by induction of a characteristic ganglionopathy and by increasing CIPN even after cessation of chemotherapy ("coastline" phenomenon).

A multitude of interplaying factors like cumulative dose, dose per cycle, duration of infusion and combination regimens are the most relevant determinants of neurotoxicity. Moreover pre-existing neuropathies especially in older patients with diabetic, alcoholic, inflammatory or hereditary polyneuropathies display risk factors for the development of severe CIPN. Prevention is essential as restorative and neuroprotective approaches with various drugs frequently failed. Results of two premature stopped studies investigating Mg/Ca infusions against cumulative oxaliplatin-induced CIPN in colon cancer patients are controversial discussed. Moreover, subcutaneous instead of i.v. administration of bortezomib may be less neurotoxic but equally effective.

Neurological as well as electrophysiological evaluation is useful by differentiating CIPN from pre-existing and other causes of neuropathies (i.e. paraneuroplastic, neoplastic, radiation induced, neurolymphomatosis, radiculopathies), which has a great impact on further therapeutic strategies. The assessment of the acuity of symptom development, distribution and affected compartment of the peripheral nervous system (axonal versus demyelinating pattern) greatly contributes to the correct diagnosis.

As CIPN is a long lasting and sometimes irreversible side effect of antineoplastic therapy an interdisciplinary approach between oncologists and neurologists is helpful for the optimal management of cancer patients.

Disclosure: No conflict of interest disclosed.

Fortbildung Ösophaguskarzinom

V419 Overview of current treatment strategies for locally advanced esophageal carcinoma

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Recent progress in the prognosis of patients with esophageal cancer appears rather to reflect better patient selection and improved peri-operative management than improved efficacy of the therapy. There are still a lot of controversies regarding treatment strategies due to lack of studies with adequate patient numbers and inclusion of an homogenous study population. Surgery alone showed high rates of locoregional and distant failure for patients with locally advanced stages and consequently a 5-year survival rate of only 15-20%. Postoperative radiotherapy and chemotherapy were usually not feasible due to the morbidity of the esophagectomy. Preoperative chemotherapy showed a significant but only modest improvement in overall survival over surgery alone. Most studies investigating neoadjuvant chemoradation in squamous cell carcinoma and adenocarcinoma were under-powered and showed no significant difference, however, in several meta-analyses a significant reduction of tumor related death rate could be shown at the expense of an increased postoperative mortality. Two European trials randomized mainly squamous cell cancer patients to chemoradiation with or without surgery. The designs of these two trials were slightly different. Both showed an increased mortality rate linked with surgery, a better locoregional control with surgery and no significant difference of overall survival according to their prospective hypothesis. The results of these trials have been controversially discussed. No general guideline of a treatment strategy is possible, because too many variables have to be considered like histology, tumor localization, response to neoadjuvant therapy, co-morbidity, performance status and age of the patient. The adenocarcinoma of the distal esophagus or the esophago-gastric junction should be resected whenever possible. These tumors respond less to neoadjuvant therapy and the risk of surgery is lower. The controversy refers here more about neoadjuvant chemotherapy versus chemoradiation. Personal conclusions and recommendations of the author were discussed.

Disclosure: No conflict of interest disclosed.

V421 Esophageal cancer: Systemic treatment in the neoadjuvant and palliative setting

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Esophageal cancer is frequently diagnosed at a locally advanced or metastatic stage. Due to it’s unique submucosal lymphatic drainage lymphatic spread occurs early in the course of the disease. As the prognosis for patients with nodal involvement is very poor barely exceeding a 5-year survival rate of more than 20 percent, combined multi-modal approaches have been studied. The theoretical advantages of adding chemotherapy to the treatment of esophageal cancer are potential tumor down-staging prior to surgery, as well as targeting micrometastatic disease, and thus decreasing the risk of distant spread.

Neoadjuvant Neoadjuvant chemotherapy has been investigated in two adequately powered phase III trials. The US Intergroup trial 0113 [1] including all histologies showed negative results for three cycles of cisplatin plus 5-fluorouracil (5-FU) followed by surgery followed by two cycles of chemotherapy compared with surgery alone (overall survival OS 14.9 versus 16.1 months). In contrast the UK MRC trial [2] assessing a similar treatment approach including all histologies with two cycles of cisplatin plus 5-FU followed by surgery followed by chemotherapy compared to surgery alone revealed an advantage for OS (16.8 vs 13.3 months, HR 0.79, p=0.004). These conflicting results might in part be the result of different dosages and different time intervals of preoperative chemotherapy. The toxicity observed in the US trial contributed that only 80% of the patients proceeded to surgery versus 92% in the MRC trial. In both trials responding patients fared a better prognosis than non-responding patients. At least four separate trials have compared cisplatin-based perioperative regimens (neoadjuvant and adjuvant chemotherapy) to surgery alone in esophageal cancer [1, 3-5]. Those that focused solely on esophageal cancer did not reveal survival benefits[1,3], whereas the two that included patients with adenocarcinoma (AC) of the stomach and GEJ did show such a benefit [4,5]. In the MAGIC-trial [4] approximately 26% of the patients enrolled had AC of the GEJ or distal esophagus. Despite the fact that 58% of patients were unable to undertake all six cycles of chemotherapy, the perioperative chemotherapy group had a statistically significant higher likelihood of OS compared to those treated with surgery alone (HR: 0.75, 95% CI: 0.60-0.93, P=0.009), with an improved median OS (24 mo vs 20 mo) and 5-year OS (36% vs 23%). Gebski et al have evaluated neoadjuvant chemotherapy compared to surgery alone in a meta-analysis [6]. Although the two neoadjuvant chemotherapy and chemoradiotherapy data pools are not directly comparable, the absolute survival benefit of chemotherapy appears to be less than that of chemoradiotherapy (7% vs 13% at 2 years).

Palliative chemotherapy: Chemotherapy as a single modality has largely been used for palliation of patients with advanced esophageal cancer. The cumulative response rate for any one drug is low, of the order of 15% to 35%, and there is no indication of a survival benefit. Cisplatin-based combinations appear to be the best studied and demonstrate the most favorable response activity. On the basis from recent phase II trials indicating the activity of agents such as paclitaxel, docetaxel and irinotecan in recurrent or metastatic esophageal cancer, these agents are now being incorporated into combined modality regimens. Recent uncontrolled studies with combination chemotherapy plus cetuximab in esophagogastric adenocarcinomas as well as aquamous carcinoma showed good tolerability and rates of tumor remission.

Literature:

Disclosure: Wolfgang Eisterer: Financing of Scientific Research: Merck, Amgen, Roche, Novartis; Expert Testimony: Merck, Amgen, Roche
Pitfalls in multimodal treatment of esophageal cancer

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Based on results of randomized trials and meta-analyses multimodal treatment of localized esophageal cancer has gained increasing acceptance. However, in daily practice the combination of different treatment modalities, e.g. chemotherapy, radiotherapy, and surgery applied by different medical specialists may raise a couple of pitfalls that need to be realized.

First of all the indication of multimodal treatment has to be taken correctly based on a complete diagnostic work-up. If a tumor infiltration of the tracheobronchial tree or peritoneal metastases can not ruled out by appropriate diagnostic procedures prior to preoperative therapy, they may be proved at the time of surgery. This will lead to confusion about the efficacy of preoperative therapy and usually will leave a patient overtreated who should have got palliative therapy without surgery. Moreover, if a patient is medically unfit for major surgery, this condition should be recognized before the start of any therapy and at the time where surgery appears to be the last treatment option. These pitfalls may be avoided if the patient is supervised by an oncologist with experience in upper GI-cancer and if major treatment decisions are taken by a group of specialists within a tumor board.

When the treatment plan is agreed upon all specialists the time schedule of this treatment needs to be communicated with the patient and with all physicians involved. Any delays within the treatment plan which are not forced by side effects should be avoided, since they will hamper the efficacy of radiochemotherapy and probably also that of chemotherapy within the multimodal concept.

When multimodal therapy shall be applied to a patient and a treatment concept is within a tumor board it is very important that all the medical disciplines involved are familiar with the treatment schedule and have experience in handling treatment related complications. Otherwise the risk for toxic side effects in general and the risk for life threatening complications will significantly increase.

All these pitfalls will be demonstrated by clinical cases.

Disclosure: No conflict of interest disclosed.

V427
Telomere elongation in vivo and clinical response upon androgen treatment in a patient with aplastic anemia and a heterozygous hTERT gene mutation

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Acquired bone marrow failure syndromes (BMFS) are thought to result from (e.g. auto-immune mediated) damage to the hematopoietic stem and progenitor cell compartment leading to a severely diminished replicative hematopoietic stem cell reserve in the bone marrow or insufficient numbers of early progenitor cells without the absence of hematopoietic stem cells. As a consequence, acquired BMFS are characterized by significantly shortened telomere length in peripheral blood cells. Telomere shortening can be counteracted by the telomerase complex. Mutations in subunits of human telomerase reverse transcriptase (hTERT), as well as genes encoding for telomerase-binding proteins as well as in members of the shelterin complex have been described both in inherited and acquired bone marrow failure syndromes.

Here, we report on a patient with moderate, acquired aplastic anemia and a non-synchronous variation of codon 1062 of the hTERT gene (pAla1062Thr).

The patient was found to have dramatically shortened telomere length of cancer-derived lymphocytes. In addition, the patient’s peripheral blood cells revealed a heterozygous hTERT gene mutation in vivo.

This case demonstrates the current state of our knowledge of telomere biology and its clinical implications. Possible strategies for telomere length restoration in vivo are discussed.

Disclosure: No conflict of interest disclosed.
granulocytes and lymphocytes (below the 1% percentile of normal individuals).

Based on clinical presentation, we decided to start androgen treatment (testos- 
teron undecanoat; Andriol®) first rather than to give standard immunosuppres- 
sion. The initially red blood cell transfusion dependent and thrombocytopenic 
patient became completely transfusion independent after twelve months of 
ongoing androgen treatment. His platelet count increased steadily and signifi- 
cantly and became stable. After 4 years of continued treatment hemoglobin 
and platelets reached normal levels. Excitingly, the clinical and hematological 
improvement during androgen therapy was mirrored by a continuous and 
persistent lengthening of telomere length in total peripheral blood mononu- 
clear cells (MNCs) measured by quantitative PCR. Furthermore, telomeres 
became significantly elongated in both major leukocyte subpopulations, i.e. 
lymphocytes and granulocytes measured by Flow-FISH indicating that telom- 
ere lengthening has occurred in vivo in the multipotent hematopoietic pro- 
genitor compartment. In summary, we describe here for the first time the 
time coincidence of blood cell recovery mirrored by elongation of telomere length in 
hematopoietic cells in vivo in a patient with an aplastic syndrome under 
treatment with androgen.

Disclosure: No conflict of interest disclosed.

V428
Platelet kinetics in ITP patients treated with thrombopoietin receptor agonists
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Introduction: Thrombopoietin receptor agonists (Tpo RA) increase platelet 
counts in the majority of chronic autoimmune thrombocytopenia (ITP) 
patients. To date, there is limited information available on platelet kinetics 
(PK) in these patients.

Methods: In order to determine platelet survival (PS), autologous platelets 
were labeled with11In oxine and transfused in six patients undergoing treat- 
ment with Tpo RA (romiplostim n = 3; eltrombopag n = 3).

Results: Stable platelet counts of greater than 100 x 10^9/μL were observed in 
all six patients. Platelet survival was decreased in all cases (mean 2.10 d; range 
0.13 – 3.73 d). No correlation was found between platelet count and PS. 
Similarly, there was no significant relationship either between platelet turn-
over and platelet counts or PS. However, a high platelet turn over, exceeding 
25 and three times the norm, respectively, was observed in two patients who 
presented the lowest PS (0.13 and 0.83 d, respectively). Two patients had a 
moderately shortened PS (1.91 d and 2.42 d), and, correspondingly, a moder- 
ately increased platelet turnover rate (63,072 and 72,872 pl/h/dL), respecti-
vely.

Conclusion: These results indicate that Tpo RA may not only overcompensate 
platelet destruction in ITP; but may interfere with other mechanisms which, in 
some cases, results in a reduced platelet destruction rate.

Disclosure: Oliver Meyer: Financing of Scientific Research: Amgen, 
GlaxoSmithKline
Abdulgabar Salama: Financing of Scientific Research: Amgen, 
GlaxoSmithKline

V429
Retrospective analysis of therapy with rabbit thrombocytopoietin globulin (rATG) in acquired aplastic anemia (AA)
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Introduction: Several clinical trials established treatment with horse ATG 
(hATG) and cyclosporine A (CsA) as standard treatment of AA in patients 
(pts) who are not candidates for stem cell transplantation (SCT). However, the 
hATG brand Lymphoglobulin® was withdrawn from the market. As the only 
other hATG brand ATGAM®, is not approved in Europe, hATG was replaced 
by rabbit ATG (rATG). Recent data comparing hATG/CsA and rATG/CsA in 
untreated AA showed significantly lower response rates and survival using 
rATG. We analyzed retrospective data of 1st line rATG therapy to prove these 
findings with a longer follow up (FU).

Methods: Retrospective analysis of 1st line rATG treatment of AA.

Results: Up to now data of 30 pts from 9 centers in Germany were analyzed.

Characteristics of the pts: 13 male, 17 female; median age 58 (6-79) years; 
29/30 pts had idiopathic AA. 47% of the pts had severe AA, 40% a very severe 
AA and 13% showed a non-severe AA. Median granulocyte count was 0.3 G/L. 
29 pts required red blood cell concentrate and 90% platelet concentrate trans-
sfusions. 71% of pts received Thymoglobulin® and 29% Fresenius ATG S®. 
29 pts required red blood cell concentrate and 90% platelet concentrate trans-
fusions. 71% of pts received Thymoglobulin® and 29% Fresenius ATG S®. 
 Median daily dose of Thymoglobulin® was 3.5 mg/kg (range from 2.5-3.75 
mg/kg) for 5 days. Response rates (at time of best response): CR 19%, PR 22 
% and NR 59% (not available in 3 pts). Relapses occurred in 2/11 responders 
and clonal evolution (PNH) was observed in 2 pts. The median FU for all pts 
was 296 days (range, 20-2127) and for surviving pts with PR/CRC 822 days 
(range, 122-2127). Two pts received allogeneic SCT. 30% of 23 pts with avail-
able FU died. In 5 pts death was caused by infections; 2 cases remained 
unclear. Adverse events were reported in 60% of pts consisting of anaphylaxis/allergy 
in 17%, serum sickness in 7%, fever/chills in 20%, and bacterial/viral/ fungal 
infections in 50% of pts.

Conclusions: Response rate and survival after rATG+CsA in this retrospective 
analysis are lower than in historical controls (e.g. hATG+CsA treatment in 
previous studies of the German AA Study Group) and the rate of (early) infec-
tions seems to be high. Our results are in accordance with recent reports from 
other groups. This retrospective data collection is ongoing and will be updated. 
Existing evidence suggests that hATG should still be considered as “gold 
standard” for AA treatment. If hATG is not available, treatment with rATG 
should be considered instead of no treatment. There is need for action to 
achieve availability of hATG.

Disclosure: Britta Höchsmann: Financing of Scientific Research; ja; Expert 
Testimony: ja
H. Schrezenmeier: Advisory Role: ja; Financing of Scientific Research: ja; 
Expert Testimony: ja

Oncologie 2011;34(supp1 6):1–305
Abstracts

114
Role of bone marrow failure in paroxysmal nocturnal hemoglobinuria (PNH) patients chronically treated with Eculizumab

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Background: The monoclonal antibody eculizumab blocks the complement-mediated intravascular hemolysis in PNH leading to marked decrease of anemia, transfusion requirements, fatigue, and thromboembolic events. Some patients, however, still require blood transfusions to various degrees. In this retrospective study we correlated the change in hemolytic parameters, need for transfusions and reticulocyte production index (RPI) in PNH patients treated with eculizumab.

Patients: Nineteen PNH patients were chronically treated with eculizumab. The median therapy duration was 16 months (range, 6–46 months). Hemolysis, requirement of transfused packed red blood cells (PRBCs), erythropoietic response by reticulocyte production index, serum iron parameters and monospecific direct agglutination test (DAT) were analyzed over time.

Results: Eculizumab effectively inhibited intravascular hemolysis in all PNH patients as evidenced by an 85% decrease in median LDH levels. Persistent elevation of reticulocytes, bilirubin, decreased levels of haptoglobin and hemopexin along with positive DAT were observed in most patients suggesting continuing extravascular hemolysis. Stratification for an RPI > 2 (n=9), indicating an effective erythropoietic response, revealed a reduction of transfused PRBCs from median of 20 to 1 units/year (p=0.008). In patients with an RPI < 2 (n=10), demonstrating an insufficient bone marrow response, median transfusional need remained unaffected. However, half of those patients (n=5), showed at least a reduction in transfusional need. A significant increase in ferritin levels from a median of 203 μg/l before to a median of 1360 μg/l (p=0.011) after initiation of eculizumab for patients with an RPI < 2 and from a median of 36 μg/l to 209 μg/l for an RPI > 2 was observed.

Conclusions: Intravascular hemolysis is effectively controlled with eculizumab and is associated with a concomitant improvement in anemia while some extravascular hemolysis may persist. The degree of the underlying bone marrow failure, however, is reflected by the RPI and determines the need for blood transfusion in treated PNH patients. Iron should not be routinely supplemented in PNH patients treated with eculizumab without close monitoring of iron parameters, and iron depletion therapy should be considered in case of overload.


Synergism of Suv39h1 deficiency and ectopic NRas expression in a murine model of leukemogenesis

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With the aim of analyzing the potential cooperative effects of the epigenetic modification of histone methyltransferase (Suv39h1) deficiency on NRas-induced leukemogenesis, we retrovirally transduced lineage negative (lin-)) wild-type (wt) or Suv39h1-deficient bone marrow (BM) cells with vectors expressing wt NRas (NRaswt) or eGFP only as control, and transplanted the cells into lethally irradiated C57BL/6 recipients. Expression of NRaswt in wt BM induced myelomonocytic leukemias (ML) approximately three months after transplantation and histiocytic sarcoma (HS) with a prolonged latency in a subset of mice (30%). ML were characterized by leukocytosis, anemia, thrombocytopenia and hepatosplenomegaly. Leukemic cells isolated from the spleen expressed monocyte/macrophage markers (CD11b/CD45/80) or granulocytic markers (CD11b/Gr1). In contrast, histiocytic cells were either positive for monocytic/macrophage markers (CD11b/CD45/80) or dendritic markers (CD11c/MHCII).

NRaswt expression activated Erk and Akt levels equally aberrant as induced by mutually activated NRasG12D, Consistent with primary human myelomonocytic leukemia cells, we also detected Stat5 activation in murine leukemic cells in vivo. Clonal evolution in NRaswt-induced leukemias included expansion of clones with activating vector insertions in the oncogenes Evi1 and Pim1. Evi1 overexpression coincides with NRAS mutations in human AML, indicating that our model mimicks important aspects of human leukemogenesis.

In contrast, NRasG12D expression in Suv39h1-deficient cells induced polyclonal HS only, with a latency of three weeks which was similar to HS induced by mutually active NRas. Clonal numerical or structural chromosomal aberrations were not observed independent of the genetic background. The response of Suv39h1-deficient and wt cells to NRasG12D expression in vitro did not differ regarding apoptosis, senescence or proliferation. Nevertheless, Suv39h1-deficient lin- BM cells
had an increased potential to form colonies in semisolid medium in comparison to Wt cells. In conclusion, overexpression of wild-type NRas is sufficient to activate Ras signalling and to induce myeloid leukemogenesis. In addition, Suv39h1 deficiency collaborates with activated NRas signalling. We propose that the loss of repressive complexes due to Suv39h1 deficiency contributes to NRas-induced leukemogenesis by differentially regulating genes involved in (de-) differentiation processes.

Disclosure: No conflict of interest disclosed.

V435

Neutropenia-induced feedback G-CSF production is regulated on the transcriptional level independent from the presence of commensal germs

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Introduction: The mechanism whereby neutrophils are kept constant in the steady-state remains incompletely understood. G-CSF has been found to be the major cytokine, and feedback regulation of G-CSF synthesis has recently been suggested to rely on a subset of T-lymphocytes. Because emergency granulopoiesis targets like MEK 1/2 then ERK 1/2 and therefore cell proliferation. This activation after the stimulation took place followed by a decrease after additional 15 minutes, AMD3100 lead to a sustained production of G-CSF. This activation after SDF-1 and AMD3100 treatment generated phosphorylation of different targets like MEK 1/2 then ERK 1/2 and therefore cell proliferation.

Conclusion: In this work we demonstrate that AMD3100 should not be considered as a complete antagonist as it is mostly found in the literature. Therefore we suggest to regard AMD3100 as a "partial agonist of functions". The conformational changes induced by AMD3100 are not the same like those induced by SDF-1, nevertheless both changes have intracellular consequences, which have to be further characterized.

Disclosure: Abraham Zepeda-Moreno: No conflict of interest disclosed.

Anthony Ho: Advisory Role: Is on the Advisory Board of Genzyme.

V437

Cerebrospinal fluid analysis – a comparison of flow cytometry and conventional cytology for patients with hematologic diseases at risk for central nervous system involvement

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Introduction: CNS involvement in patients with hematologic diseases is a rare but often fatal event. Sensitive and predictive methods are needed to improve the therapeutic options for these patients. Most CSF samples are paucicellular and cells show rapidly decreasing viability. These problems limit the application of Flow Cytometry in day to day practice and multi centre clinical trials.

Methods: 260 CSF samples obtained from 130 patients treated in 13 centres all over Germany stabilized in Transfix Medium were sent to central laboratory. Predefined antibody panels in accordance with the suspected diagnosis were applied (FC). Simultaneously, conventional cytomorphologic (CC) analyses were conducted.

Results: Stability tests of several samples confirmed the feasibility of the test method. Among all positive samples (55/260=21%) 38% were found positive by FC and CC, 58% were detected by FC alone, and 1% were suspicious by FC only, respectively.

Results: No conflict of interest disclosed.

V436

SDF-1 vs AMD3100: an experimental review

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Introduction: SDF-1 is the natural ligand of the CXCR4 receptor . This axis is an important promoter of migration, growth, retention and homing of hematopoietic stem cells (HSCs). For a long time, it was thought that blocking the CXCR4 receptor for example with AMD3100 would lead to mobilization of HSCs or leukemic cells out of their protective niche and this could therefore be used for stem cell transplantation after mobilization or better treatments of these oncologic entities. But is AMD3100 only occupying the receptor without any other effect? Here we present a comparative study which involves the characterization of cellular effects of SDF-1 and AMD3100.

Methods: Following our CXCR4 model described previously, Jurkat cells were used in this study. Flow cytometry was used to characterize the surface expression of CXCR4 in the presence of SDF-1 and AMD3100 and to measure the intracellular calcium levels in response to the activation of the axis by these two components. As a second step, cAMP was measured by ELISA 5, 15 and 30 minutes after stimulation. To document the activation of downstream signalling pathways we used a proteome profiler array to detect the activation of several human phospho-kinase targets. Proliferation assays were performed to measure the effect of the intracellular signalling pathways activation.

Results: The FC analyses showed that while SDF-1 was able to internalize the receptor, AMD3100 did not produce the configuration changes necessary to induce the same effect. As a consequence of this configuration differences, SDF-1 induced the release of intracellular calcium while AMD3100 did not. On the other hand, both agents could induce the activation of adenyl cyclases but in different manners. While SDF-1 caused a peak of cAMP 15 minutes after the stimulation took place followed by a decrease after additional 15 minutes, AMD3100 lead to a sustained production of cAMP. This activation after SDF-1 and AMD3100 treatment generated phosphorylation of different targets like MEK 1/2 then ERK 1/2 and therefore cell proliferation.

Conclusion: In this work we demonstrate that AMD3100 should not be considered as a complete antagonist as it is mostly found in the literature. Therefore we suggest to regard AMD3100 as a "partial agonist of functions". The conformational changes induced by AMD3100 are not the same like those induced by SDF-1, nevertheless both changes have intracellular consequences, which have to be further characterized.

Disclosure: Abraham Zepeda-Moreno: No conflict of interest disclosed.

Anthony Ho: Advisory Role: Is on the Advisory Board of Genzyme.
57% of all patients with synchronous MRI or CT scan had positive FC results but normal radiologic findings.

**Conclusion:** Flow Cytometry of cerebrospinal fluid shipped over long distances is feasible in a multicenter setting and improves the diagnostic sensitivity to investigate CNS involvement in hematologic diseases. The absence of symptoms or typical radiologic findings does not exclude CSF-involvement in a surprisingly high proportion of patients.

**Disclosure:** No conflict of interest disclosed.

V438

**Computed tomography follow up in patients with malignant lymphoma: does semi-automated volumetry of lymphoma improve therapy response classification compared to manual linear measurements?**

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**Introduction:** Impact of semi-automated volumetry compared to unidimensional measurements of lymph nodes on therapy response classification in CT follow-up of malignant lymphoma.

**Methods:** MSCT scans of 65 patients with malignant lymphoma prior to therapy (baseline) and after 2 cycles of chemotherapy (follow-up) were included. A total of 313 target lymph nodes (55 cervical, 131 thoracic and 126 abdominal) were evaluated by two radiologists independently. Long axis diameter (LAD), short axis diameter (SAD), WHO-square and volume were determined manually and using semi-automated segmentation software. Time for manual and semi-automatic segmentation was evaluated. Therapy response was calculated for each parameter based on „IWC“ lymphoma-guidelines and „RECIST“-adapted guidelines. Mean of metric and volumetric measurements served as the reference standard. Statistical analysis encompassed intraclass correlation coefficients (ICC), t- and McNemar-test.

**Results:** Over all regions mean lymph node size in baseline/follow-up was 23.8±10.3 mm/17.0±2.2 mm for LAD and 7.2±13.5 ml/3.4±5.9 ml for volume. Mean evaluation time for semi-automated segmentation without need for correction was shorter (16.6±11.7 sec) than for manual measurements (29.0±14.5 sec). In 65% of all lymph node correction was necessary and evaluation time increased to 39.5±25.9 sec. Regarding therapy response, semi-automated volumetry obtained significantly more accurate classifications than semi-automated and manual LAD and SAD (e.g. volume 87.8% vs. semi-automated LAD 83.8%, manual LAD 78.9%, all p<0.05).

**Conclusions:** Semi-automated lymph node volumetry is more accurate for therapy response classification in patients with malignant lymphoma as compared to established linear measurements (e.g. LAD).

**Disclosure:** No conflict of interest disclosed.

V439

**Lenalidomide-based regimen proved effective in multisystem Langerhans cell histiocytosis: case report**

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**Introduction:** Langerhans cell histiocytosis is a rare idiopathic disease with diverse clinical manifestations ranging from a single osteolytic lesion to generalized disease. Various treatment regimens have been proposed, however, with inconsistent outcomes. Herein we are the first to report on a therapy effect of lenalidomide (Revlimid™)-based regimen.

**Materials and Methods:** A male, born 1973, was diagnosed with multisystem Langerhans cell histiocytosis affecting the lymph nodes, skin and lungs at the age of 35. The symptoms reminded a lymphoma with expressed B-symptoms and generalized lymphadenopathy. Initially, the patient was treated with 6 cycles of cladribine-based regimen combined with radiotherapy of the perianal area, this led to complete remission. However, in two months the disease relapsed, newly also with bone involvement. The second line treatment consisted of 4 cycles of CHOEP regimen (cyclophosphamide, doxorubicin, vincristine, etoposide, prednisone) completed in March 2010 with high-dose BEAM (carmustine, etoposide, cytarabine, melphalan) chemotherapy followed by autologous peripheral blood stem cell transplantation, which put the disease into complete remission again. Nevertheless, after 5 months the 2<sup>nd</sup> relapse was diagnosed and the patient was started on lenalidomide (25 mg orally daily 1-21 of a 28-day cycle) with dexamethasone (40 mg orally once a week) treatment in October 2010 (8 planned cycles).

**Results:** Within several doses of lenalidomide, fatigue and other B-symptoms receded gradually, which was followed by a decrease of blood inflammatory markers. A restaging PET/CT examination during the 4<sup>th</sup> cycle showed reduction in the size of affected lymph nodes and their glucose uptake as well as generally reduced extent of the disease. Moreover, through a series of several ultrasound examinations, we documented gradual regression of enlarged lymph nodes during the therapy. To date, 6 cycles have been carried out.

**Conclusions:** Lenalidomide-based regimen proved effective in a patient with the repeatedly relapsed aggressive form of multisystem Langerhans cell histiocytosis as demonstrated in laboratory and radiological data enclosed.

**Disclosure:** No conflict of interest disclosed.
Freie Vorträge
Mammakarzinom

V440
Everolimus in combination with carboplatin: a promising treatment with low toxicity in heavily pretreated metastatic breast cancer

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Introduction: Preclinical studies in breast cancer suggested that combining RAD001 with carboplatin may produce higher activity than each drug alone. The mTOR inhibitor everolimus is a promising drug in the treatment of solid tumors. We conducted a phase I trial with everolimus and carboplatin with the goal to assess its safety in pretreated patients (pts) and to determine the maximum tolerated dose (MTD).

Methods: Pts with metastatic breast cancer were treated with oral everolimus at different dose level (level I: 2.5mg; II: 5mg; III: 7.5mg; IV: 10mg/d) in combination with weekly carboplatin (AUC2) in a 21-day cycle. 3 pts were assigned to dose level I-III, 6 to dose level IV. Dose levels were escalated strictly step by step. If no DLT occurred in 3 pts, following patients were assigned to the next dose level.

Results: The median number of previous chemotherapies was 4 (range: 1-11). Overall, the study has enrolled 15 pts (median age 58). Dose level was escalated to level IV as no DLT occurred at level I-III. Pts received a median of 4 cycles (range 1-13); 9 completed at least 4 cycles, 3 completed at least 8 cycles, and one patient 13 cycles. There were no DLTs at dose level I-IV during the first cycle. Based on the pre-determined definition of MTD, the maximum planned dose level IV was selected as the MTD. Overall, toxicities have been manageable. Most frequent G3/4 toxicities included thrombocytopenia in 5 pts, leukopenia in 4 pts, infection in 2 pts, dyspepsia in 1 pt. Response rates in 14 pts evaluable for response were as follows: 14% PR, 43% SD, and 43% PD. In 9 pts who completed 4 cycles defined in the protocol for efficacy assessment, response rates were: 2/9 PR, 6/9 SD.

Conclusion: Everolimus + carboplatin are well tolerated in heavily pretreated metastatic breast cancer. 10 mg/d Everolimus + carboplatin AUC 2 weekly is defined as MTD. The 10 mg dose of everolimus is consistent with the doses recommended in other combinations, and it is currently used in an ongoing phase II trial at our institution.

Disclosure: Sandra Schwarzlose-Schwarz: No conflict of interest disclosed.

V442
Enrichment of peripheral blood CD90/Thy-1-positive cells from cancer patients as a tool for prognostic and single cell analyses

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Introduction: The cross-talk between the tumor microenvironment, e.g. stroma fibroblasts and the epithelial tumor cells is of crucial importance for the development and progression of malignancies not only at the primary site but also in the periphery and at the metastatic site. We investigate the feasibility of an effective enrichment of peripheral blood CD90-positive cells from cancer patients and analyze them on a single cell level.

Methods: CD90/Thy-1-positive cells were detected in leukocyte fractions from patients suffering from gynecological tumors by fluorescence scanning cytometry. CD90/Thy-1-positive cells were selectively enriched using immunomagnetic cell separation technology (ROBOSEP®). The CD90/Thy-1-positive fraction and FFPE-tissue samples were analyzed by immunofluorescence and immunohistochemistry. Individual CD90/Thy-1-positive cells were selected with the aureka® system and expression analysis was performed by one-step multiplex RT-PCR.

Results: The number of CD90/Thy-1-positive cells in the leukocyte fractions from 72 tumor patients was estimated at various time points during treatment. Up to 17 samples/patient were collected. The amount of CD90/Thy-1-positive cells ranging from 1 to more than 50,000 cells/ml peripheral blood. Immunohistochemical analysis of formalin-fixed tissue sections from the corresponding primary tumors showed a localized expression of CD90/Thy-1 in the tumor-associated stroma. Comparing the 290 individual blood-derived samples a CD90/Thy-1 content higher than 1,000 cells/ml peripheral blood was observed in 65% of the patient samples but only in 4% of the samples from healthy volunteers. Furthermore, patients with high levels of CD90/Thy-1-positive cells show a tendency to form metastases (p=0.089). For molecular analysis CD90/Thy-1-positive cells were immunomagnetically enriched and 52 individual vital cells were isolated. Multiplex RT-PCR demonstrated a pronounced expression of SCF (80%) in these cells.

Conclusions: Remarkable concentrations of CD90/Thy-1-positive cells were detected predominantly in the peripheral blood of tumor patients. Preliminary molecular analyses demonstrated a robust expression of SCF mRNA, a marker for both tumor-stroma associated fibroblasts as well as endothelial cells.

Disclosure: No conflict of interest disclosed.
Global gene expression analysis of heterotypic interaction between cancer cells and osteoblasts to identify signaling pathways relevant for bone metastasis in breast cancer patients

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Introduction: Bone metastasis is a main cause of morbidity in breast cancer. Since breast cancer is a heterogeneous disease, the interactions of cancer cells with the skeletal host cells might also be diverse. So far, on a genome-wide scale gene expression alterations caused by interactions between tumor cells and osteoblasts have not been well characterized. We hypothesized that gene expression signatures induced by tumor-osteoblast interaction might be of clinical relevance.

Methods: We established an ex vivo co-culture model using benign breast epithelial cells or a panel of 5 malignant breast epithelial cells in combination with primary human osteoblasts and determined associated gene expression changes with HEEBO microarrays. Pretreatment gene expression profiles of 295 early stage breast cancers published from the Netherlands Cancer Institute with a median follow up of 12.6 years allowed evaluating in vitro effects in the in vivo situation.

Results: The effects of the interaction between osteoblasts and breast cancer cell lines of different origin were very heterogeneous. HS578T cells started to proliferate in co-culture with osteoblasts, SKBR-3 induced a TGF-β response and MDA-MB231 cells showed two distinct sets of up-regulated genes: A set of interferon response genes associated with an up-regulation of STAT1 was remarkably coherent providing a basis for segregation of tumors into two groups. In a uni-variate analysis, early stage tumors with high expression levels of this gene set had a significantly lower overall survival rate (p=0.005) (63% at 10 years) than tumors with low expression levels (n=159) (overall survival: 77% at 10 years). The second gene set was associated with bone metastasis-free survival (p=0.049; 74% vs. 83% at 10 years).

Conclusion: An IL-6 gene expression pattern induced by heterotypic interaction between cancer cells with osteoblasts in vitro is associated with a higher rate of bone metastasis in vivo. Targeting the IL-6 pathway might be a strategy to prevent bone metastasis in breast cancer.

Disclosure: No conflict of interest disclosed.

Impact of the Oncotype DX® Recurrence Score® Assay on therapy recommendations for ER-positive (ER+), node negative (N0) and node positive (N+) early breast cancer – Results of an interim analysis of the German decision impact study

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Introduction: Oncotype DX has been validated as a prognostic and predictive marker in ER+ early breast cancer (EBC). Results regarding its clinical use and impact on treatment decisions in the adjuvant setting have been published.

Fig. 1. IL-6 signature predicts bone metastasis (for Abstract V443).
Abstracts

Frauenklinik, Olten, Switzerland, 3Kantonsspital Olten, Chirurgie, Olten, Switzerland.

Methods: Patients (pts) with ER+, HER2-negative N0 and N+ (1-3 positive lymph nodes) EBC and no contraindication for adjuvant chemotherapy were included in the study. Physicians' adjuvant treatment recommendations and their confidence in these recommendations were assessed before and after knowledge of the results of the test using standardized questionnaires. Treatment data were collected to perform pharmacoeconomic analyses.

Results: The study closed recruitment in April 2011. Data from 197/379 pts were available for this analysis. Of these, 149 (75.6%) were N0 and 48 (24.4%) N+. Overall, 55.8% had low, 36.5% intermediate and 7.6% high RS values. For N0 disease, the distribution of RSs was 55.7%, 38.3%, 6% and for N+ disease 56.3%, 31.3% and 12.5%, respectively. Initial treatment recommendation changed in 38.1% overall; 34.9% in N0 and 47.9% in N+ disease, (40.9% for pts with low, 34.7% with intermediate and 33.3% with high RS). In 24.4% of all pts, a recommendation for adjuvant chemotherapeutic therapy (CHT) was changed to endocrine therapy (HT). 13.2% of recommendations changed from CHT to HT and 12.5% of recommendations changed from HT to CHT. Net reduction in adjuvant chemotherapy usage was 17.2% (from 52.2% to 35.0%) overall, 13.4% (from 46.3% to 32.9%) in node negative and 29.2% (from 70.9% to 41.7%) in node positive disease. Pharmacoeconomic analyses will be performed when data of all pts are available.

Conclusions: Results of this interim analysis that will be updated on 379 pts suggest an impact of the RS on adjuvant treatment decision making in German clinical practice resulting in a significant net reduction of adjuvant chemotherapy usage. The impact appears to be more pronounced for patients with node positive disease.


V445 Retrospective analysis of the curative management for stage I – III A breast cancer patients between 2005 – 2010 in a Swiss cantonal hospital

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Methods: The study included all patients with a newly diagnosed invasive breast cancer stage I – III A who were treated with curative intent. Recommendations for radiotherapy and systemic treatment were given according to the current guidelines of the St. Gallen International Expert Consensus on Primary Therapy of Early Breast Cancer (2005 / 2007 / 2009). The data sets were extracted from the hospital’s medical files and compared to the results of the Patterns of Care Study from the Swiss Cancer Registries on indicators of quality in breast cancer treatment.

Results:

Table 1.

<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Indicators of Quality in Breast Cancer Treatment</td>
<td>Age &lt; 80 years N = 169 Median follow-up 64 (36 – 79)</td>
<td>Age &lt; 80 years N = 3499 Median follow up n.d.</td>
</tr>
<tr>
<td>Surgery – performed according to guidelines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FNP or core-biopsy</td>
<td>93%</td>
<td>76%</td>
</tr>
<tr>
<td>Axillary dissection with ≥ 10 LN</td>
<td>88%</td>
<td>81%</td>
</tr>
<tr>
<td>Sentinel procedure for cN0</td>
<td>81%</td>
<td>67%</td>
</tr>
<tr>
<td>R0-resection during initial surgery</td>
<td>70%</td>
<td>76%</td>
</tr>
<tr>
<td>Final R0-resection</td>
<td>98%</td>
<td>91%</td>
</tr>
<tr>
<td>Full pathology work-up done</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNM stage, grading, ER/PR-immunoreactivity</td>
<td>100 % (e.g. data available on all patients)</td>
<td>98%</td>
</tr>
</tbody>
</table>

Table 2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cantonal Hospital con’t</th>
<th>Swiss Cancer Registries con’t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiotherapy recommended</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjuvant radiotherapy for breast conserving therapy</td>
<td>97%</td>
<td>96%</td>
</tr>
<tr>
<td>Adjuvant post-mastectomy radiotherapy, if indicated</td>
<td>100%</td>
<td>96%</td>
</tr>
<tr>
<td>Systemic therapy recommended</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocrine therapy, if indicated</td>
<td>100%</td>
<td>95%</td>
</tr>
<tr>
<td>Chemotherapy, if indicated</td>
<td>96%</td>
<td>91%</td>
</tr>
</tbody>
</table>

Conclusions: Our single centre analysis showed better results for most of the indicators of quality compared to the Patterns of Care Study [2]. Even with a lower case load the quality of treatment can be excellent, if the expertise of the health care professionals is up to date.


Disclosure: No conflict of interest disclosed.
**Freie Vorträge Bronchuskarzinom**

**V446**

**Tumor epidermal growth factor receptor (EGFR) expression as a predictive biomarker of survival in FLEX study patients with advanced non-small cell lung cancer (NSCLC) receiving chemotherapy plus cetuximab as first-line therapy**


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**Introduction:** The phase III FLEX study demonstrated that the addition of cetuximab to first-line chemotherapy (CT) statistically significantly improved overall survival (OS) in patients with EGFR-expressing, advanced NSCLC. Tumor immunohistochemistry (IHC) data, collected prospectively to determine FLEX study patient eligibility, were used to investigate whether EGFR expression status was predictive of clinical outcome.

**Methods:** Tumor EGFR expression data were available for 1121 of 1125 (99.6%) FLEX study patients. A continuous scoring system (scale of 0-300) was used to determine the level of EGFR expression according to the proportion of positive cells and intensity of membrane staining. A discriminating threshold IHC score of 200 was selected on the basis of response data and used to define groups with low (IHC score < 200) and high (IHC score ≥ 200) EGFR expression. Clinical outcomes were further analyzed in each group.

**Results:** High and low EGFR expression were scored for 345 (30.8%) and 776 (69.2%) patients in the high EGFR expression group. OS time was prolonged for those in the CT plus cetuximab compared with CT alone (median 12.0 vs 9.6 months; hazard ratio 0.73; p=0.011). The OS benefit was prolonged for those in the high EGFR expression group compared with CT alone (69.2%) patients. For patients in the high EGFR expression group, OS time of comparison was 12.0 vs 9.6 months; hazard ratio 0.73; p=0.011). The OS benefit was prolonged for those in the high EGFR expression group compared with CT alone. The difference in the hazard ratios for OS between the EGFR expression groups was apparent across histological subtypes. In contrast, analysis of tissues from other cancer entities of the upper aero-digestive tract such as larynx carcinoma (n=57) or oral cancer (n=54) showed only a faint II-22 expression in 15 % of the analyzed samples. The heterodimeric II-22 receptor 1 (IL-22-R-1) was also apparent in relation to response and time to treatment failure for patients with high EGFR expression. Analysis by EGFR expression level did not show any meaningful differences in the safety profile of CT plus cetuximab.

**Conclusions:** The addition of cetuximab to first-line CT substantially prolonged OS in patients with advanced NSCLC whose tumors expressed high levels of EGFR. EGFR expression is a disease-related biomarker that may facilitate the tailoring of first-line treatment with CT plus cetuximab to those patients most likely to have a clinically meaningful benefit.

**Disclosure:** No conflict of interest disclosed.


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**V447**

**Interleukin-22 is expressed by lung cancer cells**

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**Introduction:** Interleukin-22 (IL-22) is a recently discovered cytokine with unique functions and an expression profile mostly restricted to hematopoietic cells. In lung cancer, IL-22 has been proposed to have proliferative and anti-apoptotic effects. However, the source of IL-22 within the tumor microenvironment and the functional consequences of IL-22 signaling in cancer patients are unknown.

**Methods:** Using tissue microarray (TMA) technology, 215 human lung cancer samples from different histological subtypes were screened by immunohistochemistry for IL-22 expression. Human lung cancer cells lines A549, HCC and H1339 were screened for IL-22 receptor expression by Western blot and RT-PCR.

**Results:** IL-22 expression was heterogeneous within the different histological subtypes: 22% of squamous cell (n=59), 44 % of large cell (n=45), 45 % of adenocarcinoma (n=71), 54 % of bronchiolaralveolar (n=15) and 84 % of small cell carcinoma (n=15) expressed IL-22. In contrast, analysis of tissues from other cancer entities of the upper aero-digestive tract such as larynx carcinoma (n=57) or oral cancer (n=54) showed only a faint II-22 expression in 15 % of the analyzed samples. The heterodimeric IL-22 receptor 1 (IL-22-R-1) was expressed in all lung cancer cell lines analyzed as assessed by RT-PCR and Western blot. Remarkably, IL-22 stimulation of lung cancer cells induced STAT3 phosphorylation, indicating a functional IL-22-R-1-dependent signaling pathway.

**Conclusions:** Our present study shows that IL-22 is frequently expressed in lung cancer with significant differences between the histological subtypes. Expression levels were found in small cell and bronchiolaralveolar carcinoma. Expression of functional IL-22-R-1 on lung cancer cell lines indicates that IL-22 may have an autocrine or paracrine influence on cancer cells. To address a potential role of IL-22 signaling in lung cancer, we will correlate clinical data with IL-22 expression and investigate whether IL-22 is secreted and reaches significant systemic levels. Ultimately, the identification of IL-22 as a target in the tumor environment may provide a new target for anti-tumor therapies.

**Disclosure:** No conflict of interest disclosed.

Methods: The exonic expression profiling of 42 bronchoscopic biopsies was investigated using Affymetrix exon arrays. Unsupervised multivariate approaches, including principal component analysis (PCA) and hierarchical cluster analysis were used to describe the exonic variations within EGFR and KRAS among patients having various EGFR/KRAS mutational status. All analyses were blinded for the mutational status and clinical outcome.

Results: This analysis targeted all probe sets measured within EGFR (n=451) and KRAS (n=261). Fifty-one and 11 exonic probe sets were available within EGFR and KRAS respectively. PCA revealed a gradient of expression among patients which was homogeneous among the different EGFR exons (average within-patient coefficient of variation=0.28), whereas larger exonic variations were found within KRAS (average within-patient coefficient of variation=0.43). In contrast to KRAS, there was a trend showing an association between gender and the expression of EGFR (p=0.06).

Conclusion: Using the exon array technology, it was possible to assess the exonic expression profiling of 2 key genes involved in non-squamous NSCLC growth pathways. EGFR expression level was homogeneous among exons, advocating independence between the exon intensity level and the patient mutational status. Conversely, KRAS proved to exhibit larger within-patient variation in the exonic expression level. We hypothesize that the exonic heterogeneity within KRAS provides additional prognostic information complementary to the patients’ mutational status. These results will be provided after the unblinding of the trial.

Disclosure: No conflict of interest disclosed.

V450 Randomized phase 3 trial of amrubin vs topotecan as second-line treatment for small cell lung cancer (SCLC)


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Introduction: Amrubin (AMR), a 3rd generation anthracycline and topoisomerase II inhibitor, has shown promising activity in SCLC. The ACT-1 trial compared the safety and efficacy of AMR with topotecan (Topo) for second-line treatment of SCLC.

Methods: Patients with SCLC received either AMR 40mg/m² IV on day 1-3 (n=242) or Topo 1.5mg/m² IV on days 1-5 (n=231) with prophylactic WBC growth factors and antibiotics required in last 1/3 of trial. Study endpoints included overall survival (OS), response rate (RR), progression-free survival (PFS), and safety. Subgroup analyses used protocol-defined definitions: refractory patients had PD as best response to first-line therapy or progressed <90 days. The mean change in Lung Cancer Symptom Scale (LCSS), Symptom Burden (LCSS SB) score, and EQ-5D were used to assess quality of life (QoL).

Results: Both the AMR and Topo groups had similar baseline characteristics: median age 62 vs 61 years; patients <65 years 60% vs 65%; men 58% vs 60%; performance status 0 30% vs 34%; refractory 47% vs 45%. The Cox model covariates were PS 0 (yes, no), age, response to first-line platinum-based therapy (refractory, sensitive), and disease stage (limited, extensive). AMR treated patients had better symptom control and QoL than Topo treated patients: LCSS 0.2 vs 5.6, and LCSS-SB -0.1 vs 5.2 for AMR and Topo respectively, all P<0.05. The relative grade 3/4 adverse events in AMR and Topo groups were: neutropenia (41% vs 53%), thrombocytopenia (21% vs 54%), anemia (16% vs 30%), infections (16% vs 10%), febrile neutropenia (10% vs 4%), all P<0.05, and cardiac disorders (5% vs 5%; P=0.84).

Conclusions: AMR showed clinical efficacy in second-line treatment of SCLC, and significantly improved RR and PFS compared with Topo. The OS trended in favor of AMR (HR 0.88), especially in refractory patients (HR 0.77). Preliminary QoL results favored AMR.

**Introduction:** An estimated 10% of Caucasian patients diagnosed with advanced non-small cell lung cancer (NSCLC) show somatic mutations in the epidermal growth factor receptor (EGFR) gene. These mutations are positive predictors of sensitivity to EGFR tyrosine kinase inhibitors (TKI).

**Methods:** REASON is an AstraZeneca sponsored registry (ClinTrials ID: NCT00997230). The primary objective is the epidemiological survey on the EGFR mutation status in a predominantly Caucasian population, thus enabling the correlation with patient characteristics like gender, smoking history, histology and tumour anamnesis. Patients with histologically confirmed stage IIIB/IV NSCLC and information regarding their EGFR mutation status were enrolled at over 150 participating sites throughout Germany. EGFR mutation analyses were performed at 67 pathology laboratories.

**Results:**

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>OS sensitive pts (mo)</th>
<th>OS refractory pts (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS refractory pts (mo)</td>
<td>295</td>
<td>6.2</td>
</tr>
<tr>
<td>12-mo OS (%)</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>18-mo OS (%)</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>PFS refractory pts (mo)</td>
<td>295</td>
<td>2.8</td>
</tr>
<tr>
<td>ORR sensitive pts (%)</td>
<td>342</td>
<td>41</td>
</tr>
<tr>
<td>ORR refractory pts (%)</td>
<td>295</td>
<td>20</td>
</tr>
<tr>
<td>Hazard ratio (HR) &lt; 1 and odds ratio (OR for RR only) &gt; 1 favors amrubicin; CI=confidence interval.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Results: To date, demographic and clinicopathological data of 3,155 patients is available. The majority of these patients (89.1%) were newly diagnosed with advanced NSCLC and displayed already symptomatic (87.8%) stage IV disease (85.2%). Of all recruited patients, 2,845 (90.2%) show no mutation predicting TKI sensitivity. In this EGFR wild type subgroup (mut−), 65.0% of patients were male and 35.0% female, most patients have a smoking history with 84.5% ever-smokers and 15.5% non-smokers. Adenocarcinoma histology is most frequent (66.2%), followed by squamous epithelial carcinoma (21.4%). Only a minor contingent of tumour tissue samples were not evaluable (3.6%) and are subsumed in the EGFR mut+ population. The rate of EGFR mutations predicting TKI sensitivity (mut+) is 9.8% (310 out of 3155 patients). The gender distribution in mut+ patients is shifted towards female patients (62.3%) and this subgroup displays a higher rate of non-smokers (46.5%). Adenocarcinoma histology is even more dominant in the EGFR mut+ population (88.4%), whereas squamous epithelial carcinoma histology is less frequent (4.5%).

Conclusion: The REASON database can help identifying patient subgroups likely to be eligible for first line TKI treatment by providing associations between patient characteristics and positive EGFR mutation status.


Posterdiskussion
Psychoonkologie / Betreuung von Patienten und Survivors / Prävention / Ethik

P452
Implementation of a computer-based, standardized assessment of psychosocial and physical distress in patients with hematologic malignancies

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Introduction: The purpose of our project is the computer-based assessment of individual quality of life data in clinical routine (CA-QoL-CLR) and the development of strategies and approaches for implementation of such an assessment in clinical practice. A standardized assessment of psychosocial and physical distress in patients with hematologic malignancies with paper-and-pencil technique is already in place in our department since 2004. Diverse data collection systems using CA-QoL-CLR have emerged in the last years with the development of innovative computer technologies. The main advantages of such an approach are:

1) the reliable and economical data input
2) the ad-hoc-generation of clinical reports for physician and patient,
3) the integration of computerized QoL-assessments which can improve oncological treatment: e.g. at solving problems with adverse events.

Methods: We had to address the following topics for the implementation of a solid technological and administrative working structure:

1) hardware,
2) software,
3) data processing link between the clinical information system and CA-QoL-CLR hardware,
4) data transfer,
5) plausibility checks to ensure reliable data transfer.

Results: As hardware we employ a fully rugged mobile tablet PC, easy to disinfect, waterproof and able to withstand drops. It can be used for anything from reading patient notes to accessing medical databases. We apply different QoL-questionnaires using the CHES (Computer-Based Health Evaluation System) software. The reliable data input and the ad-hoc-generation of individual patients’ reports (ward round charts) worked properly in clinical routine structures. Dealing with data processing-problems required an interdisciplinary team.

Conclusion: In future, we will focus on the scientific evaluation of this CA-QoL-CLR system regarding acceptance and benefit for different clinical situations in an acute care hospital.

Disclosure: No conflict of interest disclosed.

P453
Criticizable claims for the validity of communication acts in biological systems: Therapeutic implications in cancer

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Introduction: Basis for the comprehension of biological systems are experimentally, and in the case of metastatic tumors also therapeutically derived data, mirroring the context-dependent validity of communicatively integrated systems objects (modules, pathways, cells etc.). Validity claims of experimentally defined references in terms of systems objects seem to be routinely transferable into arbitrary evolving systems, irrespectively of the self-evident assumption that novel systems functions may spin off and that those tumors show novel compositions of acquired chromosomal and molecular-genetic aberrations.

Methods: The attempt to reconstruct biologic communication processes and to show how to uncover and monitor these processes for therapeutic purposes cannot constitute a comprehensive concept of a tumor’s communicative tool of normative contents. What is at issue here is to discuss the daily diagnostic and therapeutic challenges timed at broadening the therapeutic instruments on the basis of comprehensive and evaluable communicative presuppositions, which have been shown to be inevitable for the continuous and non-circumventable process of reaching communicative understanding as well as for therapeutic communicative interventions.

Results: Reconstruction of prepositional reasons for differential rationalizations of systems within distinct evolutionary stages and the parallel uncovering of the respective situative procedural constitution of rationalization processes are of pivotal interest for broadening therapeutic options. Corresponding to the pragmatic functions of communicative expression, four aspects which are involved in communicative expression, may be therapeutically modified:

1) the tumours’ relation to their external reality (host organ),
2) the internal reality, characterized by tumor- and stage-specific rationalizations of tumor functions,
3) the intersubjective reality comprising the intentions of systems objects, and
4) the normative systems structure (robustness, reproduction, etc.).

Conclusion: Novel hypothesis-driven tumor models may serve as challenge to reinterpret the myriad of available biological data in a communicative context and to further personalize tumor therapy. The main task remains to reconstruct observable communicative interactions on the expressive level and to select and extend methodologies, which have the capacity to monitor functional changes of cell systems in response to (therapeutic) perturbations.

Disclosure: No conflict of interest disclosed.

Abstracts
Onkologie 2011;34(suppl 6):1–305

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Limitation of medical treatment at the end of life: findings of a qualitative study on the perception and ethical views of oncologists in Germany and the United Kingdom

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Introduction: Limitation of treatment is part of the care for patients with incurable cancer. We explored the perception and ethical views of oncologists working in Germany and the United Kingdom regarding these decisions.

Methods: Qualitative semi-structured interviews with physicians working in oncology in Germany and England were carried out. Interviews were audiotaped and transcribed. Transcripts were coded by identifying major themes of the interviews using constant comparison, in order to examine similarities and differences between oncologists across the whole sample.

Results: 17 (Germany) and 12 (United Kingdom) research interviews were analysed. Interviews varied in length between 27 and 73 minutes. Respondents from both countries named a variety of treatment modalities which may be limited in the context of care for patients with incurable cancer. After standard clinical criteria for decision-making (e.g. performance status), physicians’ perception of the life circumstances of the patient (e.g. being a mother of young children) as well as highly individual aspects of the physician-patient relationship (e.g. parallels between physician’s and patient’s biographies) were reported to most influence these treatment decisions. “Discussions with colleagues” and “the multidisciplinary team” were emphasised as “correctives” to make the decisions less subjective; these were emphasised as strategies by oncologists working in the United Kingdom. Physicians in both countries reported that “non-harming” treatment was given to patients who did not accept oncologists’ initial recommendation to stop treatment. The duty “not to harm” was cited as the rationale to limit treatment even though patients’ wished to receive further interventions.

Conclusions: This study indicates that decisions about limitation of medical treatment are based on numerous medical and non-medical factors. Potential strategies for dealing with clinical and ethical challenges in end stage cancer will be discussed.

Disclosure: No conflict of interest disclosed.

Predictive factors for the need of child centered counseling in parents with cancer – results from a single center analysis

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Background: A parent with cancer faces multiple problems. Besides distress due to treatment and prognosis concerns about the children are of additional relevance but often neglected. The purpose of the study was to evaluate the need of child centered counseling in patients with cancer and to identify factors that might predict the individual need for support of patients and their families.

Patients and Methods: Over a period of one year 66 of 689 inpatients treated at the Dept. of Hematology and Oncology had at least one child up to 18 years. 49 of them were seen by a child psychiatrist and particularly informed about the need for counseling. Over a period of one year 66 of 689 inpatients treated at the Dept. of Hematology and Oncology had at least one child up to 18 years. 49 of them were seen by a child psychiatrist and particularly informed about the need for counseling. 22 (45%) of the informed patients requested the counseling service more than once. 18 of them required an intervention with up to 4 appointments, 4 families needed intensive support from 7 up to 20 times. We detected neither a correlation between the consultation need and the distress measured by the DT nor the time between first diagnosis and first contact with the child psychiatrist or treatment intensity including bone marrow transplantation. In contrast the level of social distress measured by the SDS showed a correlation with the number of required appointments: patients with a score of 5 only needed one, 60% of the patients with a score over 10 needed at least three appointments. The 4 most intensively supported patients had a score of 12 and higher.

Conclusion: Many parents suffering from cancer avail themselves of a child centered counseling during hospital stays. While the DT does not predict the need for counseling the extent of social distress captured by the SDS correlates with the requested support but requires prospective validation.

Disclosure: No conflict of interest disclosed.

Learning to live with cancer – An European education and support program

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‘Learning to live with cancer’ is an evidence-based, structured educational and support service for people and their families who are suffering from cancer. Based on a research project, this program was developed by Prof. G. Grahn (Sweden) and has been implemented in many European countries including Switzerland (1998). Since 2000 courses have been offered to train experienced oncology nurses and other professionals of oncology. In 2002, the ‘Swiss Association learning to live with cancer’ was founded with the goal to promote quality and protect the original concept. The Swiss Association currently has 50 active members. Besides providing the participating persons with informations about cancer diagnosis and treatment, the service ‘Learning to live with cancer’ aims at helping the participants to adapt to their situation and to enhance their knowledge about how to cope with it.

The course program is taught in groups, containing nine units of 2 ½ hours. These are offered on weekly intervals, based on the principles of adult education and take into account cognitive, emotional and social needs. Themes include the human body, the development of cancer, diagnosis, treatment options, complementary methods, research, nutrition, dealing with side effects and crisis management. Methods are taught on how to relax the mind and body as well as learning how to express feelings through art therapy. Social support is offered through group therapy. This course program aims to achieve the best possible level of wellbeing, within the existing boundaries of the disease. In this sense it is a rehabilitation program. However it is offered at any stage of the disease. The Cancer League Graubünden offers the course since 2008. The participants’ feedbacks are highly positive and show that it is a much needed complementary service.

Further information can be found at: http://avac.ch

Disclosure: No conflict of interest disclosed.

Population based screening in breast, colon, prostate and lung cancer: Pros and contras

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Introduction: Cancer is the second leading cause of death. In females breast cancer followed by colon and lung cancer are responsible for nearly 50% of...
all cancer related deaths in Germany. In males, lung cancer followed by colon and prostate account for app. 50% of the cancer related mortality. Due to a constantly increasing life expectancy and health consciousness, early cancer diagnosis and cancer prevention have become topics of special interest in the healthy population. This is also reflected by the frequent presentation of cancer prevention issues on television, radio, Internet and print media. Moreover, healthy persons get increasingly more confronted with individualized diagnostic cancer prevention programs by general practitioners such as tumor marker screenings.

Methods: The largest recently published trials and metaanalysis for the above mentioned cancer types were carefully reviewed with special regard to relative and absolute survival benefits as well as number needed to treat and number needed to screen. The focus was to enable healthy individuals to realistically assess their risk reduction by participating in screening programs.

Results: Within the last years several extensive studies for these most frequent cancer types, including several hundred thousands of asymptomatic individuals, have evaluated the predictive value of mammography, colonoscopy, ct-scans and serum marker determination for prostate specific antigen (PSA).

Although most of these trials showed a significant statistical superiority for the investigated screening approaches, the absolute benefit regarding cancer specific survival remains negligible for the individual. Moreover, by participating in cancer screening programs, asymptomatic persons are at significant risk for over diagnosis and over therapy. Finally, elevated absolute numbers of cancer diagnosis by such screening approaches are likely to burden future public health expenses.

Conclusions: Within this talk we will give a contemporary overview about the actual data and controversies in population based screening studies for breast, colon, lung and prostate cancer. Pros and cons will be discussed.

Disclosure: No conflict of interest disclosed.

P458
Remarkable days in court: The lethal lawsuit against Dr. Mechthild Bach

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Background: On January 24th 2011 Dr. Mechthild Bach, a specialist for internal medicine, committed suicide after a lawsuit of more than 7 years. She had been working in a community hospital in the Hannover region and was indicted for the killing of thirteen patients, mostly cancer patients, with overdose of opiates and sedatives. The lawsuit ended at the moment of Dr. Bach’s suicide. There won’t be a legal judgement about whether or not she killed – either negligent or intentionally, maybe even as a murderer. But besides the legal questions that now will remain unanswered forever there are other more general questions about the standards of palliative care and the legal status of end-of-life decisions which request further reflection.

Methods: For the investigation of the case we have to follow the chronology of the lawsuit and to study the different medical expertise that either incriminated or discharged Dr. Bach. We analysed some of the specific problems about the compatibility of legal and medical perspectives. In this particular case we study how end-of-life decisions appear in both perspectives. As far as accessible we studied the court files and the expertises in particular.

Results: To the time when the incriminated cases took place the number of beds for residential palliative care in the Hannover region was zero. This changed since 2005. Thus Dr. Bach had no option referring her patients in critical condition to this kind of specialized palliative care facilities. As far as we can know, she had no networking with peers and colleagues. Her records about the information she gave the patients and about her patients own decisions are as patchy as her documentation of the medical decisions about breast, colon, lung and prostate cancer. Pros and cons were discussed.

Disclosure: No conflict of interest disclosed.

P459
Vaccination of patients with indolent lymphatic malignancies. A survey in 354 patients in 2009/2010

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Introduction: A nationally (STIKO) and internationally (WHO) propagated goal is vaccination of about 75% of elderly people and at risk patients (with e.g. chronic diseases or immune deficiencies). In this definition, patients with indolent lymphatic malignancies are included. However, no data of vaccination frequencies in these patients are available at present.

Methods: From January 1, 2010 until November 30, 2010, 354 consecutive patients treated at the authors hematology practice were surveyed regarding their vaccination behavior. The survey focussed on several diagnosis-related groups: – Indolent lymphoma (iL): CLL, follicular lymphoma, mantle cell lymphoma, hairy cell leukemia, immunocytoma;

– Secretary gammopathies (sGP): MGUS, plasmacytoma, Waldenström’s macroglobulinemia, light chain myeloma;

168 female patients (123 iL; 45 sGP) and 186 male patients (116 iL; 70 sGP) were assessed. 79 % of all patients were over 60 years of age. The following vaccinations were assessed:

– Influenza season 2009/2010?

– Influenza in one (or more) previous seasons?

– Swine influenza (‘swine flu’) 2009?

– Pneumococcus (any time)?

Results: 50.8% of all patients reported seasonal influenza vaccination in 2009/2010, while swine influenza vaccination was reported by 9.5%. Immunization against Pneumococcus had been performed in 14.4%, in 6 of these patients in the context of splenectomy. Focussing on patients over 60 years of age (born before January 1, 1950), the following results were found: 57.3% of these patients reported receiving seasonal influenza vaccination in 2009/2010, while swine influenza vaccination was reported by 10.4%. Immunization against Pneumococcus had been performed in 16.1%, in 4 of these patients in the context of splenectomy.

Discussion: In practice vaccination behaviour in patients is currently not significantly different compared to the normal population (REUSS,A.M. et al., Dtsch. Arzteblatt 107, 2010, pp 845-50).

Contrastingly, awareness of the possibly impaired immune status and interest in vaccination counseling is high. Sound information- including gaps of objective knowledge- is needed in this case, while propaganda campaigns (like e.g. “swine flu”) will result in skepticism. A key role in consulting lies with the GP in vaccination counseling is high. Sound information- including gaps of objective knowledge- is needed in this case, while propaganda campaigns (like e.g. “swine flu”) will result in skepticism. A key role in consulting lies with the GP.

Disclosure: No conflict of interest disclosed.

P460
“FlexiDoc” – a flexible tool for processing data from health services research in ambulant oncology

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Introduction: Health services research is becoming increasingly important in Germany. Its aim is to improve the medical care for individual patients, as well as to contribute to developing a health care system that can be agreed upon by society as a whole. This is done by developing and evaluating new conceptions of medical care. Their applicability to the daily routines of practices is of par-
Addressing the needs of cancer patients in a rural area: the Network for Oncology and Palliative Care Medicine Landshut – an innovative patient centered care strategy

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Interdisciplinary cancer centers have been founded throughout Europe, almost all of those being located in big cities, mainly at academic centers. Most of the cancer and palliative care patients living in rural areas do not benefit from these institutions due to the distance between their homes and the centers. Long-distance drives during chemo- or radiation therapy or in the palliative care setting are not tolerable in most cases and have a negative impact on the patients‘ and their relatives‘ quality of life. Furthermore it can be assumed that patients from rural areas are underrepresented in clinical trials as most of them are conducted at metropolitan centers.

The Network for Oncology and Palliative Care Medicine Landshut (Onkologisches und Palliativmedizinisches Netzwerk Landshut) is the first in Germany to address the special needs of cancer and palliative care patients in a rural area, combining interdisciplinary hospital based and ambulatory institutions. It is accredited by the ESMO (ESMO Designated Center of Integrated Oncology and Palliative Care) and the DGHO.

In order to compensate for the low frequency of specialised medical institutions and the insufficient public transport system, innovative solutions for patient care have been realised. The network offers in-patient and ambulatory care for hematological, cancer and palliative treatment for the rural area of Lower Bavaria. The specialised ambulatory palliative service for home-care (SAPV) is organised through the oncology day care unit. There is a 24 hours/7 days telephone hotline to the oncologist and the palliative care nurse on call available for patients, relatives and medical staff within the network.

Interdisciplinary tumor boards are held weekly. An interdisciplinary center for pain therapy and management provides ambulatory and in-patient treatment. The center’s own academy for oncology, hematology, palliative medicine and hospice work organises broad teaching activities for medical and nursing clini-
Conclusion: Our study shows that several port systems are totally or partially radiolucent and therefore several port complications may go undiagnosed for a prolonged time period. As a consequence, it should be taken into consideration to use TIVDs of exclusively radiopaque materials. This allows a sufficient visibility on thoracic x-ray images of each port component in the therapy of patients with malignant diseases.

Disclosure: No conflict of interest disclosed.

P463

Database to generate and collect feasibility documents for participation of investigators and study sites in clinical trials (QualiPRO)

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Academic non-commercial trials are of substantial relevance for both patient care and progress in clinical research. Since the 12th amendment of the German drug law application of clinical trials at the ethic-committees (EC) are much more cost- and time-intensive. This is particularly evident for Multicenter Therapy Optimization trials (TOS). To activate a trial up to 51 EC have to be addressed to confirm suitability of study sites and staff. The process is repeated for each trial although investigators and sites may be the same. EC ask for current curricula vitae (CV), certificates on good clinical practice (GCP) and regulations (1 or 2 days, also updates), financial disclosures and a feasibility declaration, all in hard-copy and as electronic version. In the nationwide TOS 100.000 paper copies or more have to be produced. Main goal of the project was to set up an open-source web-database QualiPRO to collect and store electronically documents for the suitability confirmation of investigators and sites in TOS. The project partners developed a SQL-data and document base with specified roles and rights, processes of authentication and relevant entry fields. Content and architecture of the database was created according to EC recommendation and ICH-GCP. QualiPRO provides community-oriented utilizations for investigators, their clinical trial units and coordinating centres of TOS. After registration of the unit and membership of the team, study staff members are able to enter and edit relevant personal data, create and print a “trial”-CV. CVs, GCP-certificates, licences to practice medicine and more can be uploaded and coordinating centres of TOS are able, after consent of the investigators, to download documents. In addition QualiPRO is an instrument for directors of clinical trial units and coordinating centres of TOS. To activate a trial up to 51 EC have to be addressed to confirm suitability of study sites and staff. The process is repeated for each trial although investigators and sites may be the same. EC ask for current curricula vitae (CV), certificates on good clinical practice (GCP) and regulations (1 or 2 days, also updates), financial disclosures and a feasibility declaration, all in hard-copy and as electronic version. In the nation-wide TOS 100.000 paper copies or more have to be produced. Main goal of the project was to set up an open-source web-database QualiPRO to collect and store electronically documents for the suitability confirmation of investigators and sites in TOS. The project partners developed a SQL-data and document base with specified roles and rights, processes of authentication and relevant entry fields. Content and architecture of the database was created according to EC recommendation and ICH-GCP. QualiPRO provides community-oriented utilizations for investigators, their clinical trial units and coordinating centres of TOS. After registration of the unit and membership of the team, study staff members are able to enter and edit relevant personal data, create and print a “trial”-CV. CVs, GCP-certificates, licences to practice medicine and more can be uploaded and coordinating centres of TOS are able, after consent of the investigators, to download documents. In addition QualiPRO is an instrument for directors of hospital departments, to collect efficiently and locally display data on their study activities and training status of the staff. In the future it is planned to give EC access to the database, with tools to view, download, store and confirm the qualification of the study personal and the study site. By QualiPRO qualification profiles are standardized and processes simplified. With EC-commitment extensive paper based submission could be avoided. Even without the EC study sites units and coordinating centres on TOS are supported regarding administration of study documents. QualiPRO is free of charge for interested parties (www.quali-pro.de).

Funding by BMBF/DLR 01Gl9971 and DJCLS H09/01f.

Disclosure: No conflict of interest disclosed.

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Advantages of a ‘Center of Clinical Investigations, Optimization, Standardization & Safety (CIO)’ as a central unit for Hematology & Oncology departments for clinical studies, chemotherapy management, and cancer registry assessments – Freiburg (UKF) experience

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Introduction: The departmental section unit ‘Center of Clinical Investigations, Optimization, Standardization & Safety (CIO)’ has become a principal part of our Hematology & Oncology department within the last few years. Therein, physicians, scientists, pharmacists, software engineers and study nurses provide clinical trial support, cancer registry data work and deal with all questions regarding chemotherapy (CTX)-orders – and -applications.

Methods and Results: We herein report on the advantages and challenges of our CIO that is responsible for 1. all clinical trials conducted within our department, 2. the documentation of all of our in- and out-patients using an electronic tumor based documentation system (eTBD), thereby allowing productive registry work and 3. CTX-logistics (including their error-free execution).

In close cooperation with the attendees and PIs, the CIO-office coordinates various trial tasks: from first contacts (with sponsors, CROs, ethics committees), to the scientific and internal departmental feasibility evaluation, trials’ logistics and CRF-documentation. In order to estimate highly realistic patient recruitment numbers in clinical trials, we employ our eTBD (including patients’ demographics, comorbidities, CTX-pretreatment, CTX-related side effects, progression- and survival-data). Almost 21,000 cancer patients have been recorded within our eTBD since 1997. Based on this eTBD-data, scientific issues, such as incidence rates of various novel observations, effects of specific CTXs and interventions and SAE-frequencies, can be assessed retrospectively and prospectively. Moreover, the eTBD allows realistic prognoses on clinical trial recruitment numbers. At least 500 detailed analyses on patient data for scientific and clinical trial-logistics are performed each year through our CIO. In addition, the CIO ensures the correct CTX-ordering and -application and is in charge of editing the department’s handbooks, e.g. ‘Das Rote Buch’ and ‘Das Blaue Buch’. Weekly SAE-discussions of CTX-induced AE/SAEs are also organized by the CIO. The CIO closely collaborates with the Krebshilfe-supported CCC-Freiburg and Clinical Trial Unit Freiburg and has been awarded with the 1. QM-prize UKF and Golden Helix Award.

Conclusions: In times of increasingly complex requirements for preeminent patient support, the CIO largely assists in clinical trials, cancer registry work, correct CTX-application and continuous academic education for paramount care of cancer patients.

Disclosure: No conflict of interest disclosed.

P465

Introduction of quality management causes mainly internal process optimization

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Quality assurance is mandatory in transfusion medicine. Though the resource requirements for quality management are usually obvious, the benefits are sometimes more obscure.

We report retrospectively on the first 22 months of experience with the introduction of a quality management program in a laboratory and transfusion medical institute of an university hospital. Among the introduced measures were, apart from qualification, validation and standard operating procedures, the systematic acquisition of deviations, corrective action requests, and out-of-specification (OOS) procedures as well as quality reviews.

An average of 3.0% OOS per apheresis and 5.9 corrective action requests were recorded per month since their introduction. Three types of errors became
obvious with some of them being particularly trivial. In one instance, a batch-related problem of starting materials was observed. In three instances, deviations were related to the equipment and could be compensated by adjustment settings of the equipment or by renewal of necessary components. The quantification of equipment reliance showed to be advantageous both for patient safety and economically in negotiation settings. In two instances, staff-related errors were identified that could be addressed by cutting unnecessary procedures or by targeted training measures.

The introduction of a quality management system started to create transparency and resulted in optimizations especially in procedures that involve more than one team.

Disclosure: No conflict of interest disclosed.

P466
Retrospective analysis on the influence of hepar-filtered air on infections, duration of inpatient treatment as well as antibiotic and anti fungal drug consumption in comparison to single room without hepar filtered neutropenic patients

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Introduction: According recently published recommendations of the Kommission für Krankenhaushygiene und Infektionsprävention beim Robert Koch-Institut (RKI) it should patients with a severe granulocytopenia (< 500/ul for more then 10 days), severe aplastic anaemia, autologous stem cell transplantation as well as patients with an allogeneic stem cell transplantation and/or severe Graft versus host disease grade III-IV be treated in rooms with hepar filtered air. Thus, all centers treating patients with acute myeloid leukemia should have such a air conditioning equipment to reduce incidence of severe life threatening infections in these high risk patients.

Methods: As we have the availability of a hepar filtered air ward since October 2010 we retrospectively analysed the days of fever, documented infections, suspected infections, days of inpatient treatment as well as consumption of antibiotics and anti fungal drugs in comparison to treatment in single rooms without air filtration.

Results: Between 01.1.2009 and 30.09.2010 a total of 47 patients with AML (n=17), allogeneic stem cell transplantation (n=5) and autologous stem cell transplantation (n=25) have been treated in single rooms without hepar filtration. From 01.10.2010 until 01.05.2011 11 patients wit AML, 14 patients with autologous and 5 patients with allogeneic stem cell transplantation have been treated in hepar filtered rooms. Days of granulocytopenia were comparable between groups. Median days of fever were 6 (single room) and 4.5 (hepar filtered). Documented infections were similar between groups (7 vs 8). Days of inpatients were significantly reduced (33 vs 25) in the hepar filtered group. Median days of fever were 6 (single room) and 4.5 (hepar filtered). Documented infections were similar between groups (7 vs 8). Days of infections, suspected infections, days of inpatient treatment as well as consumption of antibiotics and anti fungal drugs in comparison to treatment in single rooms without air filtration.

Conclusions: Keeping in mind the small number of patients so far, our data support the RKI recommendations to treat high risk neutropenic patients in hepar filtered rooms. An update of this data comparing a one year period will be presented at the meeting.

1: Bundesgesundheitsbl 2010; 53: 357-388

Disclosure: No conflict of interest disclosed.

P467
The tumor suppressor TNFAIP3 is frequently deleted in the CTCL Sézary syndrome

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Despite recent therapeutic improvements, the prognosis for patients suffering from Sézary syndrome (SS), a disseminated form of cutaneous T-cell lymphomas (CTCLs), is still poor. To establish more specific and targeted therapies, a better understanding of the underlying molecular mechanisms driving the aberrant proliferation is required. Using high resolution comparative genomic array hybridization we found bis- and monoallelic deletions of the tumor necrosis factor alpha induced protein 3 gene (TNFAIP3; A20) in a high proportion of SS patients as well as a biallelic deletion in the SS-derived cell line SeAx. A20 is known as one of the negative regulators of the NF-κB pathway. It’s inhibitory effect on NF-κB is based on the co-operative activity of two ubiquitin-editing domains. This function of A20 has been recognized for a number of substrates involved in transmission of signals from cell surface receptors to NF-κB, for example the receptor-interacting protein 1 (RIP1), the TNF receptor associated factor 6 (TRAF6) and the 1KB kinase (IKK). Particularly interesting is the NF-κB signaling pathway, since its constitutive activation has been identified as a key feature in CTCL, including Sézary syndrome. We demonstrate that inhibition of A20 activates the NF-κB pathway thereby increasing the proliferation of normal T lymphocytes. On the other hand, the reconstitution of A20 expression slowed down the cell cycle in SeAx cells. Recently A20 inactivation has been reported in various B-cell lymphomas. In this study we show that A20 is also a putative tumor suppressor in the T-cell malignancy – Sézary syndrome.

Disclosure: No conflict of interest disclosed.

P468
Long-term follow-up of high-dose chemotherapy with autologous stem-cell transplantation and response-adapted whole-brain radiotherapy for newly diagnosed primary CNS lymphoma: results of the multicenter Ostdeutsche Studiengruppe Hämato-Onkologie OSHO-53 phase II study

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Introduction: We previously reported the results of a phase II study for patients with newly diagnosed primary CNS lymphoma (PCNSL) treated with autologous peripheral blood stem-cell transplantation (aPBSCT) and response-adapted whole brain radiotherapy (WBRT). The purpose of this report is to
update the initial results and provide long-term data regarding overall survival, progonotic factors, and the risk of treatment-related neurotoxicity.

Methods: A long-term follow-up was conducted on surviving primary central nervous system lymphoma patients having been treated according to the "OSHO-53 study", which was initiated by the Osteoide Studiengruppe Hämatoonkologie. Between August 1999 and October 2004 twenty-three patients with an average age of 55 and median Karnofsky performance score of 70% were enrolled and received high-dose methotrexate (HD-MTX) on days 1 and 10. In case of at least a partial remission (PR), high-dose busulfan/thiotepa (HD-Bu/TT) followed by aPBSC was performed. Patients without response to induction or without complete remission (CR) after HD-Bu/TT received WBRT. All patients (n=8), who are alive in 2011, were contacted and Mini Mental State examination (MMSE) and the EORTC QLQ-C30 were performed.

Results: Eight patients are still alive with a median follow-up of 116,9 months (79 – 141, range). One of them suffered from a late relapse eight and a half years after initial diagnosis of PCNSL, another one suffers from a gall bladder carcinoma. Both patients are alive, the one with the relapse of PCNSL has finished rescue therapy and is further observed, the other one with gall bladder carcinoma is still under therapy. MMSE and QLQ-C30 showed impressive results in the patients, who were not irradiated. Only one of the irradiated patients is still alive with a clear neurologic deficit but acceptable quality of life.

Conclusions: Long-term follow-up of our patients, who were included in the OSHO-53 study show an overall survival of 30 percent. If WBRT can be avoided no long-term neurotoxicity has been observed and the patients benefit from excellent Quality of Life. Induction chemotherapy with two cycles of HD-MTX should be intensified to improve the unsatisfactory OAS of 30 percent.

Disclosure: No conflict of interest disclosed.

Can polyclonality prevent oncogenesis?

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T cell receptor (TCR) polyclonal mature T cells are surprisingly resistant to oncogenic transformation through retroviral induction of T cell oncogenes. It has been shown that leukemias/lymphomas did not occur upon transplantation of wild-type mature T cells with polyclonal T cells being transduced with high copy numbers of gammaretroviral vectors encoding potent T cell oncogenes into RAG1-deficient recipients [1]. However, further studies demonstrated that the transplantation of T cells from TCR monoclonal OTI mice that were transduced with the same protocol resulted in leukemia/lymphoma. Using a mathematical modelling approach, we challenge the arising hypothesis that polyclonality induces competition within the T cell repertoire, which in turn suppresses the emergence of a leukemic clone. As a first step, we developed a mathematical model of T cell homeostasis that is derived from a similar niche-based based model of hematopoiesis [2]. The key assumption of the novel model is that T cell survival is critically dependent on the interaction of the clone-specific TCR with self-peptide-MHC-complexes and therefore subject to competition between different T cell clones.

Based on our modelling results, we speculate about the cellular properties of the leukemic clone. Within our model framework, we are able to explain the observed phenomena under the following two assumptions about the cellular properties of the leukemic clone:

(i) The leukemic clone is less competent than other T cell clones in acquiring survival stimuli from niches.

(ii) Proliferation of the leukemic clone is less dependent on niche interaction.

From our results we conclude, that clonal competition is a possible mechanism to counterbalance clonal dominance. Our modeling results allow us to foster the design of further biological experiments. A future goal is to determine the minimum clonal complexity that is needed in order to control the leukemic clone under the given circumstances.

References:


Disclosure: No conflict of interest disclosed.

Anaplastic large cell lymphoma (ALCL) relapsed and refractory after CHOP and ICE-chemotherapy: Induction of durable complete remission with Bortezomib-CHOP

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Anaplastic large cell lymphoma (ALCL) is a rare T cell malignancy where the anaplastic lymphoma kinase (ALK) gene is fused to several partners (mainly NPM, t(2;5)) in a fraction of patients. The 5 year progression free survival (PFS) with current chemotherapy regimens (mainly CHOP-based) is about 60% in ALK-positive and 36% in ALK-negative disease. Emerging preclinical and clinical data show constitutionally activated NF-kB signalling in this T-NHL entity. NF-kB signals can be suppressed by proteasome inhibition. The combination of CHOP standard chemotherapy with Bortezomib may therefore be used to improve the effect of chemotherapy in ALCL.

We report on a patient with ALCL who was refractory to first line and salvage chemotherapy with CHOP and ICE, relapsed after radiotherapy and responded to Bortezomib-CHP chemotherapy (Cyclophosphamide, Doxorubicin, Prednisone) with a complete metabolic remission. Toxicity of Bortezomib-CHOP in combination with radiation was manageable.

Bortezomib added to CHOP chemotherapy may enhance the efficacy of the treatment of ALCL both in the frontline and relapsed setting and is a viable additional option for the treatment of ALCL.

Disclosure: No conflict of interest disclosed.

Description of the molecular mechanisms of action of cytarabine, bortezomib and temsirolimus in MCL and its good or no-good for the design of combination strategies

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Background: Mantle cell Lymphoma (MCL) is a distinct B-cell subtype characterized by the chromosomal translocation t(11;14)(q13;q32), an especially poor clinical outcome and low response to chemotherapy. The proteasome inhibitor, bortezomib, is approved for treatment of relapsed and refractory MCL and achieves a response rate of 30-40%. However complete remission rates are low and duration of response proved to be relatively short. These limitations could be overcome by combining proteasome inhibition with other single-agents showing antitumor activity like cytarabine and temsirolimus. In the present study molecular mechanisms of action of bortezomib, temsirolimus and cytarabine were investigated in MCL cell lines.

Methods: MCL cell lines were exposed to Bortezomib, cytarabine and temsirolimus as single agents at different concentrations of the drugs. Western blot and mRNA analysis were performed for various members of the PI3K/Akt/ mTOR pathway and mRNA analysis were performed for various members of the PI3K/Akt/ mTOR pathway. In the present study molecular mechanisms of action of bortezomib, temsirolimus and cytarabine were investigated in MCL cell lines.

Results:

The combination of CHOP standard chemotherapy with Bortezomib may further enhance the efficacy of chemotherapy in ALCL.

Disclosure: No conflict of interest disclosed.
3 drugs are evaluated on the base of cell proliferation analysis (WST-assy, trypan blue staining).

**Results:** Western blot analysis revealed different molecular mechanisms of action of the 3 drugs. While phosphorylation of Akt at Ser473 was most down-regulated by bortezomib treatment it was upregulated by temsirolimus. In contrast phosphorylation of mTOR on Ser2448 was downregulated by temsirolimus and bortezomib but remained unaltered upon cytarabine exposure. Interestingly temsirolimus lead to downregulation of Rictor and phosphorylated Rictor but upregulation of Raptor and phosphorylated Raptor while bortezomib downregulates both proteins. Nevertheless the 70S56 protein, a downstream member of the mTOR pathway, was downregulated by both, bortezomib and temsirolimus. CCND1 protein was downregulated after cytarabine and bortezomib. Altogether a comparison of the molecular mechanism of action of the different drugs in the cell lines based on further protein – and RNA expression data of various members of the PI3K/Akt/mTOR pathway revealed a potential additive effect of the combinations.

**Conclusions:** In this study we show the differential impact of cytarabine, bortezomib and temsirolimus on members of the Akt/mTOR pathway in MCL. The advantage of these combinations remains to be confirmed in clinical trials.

**Disclosure:** Grit Hutter: No conflict of interest disclosed.


**P472**

NK/T cell lymphoma – the importance of distinguishing between localized and advanced stage – a summary of 5 cases

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**Introduction:** NK/T cell lymphoma (NKTL) is a rare entity of Non Hodgkin Lymphoma which is in a localized stage (II/III, Ann Arbor) sensitive to radiation and potentially curable but has a dismal prognosis in advanced stage (III/IV, Ann Arbor) (Dearden et al, Br J Haematol 2011). Here we report on 2 cases of localized stage as well as on 3 cases of advanced stage EBV associated NKTL.

**Methods:** The patients (3 male, 2 female) were newly diagnosed and treated at our institution between 01/10 and 01/11. The 2 patients in localized stage nasal ENKTL were diagnosed aged 32 and 52 with low risk IPI without any severe comorbidities in a good performance status. The treatment consisted of radiation (40 Gy) concurrent to a weekly administration of cisplatin as a radiosensitizer (CCRT) followed by 3 cycles of VIPD (etoposide, ifosfamide, cisplatin, dexamethason; Kim et al, JCO 2009). The 3 patients in an advanced stage of NKTL diagnosed aged 23, 45 and 69 had an IPI of low, intermediate and high risk respectively, no severe comorbidities and a good ECOG. They were all treated uniformly analogue the SMILE protocol (first prospective multicenter trial) in which the E. coli L-asparaginase was added to already established agents in the treatment of NKTL like dexamethason and etoposide as well as to multidrug resistance-unrelated substances like methotrexate and ifosfamide (Yamaguchi et al, Cancer sci, 2008).

**Results:** Both patients in localized stage achieved an ongoing CR and similar to the experience of the original trial, our patients did not suffer from relevant side effects and treatment could be continued without any interruption. As expected the treatment of the advanced stage turned out to be more difficult. The SMILE protocol showed activity in 2 of 3 patients (ICR, 1PR), but demonstrated asparaginase associated side effects such as severe coagulopathy and allergic reactions. Remission in all treated patients was only short (5/29 mth resp.) and all patients died due to progressive disease despite further treatment.

**Conclusions:** We can therefore conclude that the CCRT in localized stage induces durable CR without any relevant side effects. Whereas for the advanced stage of NKTL asparaginase based protocol can achieve response but due to side effect related treatment delay or interruption it lacks durable remission. Further clinical studies to evaluate an appropriate dosage of asparaginase as well as an asparaginase maintenance therapy should nevertheless be considered.

**Disclosure:** No conflict of interest disclosed.

**P473**

Response assessment after 4 cycles of beacopp using FDG-PET in patients with advanced-stage Hodgkin lymphoma

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**Background:** Positron emission tomography (PET) has been proven to be a powerful prognostic marker during the treatment of Hodgkin lymphoma with ABVD. Here, we analysed the prognostic value of PET after 4 cycles of BEACOPP in patients with advanced-stage Hodgkin lymphoma.

**Patients and Methods:** Between January 2004 and February 2008, 69 patients with newly diagnosed HL in clinical stages IIb with large mediastinal mass or extranodal disease, III and IV were included in or according to the HD15 protocol of the German Hodgkin Study Group. In addition to the protocol of the HD15 trial all patients received a PET scan after 4 cycles of BEACOPP (PET-4) in the treatment consistent of 6-8 cycles of BEACOPP.

**Results:** Of the overall group (n=69), 18 patients had a positive PET-4 while 51 had a negative PET-4. At a median observation time of 55 months, 4 of the 18 patients with a positive PET-4 had progressed or relapsed, while there was one relapse in the group of PET-4 negative patients. The negative predictive value of PET-4 was 98%; the positive predictive value of PET-4 was 22%.

There was a significant arm difference between PET-4 negative and positive patients concerning the time to progression or relapse starting from the point of diagnosis (p=0.004).

**Conclusion:** The results of the present analysis underline the clinical impact of PET-4 scan in advanced-stage Hodgkin lymphoma. A negative PET-4 scan has a high negative predictive value and predicts a significant longer non-progression than PET-4 positive patients.

**Disclosure:** No conflict of interest disclosed.

**P474**

High expression and functional signalling implicate CXCR4 in T-PLL biology

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**Introduction:** T-cell prolymphocytic leukemia (T-PLL) is a rare and very aggressive mature T-cell tumor. Its molecular hallmark and putative initiating event is an over-expression of the T-cell leukemia 1 (TCL1) proto-oncogene. We previously showed a differential TCL1 expression to be of diagnostic and prognostic value and to influence functional aspects in the context of T-cell receptor (TCK) signaling in T-PLL (Herling et al., 2008). The chemokine receptor CXCR4 is implicated in stem cell homing to the bone marrow niche and leukemia cell survival. To elucidate pathogenetic cascades as potential treatment targets in T-PLL, we focus our work on histogenetic, immunophenomologic, and mechanistic aspects of this understudied disease.

**Methods:** Extensive immunophenotyping of n=10 primary T-PLL samples was performed using 10-colour flow cytometry. Serum levels of 9 chemokines were measured using ELISA. Cell signalling was assessed by flow cytometry (FACSCalibur, Becton Dickinson) after stimulation of T-PLL with a recombinant human chemokine (DuoSet, R&D Systems) and intracellular phosphorylation of CXCR4 was detected by Western blot analysis. CXCR4 expression was analyzed by immunohistochemistry on 5 primary T-PLL samples in comparison to 15 healthy lymph node control samples.

**Results:** The expression of CXCR4 on T-PLL cells correlated with the expression of TCL1, and both proteins were overexpressed in 100% of T-PLL samples. Functional assays showed a significantly higher chemotactic activity of T-PLL cells towards human CXCL12 compared to healthy lymph node control cells. The CXCR4 receptor displayed a higher intracellular phosphorylation on Ser239 in T-PLL compared to control cells. The expression of CXCR4 was confirmed by immunohistochemistry of primary T-PLL samples and healthy lymph nodes.

**Conclusion:** The high expression and functional signalling of CXCR4 in T-PLL cells is indicative of a prolymphocytic growth advantage. The overexpression of CXCR4 may be a novel therapeutic target in T-PLL.
in vitro to TCR stimulation with regard to expression of stimulation markers, cell cycle progression (DNA content analysis) and migratory capacity (trans-well).

Results: Surprisingly, we found intra-tumoral expression of TCI1 expression and also distinguish between T-PLL clones displaying a naïve or memory phenotype, with the latter predominately showing ‘central memory’ characteristics. Activation markers are consistently upregulated upon stimulation. This is accompanied by cell cycle progression and proliferation. Among the chemokine and homing receptors analysed, CXCXR4 turned out to be consistently highly expressed, physiologically regulated and functional in T-PLL cells. These data implicate a role for the CXCXR4/CXCL12 axis in T-PLL cell migration and potentially survival.

Conclusion: We show for the first time a central memory phenotype of T-PLL cells. Together with the consistently high CXCXR4 expression, this hints at the central importance of the lymph node microenvironment for T-PLL pathobiology. We are currently investigating the relationship of TCI1 activation with these observations. CXCXR4 mediated signalling has been shown to rely on the TCR proximal signalling machinery (Kumar et al., 2006). Consequently, our data on a functional response of T-PLL cells to TCR crosslinking and SDF1α provide evidence that the CXCXR4/SDF1α axis as well as TCR-mediated survival signals are potential treatment targets in T-PLL.

Disclosure: Nicole Weit: No conflict of interest disclosed.
Marco Herling: Honoraria: commercial interests in the TCL1 antibody used in this study.

P476
Aplastic anemia in association with a lymphoproliferative neoplasm: coincidence or causality?

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Introduction: Acquired aplastic anemia (AA) is a bone marrow failure syndrome resulting from an immune-mediated destruction of hematopoietic stem cells. Most cases are considered idiopathic. Rarely, AA is associated with underlying disorders, mostly autoimmune disorders.

Methods: Here, we present 3 patients with aplastic anemia associated with lymphoid neoplasia.

Results: The 1st patient is a 74 year female who presented with pancytopenia in 09/2010. Bone marrow cytology was aplastic without dysplasia. Bone marrow biopsy was hypocellular with decreased hematopoiesis, but few cellular areas were densely infiltrated with a lymphoplasmacytic lymphoma. The immunofixation showed a monoclonal IgM kappa (4.5 g/l). The patient received one cycle of rituximab and bendamustine resulting in remission of the paraprotein but worsening pancytopenia. Treatment was changed to G-CSF and cyclosporine, without improvement and the patient continues to be transfusion-dependent.

The 2nd patient is a 54 year male who was diagnosed with unclassifiable small B-cell lymphoma and pancytopenia in 11/2003. Marrow histology showed an aplastic marrow with focal, monoclonal lymphoid infiltration. The patient was treated with 2 cycles of R-CHOP and 1 cycle of rituximab monotherapy resulting in a complete remission of the lymphoma but with no effect on hematopoiesis. The patient then received an allogeneic HSCT from a HLA-identical sibling in 2004 resulting in complete remission of the lymphoma and AA. The 3rd patient is a 65 year male who was diagnosed with multiple myeloma and pancytopenia in 1987. The patient was treated initially with 2 cycles melphalan and GM-CSF with no effect on hematopoiesis. In 1988 the patient was treated with anti-lymphocyte-globulin and GM-CSF which resulted in a short-term hematological improvement but the patient died in 11/89 due to aplasia and infection. In all 3 patients, there was no growth in short term culture assays.

Conclusions: These three cases show remarkable similarities: they all presented with a lymphoproliferative neoplasm and pancytopenia, but hematopoiesis completely collapsed with short and mild chemotherapy. Immunosuppressive therapy and growth factors were without any effect on AA. This particular behavior of the AA, speaks in favor of an underlying stem cell defect rather than autoimmune, and could be an argument for causality between lymphoma and AA rather than coincidence.

[Characteristics of SAA patients]

Disclosure: No conflict of interest disclosed.

P475
Reactive tumor infiltrating T-cells predict survival in mantle cell lymphoma: an immunohistochemical study of 81 patients

Schrader, C.1, Akaltun, Ö.1, Meusers, P.2, Brittinger, G.2, Claasen, J.1, Klapper, W.3
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Introduction: The role of tumor infiltrating T-Cells in malignant B-Cell lymphomas is discussed controversial. There are only limited data on CD 8 and FOXP3 positive cells in mantle cell lymphoma.

Methods: 81 biopsy specimens of patients (64 men and 17 women) with mantle cell lymphoma and a median age of 64 years (range: 41 to 86 years) were included in this study. The slides were stained immunohistochemically with CD3, CD8 and FOXP3. Positive T-cells of 10 High power fields (HPF) were included in this study.

Results: CD 8 and FOXP3 staining showed a range of 0 to 138 positive cells per HPF with a mean value of 19.4/HPF. A high account of CD 8 positive cells was associated with a significantly longer overall survival time (42 months) compared to the group with low account (<20/HPF) of CD 8 positive cells (23 months). Kaplan Meier analysis revealed a significant difference (P=0.015) in overall survival time.

Conclusions: High number of CD 8 and FOXP3 T-Cells predicts a superior clinical outcome in patients with mantle cell lymphoma.

Disclosure: No conflict of interest disclosed.
P478
Bcl11b expression leads to chemoresistance accompanied by G1 accumulation


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Abstracts

Background: The expression of BCL11B has been reported in normal and transformed cells derived from T-lymphocytes, neurons, keratinocytes and recently in a subset of squamous cell carcinomas. Despite the rapidly accumulating knowledge concerning Bcl11b biology, the contribution of this protein to normal or transformed cell homeostasis remains open.

Methodology/principal Findings: Here, by employing an overexpression strategy and cells endogenously expressing BCL11B we revealed formerly unidentified features of Bcl11b which shed some light on the potential involvement of the protein in tumor maintenance. Two different T cell lines were forced to overexpress BCL11B which resulted in markedly increased resistance to radiomimetic drugs while no influence on death-receptor apoptotic pathway was observed. Apoptosis resistance triggered by BCL11B overexpression was accompanied by a cell cycle delay caused by accumulation of cells at G1. This cell cycle restriction was associated with upregulation of CDKN1C (p57) and CDKN2C (p18) cyclin dependent kinase inhibitors. Moreover, p27 and p130 proteins accumulated and the SKP2 gene encoding a protein of the ubiquitin-binding complex responsible for their degradation was repressed. Furthermore, the expression of the MYCN oncogene was silenced which resulted in significant depletion of the protein in cells expressing high BCL11B levels. Both cell cycle restriction and resistance to DNA-damage-induced apoptosis coincided and required the histone deacetylase binding N-terminal domain of Bcl11b. The sensitivity to genotoxic stress could be restored by the histone deacetylase inhibitor trichostatine A.

Conclusions: The data presented here suggest a potential role of BCL11B in tumor survival and encourage developing Bcl11b-inhibitory approaches as a potential tool to specifically target chemoresistant tumor cells.

Disclosure: No conflict of interest disclosed.

Table 1. (for Abstract P476)

<table>
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<tr>
<th>Pat-Nr</th>
<th>Sex</th>
<th>Age at diagnosis</th>
<th>Lymphoma Entity</th>
<th>Therapy</th>
<th>Best response</th>
<th>Alive</th>
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<tr>
<td>1</td>
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<td>74</td>
<td>lymphoplasmacytic lymphoma</td>
<td>1. 1x R-Benda 2. CSA</td>
<td>AA: PR NHL: CR</td>
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<tr>
<td>2</td>
<td>m</td>
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<td>unclassifiable small B-cell lymphoma</td>
<td>1. 2x R-CHOP 2. R mono 3. allos-HSCT 02/04</td>
<td>AA: CR NHL: CR</td>
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<td>3</td>
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<td>65</td>
<td>multiple myeloma</td>
<td>1. 2x melphalan/ GM-CSF 2. ATG/ GM-CSF</td>
<td>AA: NR NHL: SD</td>
<td>Death 11/98 in aplasia and infection</td>
</tr>
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</table>

P477
Account of tumor infiltrating macrophages is a prognostic factor for patients with mantle cell lymphoma

Schrader, C., Sirin, F., Meusers, P., Brittinger, G., Claasen, J., Klapper, W.

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Introduction: Mantle cell lymphoma (MCL) is a malignant lymphoma associated with a relatively aggressive clinical course and a median overall survival time of 3-4 years. Only limited data about tumor associated macrophages and their influence on survival in MCL exists.

Methods: We analyzed the amount of CD68 macrophages in relation to the clinical outcome in patients with MCL. Lymph node biopsies of 77 untreated patients (17 women and 60 men) enrolled in two multicenter trials (1975-1985) with a median age of 66 years (range 41-86 years) were included in this study. Biopsy specimens were investigated immunohistochemically with monoclonal antibodies against CD68 (Ki-M1P). 10 High power fields (HPF) were evaluated by random.

Results: Patients with low account (less than 10/HPF) of CD 68 positive macrophages had a median overall survival time of 38.2 months, compared to 24.2 months for patients with high (more 10/HPF) CD 68 positive macrophages. The Kaplan-Meier analysis showed a significant difference in the overall survival time (p=0.0027).

Conclusions: Patients with mantle cell lymphoma and a low number of CD 68 positive macrophages have a better prognosis and can predict outcome.

Disclosure: No conflict of interest disclosed.

Fig. 1. Overall survival FOXP 3

0.0 0.5 1.0
0 25 50 75 100
Overall survival
Months
FOX3 positive cells

Fig. 1. Overall survival FOXP 3

0.0 0.5 1.0
0 25 50 75 100
Overall survival
Months
>25/HPF <25/HPF

Table 1. (for Abstract P476)

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</table>

Fig. 1. Overall survival FOXP 3
The oncogenic miR-17-92 cluster and miR-155 define ALK status in anaplastic large cell lymphoma

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Introduction: ALCL is an aggressive T-cell-derived malignancy that expresses CD30 and represents up to 5% of all non-Hodgkin lymphomas (NHL) and ~40% of pediatric NHL (1). About half of the patients carry the t(2;5) (p23;q34) translocation which results in constitutive activation of the ALK kinase (2). In 85% the ALK kinase is linked to the NPM gene but also other fusion-partners have been described. The discovery of over-activated ALK in subsets of lung and breast cancer as well as neuroblastoma has accelerated the development of potent ALK inhibitors (3). However, ALK negative ALCL has a worse prognosis and yet is little known about the molecular drivers of this ALCL variant (4).

Methods: miRCURY LNA mRNA Arrays containing 626 different miRNAs in 8 replicates were used (Exiqon). FFPE ALCL specimens were provided by the Institute of Clinical Pathology at the Medical University of Vienna. mRNA mimics were transfected using HiPerfect reagent (Qiagen) and expression tested by fluorescently labelled RT-PCR (Applied Biosystems). Rabbit antibodies for PPIA, SHIP1, IRAK1 and C/EBPβ were used for western blotting (Cell Signaling Technology).

Results: We have recently described a set of miRNAs that deregulate in ALCL and have shown that the oncogenic miR-17-92 cluster is higher in ALK+ form (5). Now, we describe two microRNAs, miR-155 and miR-146a involved in innate immune response and hematopoietic differentiation to be defining features of ALK- ALCL. Moreover, the levels of protein targets of these microRNAs C/EBPβ, IRAK1 and SHIP1 are correlated to miRNA expression. Active reintroduction of miRNAs into ALCL cell lines was able to specifically down regulate C/EBPβ, IRAK1 and SHIP1 suggesting a active role of miR-155 and miR-146a in ALK- ALCL.

Conclusions: In the last years microRNAs have emerged as potent regulators of tumorigenesis. We identified two microRNAs which are highly expressed in ALK- ALCL: miR-146a and miR-155 and could show that important effector molecules of survival and immunity are targeted by these miRNAs in ALCL. Given their overexpression it is possible to therapeutically target these miRNAs. Engraftment of ALK+ ALCL cell lines in NOD/SCID mice will allow us to test antagonist efficacy alone or in combination with commonly used chemotherapeutics.

References:
1) Piccaluga et. al., Adv. Hematol. 2010
2) Falini et. al., Haematologica 2009
4) Savage et. al., Blood 2008
5) Merkel et. al., PNAS 2010

Disclosure: No conflict of interest disclosed.

P481
PET scans in HIV-related Hodgkin’s lymphoma (HIV-HL): results of a retrospective study

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Introduction: The role of PET-scans in patients (pts) with Hodgkin’s lymphoma (HL) is currently being investigated in prospective trials. By contrast, no data are available on the use of PET-scans in HIV-HL. The goal of the present study is to analyse consecutive pts with HIV-HL who had at least one PET scan conducted during their course of primary chemotherapy (CT).

Methods: In the ongoing prospective multicenter trial pts with HIV-HL are planned to receive 2x ABVD + 30 Gy involved field (IF) radiation (RT) for early stage (ES) favourable HL (stage I/II without risk factors), 4x BEACOPP baseline or 4x ABVD + 30 Gy IF for ES unfavourable HL, and 6-8x BEACOPP baseline for advanced stage HL with BEACOPP being replaced by ABVD in pts with far advanced HIV-infection or poor performance status. Since the role of PET-scans is not specifically addressed in this trial FDG-PET-CT is not routinely scheduled. However, as PET-scans are being performed on physician’s choice we retrospectively analyzed results and clinical impact of FDG-PET in this cohort of pts.

Results: From 03/04 to 12/10 105 pts (median age 43.9 yrs, 8 females) were included in the study. 23 pts (22%) had ES favourable HL, 14 (13%) ES unfavourable HL, and 68 (65%) advanced stage HL. 26 of 105 pts had a total of 37 PET/PET-CT scans done at some point during their course of treatment. FDG-PET was conducted as part of the initial staging and as response assessment in 8 and 28 cases, respectively. 1 pt had a PET-scan performed to rule out a late relapse of HL or a second malignancy. In 15 pts a negative FDG-PET had an impact on further management as additional RT was abandoned (n=9) or CT was reduced (n=6).
for advanced HL terminated after 6 (n=5) and 7 cycles (n=1) of BEACOPP. A PET-scan performed after 2 cycles (ABVD/PEIT2) proved negative (neg) in 3/3 pts with 2 of those not receiving IF-RT. PEIT4 proved negative in 4 of 5 pts with ES-unfavourable HL and all of those had IF-RT spared. All pts (n=6) remain disease-free after a median follow-up of 24 months. RT was also successfully spared in 3 pts with neg. FDG-PET after 6 or 8 cycles of CT.

Conclusions: Given the high negative predictive value of FDG-PET in HIV-negative HL, this data indicate that in pts with HIV-HL a negative PET-scan conducted after 2, 4 or 6-8 cycles of CT may allow sparing of additional RT or CT.

Disclosure: No conflict of interest disclosed.

P482
Follicular B-cell lymphoma in a patient with a history of a T-ALL
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T-ALL is a rare hematologic disease. Follicular lymphoma is an indolent lymphoma that originates from the germinal center of the lymph follicle. The t(14;18) translocation is detectable in all cases of the lymphoma. By this translocation the anti-apoptotic factor bel-2 gets under control of the heavy chain locus. The combination of a T-ALL and a B cell lymphoma like the follicular lymphoma has not been reported yet.

We present a 34 year old patient who underwent chemotherapy for T-ALL in 1996 and was since then in stringent complete molecular remission. Unfortunately as the treatment took place in Romania the chemotherapeutic regimen could not be followed up completely. He remained in remission for 14 years until he presented in our clinic in 2010 with newly grown tumour of the right cheek. No B-symptoms, peripheral lymphomas, cso or bone marrow infiltration could be detected. Histopathology from a skin biopsy showed a primary cutaneous follicular lymphoma (stadium I AE). The T-ALL however could not be found neither in the bone marrow nor in the peripheral blood stream at the time of diagnosis. The standard regimen of irradiation of the cutaneous lymphoma was not initiated due to the risk of irreversible skin lesions. Therefore a chemotherapy regimen with four cycles rituximab and bendamustin was initiated. The T-ALL is a rare hematologic disease. Follicular lymphoma is an indolent lymphoma that originates from the germinal center of the lymph follicle. The t(14;18) translocation is detectable in all cases of the lymphoma. By this translocation the anti-apoptotic factor bel-2 gets under control of the heavy chain locus. The combination of a T-ALL and a B cell lymphoma like the follicular lymphoma has not been reported yet.

So far, no T-ALL- B-CLL association has been reported. To our knowledge this report is the first demonstration of MCPyV-DNA by FISH analysis in the nuclei of MCPyV-positive CLL and MCC cells. Compared to the FISH signals in MCC, the specific FISH signals in the CLL cases revealed strong specific punctate but slightly more granular nuclear signals. However, the CLL specimens derived from EDTA decalcified bone marrow trephines in contrast to the non decalcified specimens of MCCs. No signals were obtained by MCPyV FISH in breast or colon cancer specimens.

Conclusions: The specific detection of MCPyV in CLL cells further supports previous reports of a possible involvement of MCPyV in a significant subset of CL. The specific punctate nuclear FISH signals in MCPyV-positive CLL cells are compatible with viral integration of MCPyV in CLL cells. This is the first demonstration of MCPyV-DNA by FISH in CLL cells.

Disclosure: No conflict of interest disclosed.

P484
Targeting the PI3K/AKT/mTOR pathway in CLL as a strategy to interrupt in TCL1A-mediated oncogenic signalling
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Background: The PI3K/AKT/mTOR pathway promotes a central role in governing malignant cell growth. In CLL, it represents a central signalling node integrating milieu-derived pro-survival stimuli. The lymphoid oncogene T-cell leukemia 1A (TCL1A), which causes CLL-like tumors in transgenic mice, could be established to augment AKT activity through an activation-induced physical interaction. Therefore, interruption in PI3K/AKT/mTOR signalling could be an efficient strategy to more selectively interrupt in CLL cell survival.

Material and Methods: Using a panel of established and novel small molecules, we aimed to specifically target PI3K/AKT/mTOR signalling in short-term cultures of isolated primary CLL cells (n=32) and cell lines. We demonstrated that, in addition to inhibiting the AKT and mTOR signals, specific PI3K/AKT/mTOR inhibitors PI-103 and BEZ235, the mTOR-inhibitor Rapamycin, and the AKT-substrate inhibitor A443654 were assessed for their "biocchemical" and "cellular" efficacy. In laser confocal microscopy we showed that such selected pathway inhibition translates well to aberrant TCL1-AKT complex formation. Generally, inhibitor-induced altered levels of pAKT/AKT paralleled Caspase-3- and PARP-mediated apoptosis. However, there was a heterogeneous response pattern across the primary CLL samples allowing distinction of responders from those which did not reach LD50 (apoptosis) or IC50 (viability) values with relevant (nano- / low-micromolar) dosages (non-responders). Healthy-donor PBMC or B-cells were by far less sensitive towards the inhibitors used.

Conclusions: A preliminary analysis reveals that lack of response to treatment with certain substances is associated with high-level TCL1A expression, confirmed by experimental introduction of TCL1A into B-cell lines. We currently investigate for correlations of CLL subsets of inhibitor sensitivity with baseline levels of TCL1A or phospho-kinases as well as with clinico-pathologic patient/tumor features.

Disclosure: No conflict of interest disclosed.
P485

Reconstitution of pro-apoptotic death-associated protein kinases (DAPK) to re-establish apoptotic sensitivity in CLL

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Background: At the cellular level treatment of chronic lymphocytic leukemia (CLL) is hampered by an abnormally low rate of spontaneous and drug induced cell death rendering the CLL cell invulnerable to a broad spectrum of currently available chemotherapeutic drugs. Therefore, strategies targeting apoptotic resistance in this heterogeneous disease are of particular interest. Death-associated protein kinase (DAPK) is thought to play a key role in conferring apoptotic resistance as DAPK1 transcription was shown to be impaired in 98% of CLL across all known molecular and clinical subsets (Raval et al., CELL 2007). Here, we report on encouraging data about the reconstitution of pro-apoptotic DAPK activity in CLL cells that warrants further study towards potential anti-leukemic treatment options.

Methods: We generated a panel of constitutively active DAPK1 and DAPK2 mutants, including DK1KD/DKD2 and that upon delivery into target cells should reconstitute pro-apoptotic DAPK activity. To achieve CLL-specific targeting of the DAPK mutants, we fused these mutants to a CD2 single chain variable fragment (scFv) SGGH and α-CD40 scFv G28-5. Their in vitro activity as compared to negative and positive controls was investigated in peripheral blood B-cells from CLL patients and those of healthy donors. Analysis included molecular markers of cell death induction (i.e. AnnexinV/7AAD staining, Caspase activation, or apoptotic protein expression) and in vitro kinase activity assay of DAPK mutants.

Results: Overall, reconstitution of pro-apoptotic DAPK activity by the delivery of novel, functional DAPK mutants into CLL is followed by cell death induction. In detail, we were able to show that (1) DAPK1/2 is absent in the majority of CLL patient samples. (2) Our novel fusion proteins DK1KD-SGGH and DK2KD-G28-5 are capable of both target-cell specific, robust binding and internalization into several CD22/CD40 antigen-positive cell lines and primary CLL patient samples and functional DAPK kinase activity. (3) Internalization of DK1KD-SGGH and DK2KD-G28-5 inhibited cell proliferation and induced apoptosis in various B-cell lines and primary CLL samples in a dose-dependent manner.

Conclusions: Reconstitution of pro-apoptotic DAPK in CLL cells shows great therapeutic potential for the treatment of CLL. Further studies will address the combination of our constructs with established drugs and determine their sensitivity in different CLL subpopulations.

Disclosure: No conflict of interest disclosed.

P486

Leflunomide induces apoptosis in Fludarabine-resistant and clinically refractory CLL cells

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Environmental conditions in lymph node proliferation centres protect chronic lymphocytic leukemia (CLL) cells from apoptotic triggers. This situation can be mimicked in vitro stimulation with CD40 Ligand (CD40L) and IL-4. Resistance to Fludarabine-mediated apoptosis was stimulated by CD40 activation alone inducing high levels of BCL-2 and MCL1. Apoptosis resistance was further enhanced by a complementary JAK/STAT signal induced by IL-4. In contrast, CLL proliferation required both, a CD40 and a JAK/STAT signal and could be completely blocked by pan-JAK inhibition. Leflunomide is an anti-rheumatic drug achieving serum concentrations of >100 μg/ml of its active metabolite A771726. A771726 antagonizes CD40L/IL-4-induced proliferation at very low concentrations (3 μg/ml) by inhibiting dihydorotate dehydrogenase. At a concentration of 10 μg/ml, A771726 additionally attenuated STAT3 phosphorylation, whereas apoptosis of CD40L/IL-4-activated (“resistant”) CLL cells was achieved with higher concentrations (IC50: 30 μg/ml). Apoptosis was also effectively induced by A771726 in clinically refractory CLL cells with a defective p53 pathway. Induction of apoptosis involved inhibition NF-κB activity and loss of BCL-XL and MCL1 expression. In combination with Fludarabine, A771726 synergistically induced apoptosis (IC50: 56 μg/ml). We thus demonstrate that A771726 overcomes CD40L/IL-4-mediated resistance to Fludarabine in CLL cells of untreated as well as clinically refractory CLL cells and represents a novel therapeutic principle for attacking CLL cells in chemoresistant niches.

Disclosure: No conflict of interest disclosed.

P487

Case report: A 62-year-old man with simultaneous diagnosis of diffuse large B-cell lymphoma (DLBCL) of the testis and chronic lymphocytic leukemia (CLL) molecular genetic confirmed as Richter’s syndrome

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A 62-year-old man was admitted to our hospital because of painless swelling of the left testis. He had been well until 2 months before presentation. The patient had no fever, night sweats or weight loss. In past medical history the patient reported only the diagnosis of a deep venous thrombosis 10 years ago. On clinical examination, ultrasound and computed tomography there were no abnormal findings with the exception of the enlarged left testis.

Laboratory Findings: White cell count (WBC) was 6.100 per mm3 (different count: neutrophils 55%, lymphocytes 34% absolute 2.074 per mm3), monocytes 8%, eosinophils 2%, basophils 1%), hemoglobin 11.5 g/dl, hematocrit 37% and platelet count 194.000 per mm3. Routine laboratory tests and serum levels for LDH, AFP and β-HCG were normal.

Pathological examination of the left testis was performed. The histologic examination showed a testicular infiltration of a DLBCL of immunoblastic / plasmoblastic subtype with expression of CD20 and CD23 but no CD5.

Routine staging procedures for testicular DLBCL ruled out enlarged lymph nodes, hepatosplenomegaly or any other extranodal manifestation. Serum protein electrophoresis and immunofixation detected a monoclonal IgM lambda serum protein. Serum level for IgM was increased (20.4 g/l). Serum β2-Mikroglobulin, haemolysis and examination of cerebrospinal fluid were normal. Bone marrow examination showed a nodal and diffuse infiltration of 30 % of small lymphocytes with clumped chromatin and scanty cytoplasm. These cells express the typical immunophenotype of CD19, CD20, CD5 and CD23 but negative for CD10. Further molecular analyses were performed. The PCR based amplification of FRA2 region of immunoglobulin heavy chain gene detected the identical clonal 230 bp peak amplification in the CLL cells of the bone marrow and in the cells of the testicular DLBCL. These results confirmed the transformation of the CLL to high grade lymphoma (Richter’s syndrome).

Summary: We describe the case of a 62-year-old patient with simultaneous diagnosis of CLL with IgM lambda paraproteinemia and a testicular DLBCL confirmed as an uncommon extranodal involvement of Richter’s syndrome.

Disclosure: No conflict of interest disclosed.

P488

The receptor tyrosine kinase-like orphan receptor 1 (ROR1) as a diagnostic tool in chronic lymphocytic leukemia (CLL) using flow cytometry

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Introduction: Flow cytometry is commonly used to establish the diagnosis of chronic lymphocytic leukemia (CLL) as a defined combination of antibodies
discriminates between normal B cells and CLL cells (CD5, CD19, and CD23). The receptor tyrosine-like orphan receptor 1 (ROR1) is an embryonic glycoprotein involved in several developmental processes. It was shown to be highly expressed on CLL cells, but not on normal B cells. Due to this fact we examined the potential of ROR1 as a diagnostic marker in initial and follow-up diagnostics of clinical heterogeneous patients with CLL.

**Methods:** Peripheral blood of 168 CLL patients in different clinical stages as well as healthy volunteers was subject to flow cytometric analysis of ROR1 surface expression. The study included 105 untreated patients with or without relevant comorbidities and 72 patients which were measured on different time points of treatment. A fluorescently labelled anti-ROR1 antibody was used. We also examined ROR1 expression in 12 patients with other B-non-Hodgkin-Lymphomas (B-NHL).

**Results:** ROR1 was expressed uniformly at high levels on the surface of CLL cells (mean 98.1%) of untreated patients, independent of clinical stage or other comorbidities. ROR1 expression was also consistently high on CLL cells from treated patients, independent of received chemotherapeutic substances and treatment cycle. In contrast, in healthy volunteers ROR1 expression on different cell subgroups was only marginal (mean 1.9%) and significantly lower than on CLL cells. Also in B-NHL patients we could detect ROR1 surface expression in different intensities. Further, in CLL patients no correlation between the ROR1 expression level and prognostic markers could be detected.

**Conclusions:** Due to the uniform ROR1 expression on CLL cells in treated as well as untreated patients we conclude that ROR1 is a suitable marker for initial and follow up diagnostics, but cannot detect CLL specifically, because of its expression in other B-NHL.

**Disclosure:** Sabrina Uhrmacher: No conflict of interest disclosed.

Karl Kreuzer: Advisory Role: Roche, Celgene, Novartis, Bayer, Pfizer; Expert Testimony: Roche, Celgene, Novartis, Bayer, Pfizer; Other Financial Relationships: Roche, Celgene, Novartis, Bayer, Pfizer.

**P490**

*p-N0 acetylsalicylic acid (p-NO-ASA) for treatment of high-risk chronic lymphocytic leukemia (CLL)*

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**Introduction:** Chronic lymphocytic leukemia (CLL) is characterized by an accumulation of long lived B-cells. It generally progresses slowly, but some patients quickly develop symptomatic disease requiring immediate therapeutic intervention. Unfortunately, most of these high-risk patients feature a distinct resistance towards standard therapy. Over-represented in this group is the incidence of del17p and TP53mut.

**Non-steroidal anti-inflammatory drugs (NSAIDs) possess anti-neoplastic activity in CLL. Interestingly, nitrosylated derivatives of NSAIDs show superior anti-neoelastic efficacy compared to their parent compounds. On this account we tested the efficacy of para-NO-ASA (p-NO-ASA) in cell-lines with selected characteristics representing bad prognosis markers for CLL and in patients with high-risk TP53-mutated CLL.

**Methods:** Primary cells were isolated from peripheral blood of CLL patients or healthy individuals. Further, the human B-cell lymphoma cell lines HUT (B-cell non-hodgkin lymphoma, TP53 mutated), U2932 (treatment resistant B-cell lymphoma), GRANTA-519 (relapsed high-grade B-non-hodgkin lymphoma, Cycin D1 activation), JVM3 (B-prolymphocytic leukemia, expresses proto-oncogene Bc2) and MEC-1 (chronic B-cell leukemia, del17p) were used. Primary cells and cell lines were incubated with p-NO-ASA in concentrations ranging from 0.01 μM to 250 μM for 24 hours. Cell viability was determined by ATP assay (Promega).

**Results:** p-NO-ASA effectively reduced ATP content in the cell lines HUT (LD₅₀ 9.28 μM), U2932 (LD₅₀ 4.81 μM) and JVM3 (LD₅₀ 3.33 μM). LD₅₀ values were comparable with those achieved for CLL cells from a mixed patient population (LD₅₀ 4.34 μM). Primary CLL cells from TP53 mutated patients (n=5) showed a slightly increased LD₅₀ of 25.56 μM, which was still significantly lower than that for healthy PBMCs (n=5) (LD₅₀ 63.72 μM). GRANTA-519 and MEC-1 were less sensitive (LD₅₀ 53.44 μM and 22.21 μM, respectively).

**Discussion:** p-NO-ASA shows potent reduction of ATP content in cell-lines derived from treatment resistant cell lines featuring several bad prognosis markers such as TP53 mutation or Bc2 overexpression. Further, TP53 mutated patients are about three fold more sensitive towards p-NO-ASA treatment as PBMCs derived from healthy individuals. Hence, p-NO-ASA is worth further evaluation as treatment for bad prognosis patients unresponsive to conventional CLL treatment regimens.

**Disclosure:** Sylvia Karlmann: No conflict of interest disclosed.

Karl Kreuzer: Advisory Role: Roche, Celgene, Novartis, Bayer, Pfizer; Expert Testimony: Roche, Celgene, Novartis, Bayer, Pfizer; Other Financial Relationships: Roche, Celgene, Novartis, Bayer, Pfizer.
been suggested to play a role in the malignant behavior of CLL cells via controlling the expression of groups of genes such as enhances the expression of apoptosis inhibitors (BCL-2), NF-κB, Wnt, MAPKs, Stats, and cytokines (IL-10). By being naturally phosphorylated on tyrosines CD5 is chronically activated in CLL cells. Moreover, the B CLL cell is also characterized by the expression of ROR1, a receptor tyrosine kinase which is highly expressed in many tissues during development but it is absent on normal adult tissues including non-malignant B-cells. In this study we elucidate whether CD5 has an effect on the surface expression of ROR1 in CLL cells.

Methods: A retrospective analysis of immunophenotype results for 71 randomly selected patients diagnosed with CLL was performed. Patients were divided into two groups depending on the mean fluorescence intensity (MFI) of CD5 (dim and moderate to bright MFI). MFI was measured using a logarithmic scale with signal intensity ranging from 10^4 to 10^10. Four degrees of fluorescence intensity were assigned using college of American pathologists recommended standard criteria, negative 0 cases, dim 16 cases (23%), moderate and bright 55 cases (77%). The average ROR1 MFI was compared between the two groups using Wilcoxon-Mann-Whitney test for independent samples with 95% confidence interval.

Result: The study showed that the average ROR1 MFI for CD5 dim group was 12.4 and 16.9 for CD5 moderate to bright group with a P-value of 0.003 which means a significant difference in the expression of ROR1 between the two groups.

Conclusion: The difference in the expression of ROR1 between the two groups might be due to the influence of CD5 on ROR1 gene which emphasizes the need for an experimental study to compare CD5 and ROR1 expression on a molecular basis.

Disclosure: Hessah Alsalami: No conflict of interest disclosed.

P492

NFAT2 is essential for normal B1a cell development

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Introduction: NFAT is a family of highly phosphorylated proteins residing in the cytoplasm of resting cells. Upon dephosphorylation by calcineurin, NFAT proteins translocate to the nucleus where they orchestrate developmental and activation programs in diverse cell types. Although identified originally as a major transcriptional regulator in T cells, it is now clear that NFAT transcription factors also possess important roles in other cells of the hematopoetic system including dendritic cells, mast cells, megakaryocytes and B cells. B1 B cells are a subclass of B lymphocytes which can be further divided into CD5 B1a and CD5 B1b subtypes. B1a cells are a phenotypically and functionally distinct population of B cells which are long-lived and typically express CD5, CD43 and high levels of surface IgM together with low surface IgD and CD45 (B220). A human B cell equivalent of the murine B1a cell has been suggested as the leukemic precursor cell in chronic lymphocytic leukemia (CLL).

Methods: Analysis of the role of NFAT2 in hematopoiesis has been complicated by the fact that deletion of this gene is embryonic lethal around embryonic day 13 because of defects in heart valve development. To circumvent this problem we generated mice with a conditional NFAT2 knock out allele (NFAT2flox/flox). To achieve NFAT2 deletion limited to the B cell lineage, we bred NFAT2flox mice to Bcl10-Cre mice, in which the Cre recombinase is expressed under the control of the B cell-specific cd19 promoter.

Results: B cells from these mice exhibited complete absence of NFAT2 expression as assessed by immunocytochemistry, western blot and RT-PCR. While the mice showed normal development of conventional B2 B cells, they exhibited significantly reduced amounts of B220* IgM* CD5* CD23* CD43* B1a B cells in the peritoneal cavity as assessed by flow cytometry, clearly demonstrating the requirement of NFAT2 in the development of this subclass. Moreover, the amount of B220* CD19* CD43* B1 progenitor cells in bone marrow and spleen were significantly reduced in NFAT2 knockout mice as compared to wild type controls.

Conclusions: In summary, our data provide strong evidence that NFAT2 is essential for B1a cell development and suggest that Ca2+/NFAT signalling substantially contributes to the regulation of B1a cell homeostasis. Furthermore, this model will enable us to investigate the relevance of B1a cells and their progenitors in the pathogenesis of CLL in vivo.

Disclosure: No conflict of interest disclosed.

P493

Percentage of smudge cells on routine blood smears predict time to first treatment and overall survival in patients with Binet stage A chronic lymphocytic leukemia

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Smudge cells are CLL cells ruptured during smear preparation. We and others have recently demonstrated that percentage of smudge cells on routine blood smears may serve as a readily available and inexpensive prognostic factor in CLL, where a percentage of smudge cells ≤20% predicts an aggressive course of the disease. Prognostic markers should be stable over time and able to identify those patients in early clinical disease stage who will progress to a more advanced disease requiring early therapy. We addressed these issues by calculating smudge cell percentages (ratio of smudged to intact lymphocytes) on archived blood smears from our monocentric cohort of 93 previously untreated Binet stage A patients. During a median follow up time of 79 months 18 patients died and 39 patients received treatment. In univariate analysis a smudge cell percentage ≤20%, CD38 expression >30%, high risk cytogenetics (11q-, 17p-) and ZAP-70 expression >20% were associated with shorter progression free survival (PFS) and overall survival (OS). On Kaplan-Meier analysis median PFS and OS in patients with a smudge cell percentage ≤20% were 73 and 160 months as compared to 124 and 297 months, respectively in patients with smudge cells >20% (p=0.029 and p=0.008, respectively). In a multivariate Cox regression analysis of OS including smudge cell percentage, CD38 expression status and cytogenetic risk smudge cell percentage ≤20% remained an independent prognostic marker of shorter OS. Sequential samples from 18 untreated patients (range 3-9 samples per patient) over time periods ranging from 7 to 38 months were analyzed to investigate whether smudge cell percentages remain constant over time. In 7/18 patients (39%) changes in smudge cell percentages crossed the 20% cutoff. In summary, percentage of smudge cells on blood smears is an independent factor predicting PFS and OS in this cohort. Its stability over time needs to be further analyzed in an expanded patient cohort.

Disclosure: No conflict of interest disclosed.

P494

Bendamustine (Ribomustin®) in the treatment of CLL patients – registry provides new aspects

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Introduction: Clinical trials prove that Bendamustine is highly effective in indolent lymphoma including CLL and MM while showing a favourable safety profile 1-3. Besides clinical trials exists a lot of experience with Bendamustine, however, not much of this is documented so far. First results of a registry documenting routine treatment of CLL patients with Bendamustine were published already at DGHO. This year, due to a larger number of patients documented, new aspects out of this ‘real life’ registry were analysed.

Methods: 58 medical practices specialised on oncological treatment in the outpatient setting in Germany participate in this registry, surveying the treatment quality in daily routine use of Bendamustine in CLL. Since May 2008, 545 patients were reported. 303 patients have been analysed so far.

Results: The analysed patient pool matches daily clinical experience: 16 (5.3%) patients had a Binet stage A, 174 (57.7%) stage B and 113 (37%) stage C before start of therapy. The median age was 73 years (42-95). Patients with ECOG 0 (48, 16%), ECOG 1 (188, 62%) and ECOG 2 (67, 22%) were...
P495
The lukast family of asthma drugs induce apoptosis in chronic lymphocytic leukemia cells due to overexpression of CysLT1, but not CysLT2 receptor
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Introduction: Heterotrimeric G protein-coupled receptors (GPCR) are involved in bone marrow tropism and survival of chronic lymphocytic leukemia (B-CLL) cells. The GPCRs cysteinyl-leukotriene receptor 1 and 2 (CysLT1 and CysLT2) recognize inflammatory lipid mediators of the cysteinyl-leukotriene (cystLT) family. Their expression and function in CLL cells has not been analyzed so far. Antagonists of CysLT1 are established in the therapy of nonmalignant, inflammatory diseases such as allergic asthma (the lukast family of drugs).

Methods: CysLT1 and CysLT2 mRNA expression was analyzed by real-time RT-PCR, calcium fluxes and actin polymerization as typical GPCR effects were studied by flow cytometry. Chemotaxis was measured using a Boyden chamber assay, and phosphorylation of p44/42 MAP kinase activity was assessed by Western blot. Apoptosis was analyzed by annexin V staining, and cell viability was determined with WST-1 reagent.

Results: CysLT1 mRNA was consistently and highly expressed in CLL cells and normal peripheral blood B lymphocytes, while only low levels of CysLT2 were present in these cells. CysLT1 was functionally active and induced intracellular calcium fluxes, actin polymerization, and a chemotactic response at a magnitude comparable to the chemokine CXCL12/SDF-1. These functional effects were completely blocked by the lukasts MK571 and LY171883. Calcium mobilization and actin polymerization were also inhibited by pertussis toxin (PTX), indicating G/o protein involvement. CysLT1-mediated signaling resulted in phosphorylation of p44/42 MAP kinase, suggesting a contribution of cysLT1s to survival of CLL cells. In line, both CysLT1 antagonists significantly induced apoptosis and reduced viability of CLL cells in vitro. PTX had no effect, suggesting the involvement of Gq rather than Gi/o.

Conclusions: CysLT1 is highly expressed and functionally active in CLL cells, representing a potential target for treatment. The lukast family of asthma drugs acting as CysLT1 antagonists may be useful as an adjunct to established CLL therapies.

Disclosure: No conflict of interest disclosed.

P496
CD44-hyaluronan interactions in the pathophysiology of chronic lymphocytic leukemia – regulation by cell activation
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Introduction: B-cell chronic lymphocytic leukemia (CLL) is characterized by the accumulation of malignant B-lymphocytes within the blood, bone marrow (BM) and lymph nodes (LNs). While the peripheral blood pool consists mainly of cell cycle arrested CLL cells, the LNs and to a lesser extent the BM contain specialized niches, supporting proliferation and survival. These centers are interspersed with CD4+ CD40L+ T lymphocytes suggesting that CLL activation by T cells supports the outgrowth of the malignant clone. Here, we determined the function of the adhesion receptor CD44 and its main ligand hyaluronan (HA) during CLL cell motility, retention, and proliferation, and analyzed CD44L-mediated alterations of these processes.

Methods: CLL cells were activated by co-culture with CD40L-transfected fibroblasts. Expression of activation markers, costimulatory molecules and specific CD44 isoforms was cytomorphometrically determined. Variant-specific RT-PCR was conducted to analyse the induction of CD44v transcripts upon CD40L-stimulation. CD44-mediated HA binding was analysed by flow cytometry. Furthermore, shear-resistant adhesion and motility of CLL cells on HA-chemokine substrates was determined by real-time and time-lapse videomicroscopy. Proliferation of CLL cells was detected by staining for Ki-67. To determine short-term homing into lymphoid organs, CLL cells were adoptively transferred to immune-deficient mice. RT-PCR, calcium fluxes and actin polymerization as typical GPCR effects were studied by flow cytometry. Chemotaxis was measured using a Boyden chamber assay, and phosphorylation of p44/42 MAP kinase activity was assessed by Western blot. Apoptosis was analyzed by annexin V staining, and cell viability was determined with WST-1 reagent.

Results: We found that CLL cells use immobilized HA as a substrate for motility when stimulated with the lymph node chemokine CCL21. CD44L-stimulation induced an activated phenotype of the malignant cells and their subsequent proliferation. This phenotype was accompanied by increased transcription and surface expression of overall CD44 as well as CD44v variants, in particular CD44v3 and CD44v6. Moreover, activation induced a high CD44-mediated binding affinity to HA. Activated CLL cells displayed strongly increased shear-resistant adhesion but impaired motility on HA/chemokine surfaces and homing into lymphoid organs, which. This could be reversed by blocking high affinity interactions of CD44 and HA with soluble HA.

Discussion: CD40L stimulation of CLL cells induces a shift from a motile to an adhesive cellular phenotype, which is caused by high-affinity CD44(v)-HA interactions. Further analysis is conducted to analyse the implications of this shift during proliferation of CLL cells in lymphoid niches.

Disclosure: No conflict of interest disclosed.

P497
Comparative assessment of the tyrosine kinase inhibitors dasatinib and bosutinib on CLL cells
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Background: In CLL cells, the Src family kinases (SKFs) Lyn and Lck are over-expressed and potentially mediate growth, survival, proliferation and anti-apoptosis by coupling with downstream B cell receptor signaling. The second generation tyrosine kinase inhibitors dasatinib and bosutinib both target SKFs and ABL, but otherwise show differing target profiles.

Design and Methods: Apoptosis induction by kinase inhibitors in primary CLL lymphocytes was monitored via phosphatidylinosine exposure and membrane integrity and associated with changes in signaling and clone-specific molecular features. The changes in cellular signal transduction were analyzed on Western blots.

Results: Using tyrosine kinase inhibitors at a concentration of 10 μM the average percentages of annexin V-positive, apoptotic cells in 11 CLL samples increased from 24 % in untreated controls to 55 % and 37 % after treatment with bosutinib and dasatinib, respectively. Both substrates efficiently reduced

Disclosure: No conflict of interest disclosed.
the basal auto-phosphorylation of SFKs at submicromolar concentrations, which resulted in decreased levels of the anti-apoptotic proteins Mcl-1 and survivin.

Conclusions: In contrast to bosutinib, dasatinib did not display a sigmoidal dose-response relationship for apoptosis induction, but attained sample-specific saturation levels. Bosutinib induced apoptosis with significantly higher efficiency than dasatinib, which calls for further investigation of its pre-clinical potential for treatment of CLL.

Disclosure: No conflict of interest disclosed.

P499
Transfection of deregulated microRNAs results in induction of apoptosis in CLL cells

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Introduction: Deregulation of microRNAs (miRNAs) plays a pivotal role in the pathogenesis of chronic lymphocytic leukemia (CLL). MiRNAs are approximately 22 long RNA molecules which are able to repress protein expression on translational level. As already published miRNAs are mostly downregulated in CLL cells which leads to an overexpression of some proteins e.g. PLAG1, TCL1, BCL2. As the origin of CLL cells is discussed controversially we analyzed the miRNA expression between different B cell subsets. MiRNAs which discriminate between CLL cells and all B cell subsets were further analyzed as they could have influence on all CLL subtypes.

Methods and Results: To elucidate the differences in miRNA expression between B cells and CLL cells we performed an Illumina Bead Chip Array compromising 752 miRNAs. We isolated different subtypes of B cells as CD19-positive periphery B cells, CD19/CD27-positive B cells and CD5/CD19-positive B cells. The determined miRNA expression was compared to the expression in CLL cells. One miRNA family was persistent upregulated in the B cells subsets isolated from healthy individuals. The targets of this miRNA family were further analyzed. One target group found with impact in CLL consists of proteins involved in apoptotic pathways. To elucidate the impact of this miRNA family on the physiology on CLL cells we transfected a representative member of this family in CLL cells and performed Annexin V Assay. The transfection was proven by RT-PCR amplifying the transfectect miRNA. The transfection leads nearly by all patients to a stronger apoptosis induction after 24h compared to miR-control transfected cells. Taken the results of all tested CLL samples (n=22) together a significant apoptosis induction after 24h and 48h is visible. To sensitize the cells for apoptosis Fludarabine, Bendamustine or AIT 737 were supplemented. These show additive effects to the transfectected miRNAs (p<0.02).

Conclusion: The results show that aberrantly expressed miRNAs have impact on the survival of CLL cells. In further experiments the direct interaction of miRNAs to miRNA of relevant proteins will be proven.

Disclosure: No conflict of interest disclosed.

P500
Sustained NF-kappa B activity in CLL is independent of genetic and epigenetic modifications of the tumor suppressor genes CYLD and A20

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Background: Prior studies revealed that cyclomorphism (CYLD) inhibits signaling via TRAF2 and c-IAP1/2, which are overexpressed in CLL. CYLD inactivation might therefore result in sustained NFkB signaling. Moreover, impaired CYLD activity leads to increased NFkB activity in multiple myeloma cells demonstrating its negative regulatory function regarding NFkB. Aside from the constitutively active CYLD, A20, a negative feedback loop regulator of NFkB, operates via induction, suggesting that both enzymes proceed at different phases of NFkB signaling. Frequent mutations of A20 – leading to sustained NFkB activity – could be shown to play a dominant role in development of different B-cell malignancies.

Methods and Results: Based on genome-wide gene expression profiling analysis of CLL samples (n=8) compared to healthy B-cells (n=5), CYLD...
expression was reduced following B-cell receptor (BCR) cross-linking (p<0.0036) contrary to A20 that could be induced after receptor stimulation (p=0.044). Methylation and sequence analysis revealed that the A20 region neither contains any methylation (63 CLL patients vs. 10 healthy donors) nor mutation (55 CLL patients) contrary to other B-cell entities. A20 expression was confirmed by immunoblotting showing comparable results to healthy B-cells. Methylation analysis of the CYLD promoter in 63 CLL patients compared to 10 healthy controls showed no epigenetic alterations. We identified that CYLD and A20 are regulated by BCR signaling. The opposed expression of both proteins after BCR stimulation might contribute to a balanced NFκB activity.

Conclusion: Our results of lacking epigenetic alteration in both enzymes and absence of mutations in A20 indicate that malignant development in CYLD differs from other B-cell malignancies, which show inactivation of either CYLD or A20.

Disclosure: No conflict of interest disclosed.

P501
Primed for death: Hypoxia sensitizes primary CLL cells towards compounds, which target mitochondrial integrity

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Background: CLL cells prefer to remain in the microenvironment since they feel safe there. CD40 ligand (CD40L)-CD40 interaction induces proliferative/anti-apoptotic genes in CLL cells, which protects them from apoptosis and most cytotoxic drugs by the microenvironment. Research interested in identifying novel drugs that effectively target CLL cells within microenvironmental niches has to consider the lymphatic microenvironment, especially hypoxia. Prior investigations never took this important factor into account. The impact of hypoxia on survival and drug-resistance remains unknown.

Methods: Therefore we have established an in vitro model, which reflects hypoxic conditions and CD40L-CD40 interaction, in order to understand the molecular basis of drug resistance of CLL cells under these conditions. CLL cells were cultured on CD40L feeder cells and kept in hypoxia (1% O₂) or normoxia (21% O₂). We applied several drugs under these conditions to investigate the difference between normoxia and hypoxia.

Results: Surprisingly, we identified drugs (e.g. ABT737), which affect mitochondrial integrity, to be even more efficient under hypoxic conditions. In contrast classical DNA-targeting drugs were inefficient to kill CLL cells cultured on CD40L feeder cells under hypoxia and normoxia. Moreover, hypoxia did not affect susceptibility of primary CLL cells towards DNA-damaging drugs.

To understand this discrepancy, we investigated the expression of several mitochondrial localized anti-/pro-apoptotic genes on protein level. We identified that the expression of ABT737 target genes are de-regulated under hypoxic conditions. The balance of anti-/pro-apoptotic molecules is disrupted under these conditions.

Conclusion: Therefore we assume that small molecules like ABT-737 (BH3 mimetic), which specifically target mitochondria, might be efficient in targeting CLL cells, protected by the microenvironment.

Development of novel in vitro models like ours, will improve in vitro testing of novel drugs and their molecular mode of action.

*LPF and MHü contributed equally to this work

Disclosure: No conflict of interest disclosed.

P502
Data quality improvement by a screening process within the CLL10 trial of the German CLL Study Group evaluating combined chemoimmunotherapy in frontline treatment of chronic lymphocytic leukemia

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Introduction: Because of the broad age spectrum the patient (pt) population in aggressive lymphocytic leukemia (CLL) is quite heterogenic due to different physical conditions and the burden of comorbidity. Moreover, protocol violations are a frequent problem in clinical trials. Primarily in order to enhance patient safety, but also to increase the data quality the GCLLSG implemented a central screening process prior to randomisation.

Patients & Methods: The CLL10 trial is an international multicenter phase III trial comparing fludarabine, cyclophosphamide plus rituximab (FCR) versus bendamustine plus rituximab (BR) in pts with low comorbidity score, normal renal function and advanced CLL requiring treatment. Pts with del(17p) are excluded and treated in a separate protocol. The CLL10 study is the first GCLLSG study with a screening process prior to randomisation. Blood samples from all pts are sent to the central laboratories for immunophenotyping and cytogenetic analysis to confirm the diagnosis of CLL and to exclude del(17p).

In addition, the pt's comorbid conditions and renal function are evaluated by checking the cumulative illness rating scale (CIRS) and concomitant medication and by recalculating the creatinine-clearance. Moreover, it is the need of treatment according to the recently published guidelines by Hallek et al. 2008 is reassessed.

Results: Between September 2008 and May 2011 664 pts were screened for participation in the CLL10-trial. 114 pts (17%) were not eligible for randomisation. Reasons for screening failure were: del (17p) 23 pts (20%), other lymphoma 23 pts (20%), impaired renal function 29 pts (25 %), comorbidity 2 pts (2%), previous therapy 8 pts (7%), no treatment indication 11 pts (10%), physician's/pts' decision 11pts (10%), other (lost, sec. malignancies) 7 pts (6%).

At the beginning of the trial, the percentage of screening failures was 25% (January 2009) and decreased now to 17% (April 2011).

Conclusions: The high rate of screening failures underlines the importance of quality assurance in clinical trials – not only to achieve comparability and transferability of results, but also for better patient-security. The decline in the rate of screening failures reflects a learning process. Moreover, these data show that central laboratory diagnostic for immunophenotyping and prognostic factors is essential in CLL.

Disclosure: No conflict of interest disclosed.

P503
DNA hypomethylation of promoter-associated CpG causes CLL specific overexpression of TOSO

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Background: We recently identified the transmembrane protein TOSO to be significantly overexpressed in chronic lymphocytic leukemia (CLL). TOSO is the only bona fide Fcμ receptor presented on B cells and is solely expressed in the lymphoid compartment. However, little is known on its regulation and the molecular background of over-expression in CLL.

Disclosure: No conflict of interest disclosed.
Methods: We determined the impact of NLCs to TOSO expression and co-incubated primary CLL cells for up to 14 days with nurse-like cells. Additionally various CLL relevant receptor stimuli were investigated to validate the TOSO upregulating signal. Since expression might be finally also controlled on epigenetic level, we determined the methylation status of the TOSO promoter in 64 CLL samples and 10 healthy B cell samples. Quantitative DNA methylation analysis was conducted using the EpiTyper application by Sequenom (San Diego, CA, USA).

Results: We identified nurse-like cells (P=0.008) and BCR cross-linking (P=0.006) to induce TOSO expression on the cell surface of CLL cells. Regarding epigenetic alterations our analyses from genome-wide screening experiments in CLL patients compared to healthy B-cells did reveal significant higher CpG methylation (p<0.001) in the promoter-associated CpG island of the toso-gene in healthy cells. In order to cover also the functional part of TOSO, a B cell specific TOSO*10-/- mouse model was created. Initial results present a clear clustering of deregulated genes compared to TOSO**/** mice.

Conclusions: Here we revealed nurse-like cells and BCR stimulation as the key components in upregulation of TOSO in the CLL cell microenvironment. Furthermore, the loss of DNA methylation at CpG dinucleotides is an epigenetic abnormality characteristic of cancer cells including CLL. Examining the cause for the CLL specific high amounts of TOSO, we firstly illustrate that DNA hypomethylation of the toso promoter is a conspicuous characteristic in CLL patients compared to healthy donors. However, the biologic significance of TOSO in CLL has not yet been elucidated. We aimed to elucidate the role of TOSO in B cell specific gene expression by creating a B cell specific knockdown mouse model. Thereby, we identified deregulated genes involved in NFkB signaling and migration, suggesting that TOSO represents an important factor in these processes. This work was supported by the Deutsche Forschungsgemeinschaft (Excellence Cluster 229).

LPF and AS contributed equally to this work.

Disclosure: No conflict of interest disclosed.

PS05

Lipid composition of mitochondria in chronic lymphocytic leukemia (CLL) cells is deregulated and might lead to altered mitochondrial function

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Purpose: Cardiolipin is an important phospholipid in mitochondria and almost exclusively found in the inner mitochondrial membrane (J. Biol. Chem. 254 (1979) 5308-5316). In addition, some studies report that cardiolipin is also located at contact sites between the inner and outer mitochondrial membrane (Biochim. Biophys. Acta – Biomembranes. 1325 (1997) 108-116; J. Bacteriol. 173 (1991) 2026-2034). Cardiolipin seems to have a role in membrane dynamics and it is able to either promote or inhibit apoptosis (IUBMB Life. 63 (2011) 160-165; Biophys. J. 77 (1999) 2003-2014). In addition, it functions as a proton trap in oxidative phosphorylation, thereby influencing cellular bioenergetics (Prog. Lipid Res. 39 (2000) 257-288). Deregluation of this lipid can lead to severe mitochondrial dysfunction, for example by increasing the survival of malignant cells. CLL cells are characterized by an enhanced longevity, rather than increased proliferation and therefore remarkable resistant to apoptosis (Leukemia 22 (2008) 635-638). Furthermore, mitochondrial function was reported to be modified in these cells (Leukemia 18 (2004) 1934-1940).

Methods: The lipid composition of CLL cells and healthy B cells was analyzed by the help of thin layer chromatography. Therefore freshly isolated primary cells were processed to gain liquid extracts. These extracts were separated on silica gel plates by using a lipophilic mobile phase and visualized by lipid staining.

Results: We show that cardiolipin is upregulated in malignant CLL cells, while all other important phospholipids namely phosphatidylethanolamine, -inositol, -serine, -choline or -glycerol are downregulated comparing CLL cells to the healthy controls.

Conclusion: We conclude that deregulation of cardiolipin in CLL cells leads to changes in mitochondrial function, especially in terms of apoptosis induction. Furthermore, it might influence survival of malignant B cells by kind of activating mitochondria. Future experiments will aim at investigating alterations in cardiolipin structure and acid chain composition, since these are crucial for the correct function of cardiolipin (J. Anim. Sci. 84 (2006) 2818-2825). In addition, alterations in pathways and processes influenced by cardiolipin will be analyzed in more detail. The work is supported by a grant from the German Cancer Aid/ Deutsche Krebshilfe (DKH109159).

Disclosure: No conflict of interest disclosed.

PS05

Posttraumatic splenosis with distinctive blood pooling mimicking malignant lymphoma with bone marrow infiltration, revealing as myelodysplastic syndrome (MDS) – a case report

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A 45-year-old male with a history of splenectomy after splenic injury 15 years earlier underwent investigations for an incidentally discovered tricystepenia with leading leucopenia. Abdominal CT scans showed a perirenal tumor mass measuring 50 x 16 mm as well as multiple retroperitoneal and mesenterial foci suggesting manifestations of malignant lymphoma. Cytopenia was considered to be related to bone marrow infiltration. Therefore, two repetitive CT-guided needle biopsies from different abdominal lesions and bone marrow aspiration were performed for further staging. Surprisingly, the pathological studies showed no malignant process but splenic tissue in the abdominal tumors, and bone marrow without pathological findings but signs of hyper-regenerative haematopoiesis consistent with a peripheral consumption. These results led to the diagnosis of splenosis. This is consistent with an auto-transplanted splenic tissue after injury or surgical manipulation, which is often confounded with malignancies and leads to extensive diagnostic procedures before diagnosis is confirmed. But could splenosis explain the observed tricystepenia? We propose a functional hypersplenism syndrome with distinctive blood pooling in spilled splenic tissue as reason for the patient’s altered blood count. We repeated the bone marrow aspiration and performed a cytogenetic profile to take the possibility of a myelodysplastic syndrome (MDS) into account. Here, we were able to detect the aberrant karyotype 46XY, del(11)q (1q41q42) consistent with the existence of MDS without dysplasia signs. This diagnosis is a rare event (~5%) but is most probably responsible for the altered blood count in this case.

Unanswered is the question, whether there is any link between splenosis and the occurring of MDS? On the one hand an increased sequestration of MDS damaged cells could explain the extended splenic formations found in the abdomen. On the other hand peripheral consumption could escalate a pre-existing MDS by giving permanent growth signals to the bone marrow. However, we opted for a surveillance strategy with regularly bone marrow aspiration to notice an eventually occurring MDS betimes. After 24 months the patient now shows no malignant process but splenic tissue in the abdominal tumors, and bone marrow without pathological findings but signs of hyper-regenerative haematopoiesis consistent with a peripheral consumption.

Disclosure: No conflict of interest disclosed.

Onkologie 2011;34(suppl 6):1–305
Abstracts

P506

Presentation of the MPN&MPNr-EuroNet (COST Action BM0902)


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Introduction: Philadelphia chromosome-negative myeloproliferative neoplasms (Ph−MPN) comprise Polycythemia Vera (PV), Essential Thrombocytosis (ET) and Primary Myelofibrosis (PMF). The JAK2 V617F plasms (Ph−MPN) comprise Polycythaemia Vera (PV), Essential

Four major working groups (WG) focus on different molecular pathological

Centralization of molecular diagnosis of rare mutations, notably EPOR, Optimization and standardization of molecular diagnosis of MPN and MPNr.

Objectives:

now, 67 biologists, haematologists and pathologists from 16 countries partici-

In 2007, a group of European biologists decided to compare and share their experience in the new molecular assays designed to detect mutations identified in MPN and MPNr. This first, informal network led to the creation of MPN&MPNr-EuroNet in November 2009 (COST Action BM0902). Until

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Unfortunately, the text is not fully visible in the image. It appears to be discussing the development and activities of the MPN&MPNr-EuroNet network, which was established in 2007 by European biologists to compare and share experience in molecular assays for the detection of mutations in myeloproliferative neoplasms (MPN). The network aims to centralize molecular diagnosis, optimize and standardize molecular diagnosis, and provide information on molecular diagnosis. The text also mentions various working groups (WG) that focus on different aspects of molecular pathology and communication. For more information, see www.mpnneuronet.eu.

Disclosure: No conflict of interest disclosed.

P507

Limited clinical activity of nilotinib and sorafenib in FIP1L1-PDGFRα positive chronic eosinophilic leukemia with imatinib-resistant T674I mutation

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Imatinib induces rapid and durable complete remissions in FIP1L1-PDGFRα (FP) positive chronic eosinophilic leukemia (CEL). Primary resistance has not yet been reported and secondary resistance seems to be rather rare. In a German-wide registry, we currently oversee the clinical course of 57 patients who were treated with imatinib for a median time of 36 months (range, 1-82). Recently, we identified the first case of secondary resistance in a 50-year-old male patient due to acquisition of a T674I point mutation in the ATP-binding domain of PDGFRα, 7 months after start of imatinib. Structurally, PDGFRαT674I is equivalent to the ABLT315I mutation. Including the case presented here, 7 patients with secondary resistance to imatinib have now been reported, 4 patients with a T674I, 1 patient with a D842V and 1 patient with a T674I followed by a D842V mutation. All patients were male with a median age of 39 years (range, 25-67). Imatinib was administered at doses of 100μg every other day (1/5), 100μg/day (2/5) or 400μg/day (2/5). The median time to imatinib resistance was 5 months (range 2-9). Marrow blasts were increased in 5/7 patients. Four patients died, 3 of them within 5 months after diagnosis of imatinib-resistance. Based on the excellent in vitro data of PDGFRαT674I towards second-generation tyrosine kinase inhibitors (TKI), our patient was treated with nilotinib 400μg BID but no response was observed. We therefore switched for 2 months to sorafenib 400μg BID which only resulted in a stable disease. Subsequently, allogeneic stem-cell transplantation (SCT) from a related donor was performed 5 months after first detection of imatinib-resistance. Twelve months after SCT, the patient is in complete hematological and molecular remission. We conclude that treatment with imatinib is associated with an excellent prognosis in FP-positive CEL in first chronic phase. Secondary resistance is rare but occurs predominantly within the first year of diagnosis. Resistance is associated with PDGFRα kinase domain mutations which are sensitive in vitro to second-generation TKI but which have only limited activity in clinical practice. Because of an aggressive clinical course and lack of alternative treatment options, allogeneic SCT should be considered in eligible patients as it might be the only effective and curative treatment option.

Disclosure: No conflict of interest disclosed.

P508

EZH2 mutations are frequently associated with other acquired mutations in myelodysplastic/myeloproliferative neoplasms (MDS/MPN) and myelofibrosis

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Introduction: We recently identified EZH2 as the major target of chromo-

some 7q acquired uniparental disomy (aUPD) in myeloproliferative neoplasm (MPN) and myelodysplastic syndromes (MDS). To determine the prevalence

Disclosure: No conflict of interest disclosed.
of EZH2 mutations we screened a total of 624 cases with myeloid disorders (MDS, n=154; MDS/MPN, n=219; AML, n=54; CML, n=40) and found 49 EZH2 mutations in 42 individuals, most commonly MDS/MPN (27/219; 12%), myelofibrosis (4/30; 13%) and MDS (9/154; 6%).

**Methods:** To determine if EZH2 mutations might co-operate with other known abnormalities or whether they might be mutually exclusive, we tested the mutational status of TET2, ASXL1, CBL, RUNX1, CEBPA, FLT3, NPM1, and WT1 in 187 of the 219 MDS/MPN cases that were screened for EZH2. We also tested an additional cohort of 52 myelofibrosis cases for both EZH2 and JAK2 V617F mutations.

**Results:** Of the 187 MDS/MPN cases, mutations were seen most frequently in TET2 (67/187; 36%), followed by ASXL1 (38/187; 20%), RUNX1 (27/187; 14%), EZH2 (25/187; 13%), CBL (22/157; 13%), FLT3 (8/187; 4%), CEBPA (7/187; 4%), NPM1 (6/187; 3%) and WT1 (2/187; 1%). Sixty six (35%) cases tested negative for mutations in all 9 genes. Of the 25 cases with EZH2 mutations, 22 (88%) had mutations in at least one other gene, most frequently TET2 (n=11) and ASXL1 (n=10). There was no significant difference in the frequency of other mutations on comparison of EZH2 mutated and EZH2 unmutated cases. Analysis of CPU-GM from one case that tested positive for both EZH2 and TET2 mutations revealed a complex pattern with an EZH2 mutation clearly preceding the sequential acquisition of two TET2 mutations. Of the 82 myelofibrosis cases, 9 (11%) tested positive for an EZH2 mutation. Of these, 5 were also positive for JAK2 V617F. In 2 cases both EZH2 and JAK2 V617F were homozygous indicating that the predominant clone must harbour both mutations.

**Conclusions:** Our data indicate a complex interaction between different abnormalities with little indication of co-operativity or functional redundancy. Whilst these observations will need to be refined by detailed analysis of single clones, they do suggest that the development of both myelofibrosis and MDS/MPN requires functional alterations in multiple pathways.

**Disclosure:** No conflict of interest disclosed.

**P509**

**Liver-MRI as a routine screening method for iron-overload in patients with myelodysplastic syndromes**


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**Introduction:** Myelodysplastic syndromes (MDS) are characterized by ineffective hematopoiesis causing anaemia, thrombocytopenia and neutropenia. Most of these patients depend on regular transfusions of packed red blood cells (PRBC) with the risk of a secondary iron overload. The monitoring of iron overload by measuring the serum ferritin level is imprecise because of influencing factors like inflammation, infection, malignancy or liver damage. Liver biopsy to estimate the liver iron concentration is difficult to perform because of the high incidence of thrombocytopenia in patients with MDS. Liver iron measurements according to Gandon’s protocol are easy to perform, reliable and reproducible. Iron is being measured as milligrams per gram dry liver weight. Levels below 5 mg/g dry weight do not require intervention. Levels above 10 mg/g dry weight indicate severe iron overload.

**Methods:** To assess iron overload in a random population of newly diagnosed MDS patients, we performed routine liver MRI in 16 patients with both low and higher risk MDS. 3 of them never received PRBC transfusions (group 1). Another 5 patients did not depend on regular transfusions but received between 2 and 13 units of PRBC (group 2). The remaining 8 patients depend on regular transfusions and received between 20 and 70 units of PRBC (group 3).

**Results:** We found a correlation between serum ferritin levels greater than 800ng/ml and liver iron concentrations (LIC). The median serum ferritin level of group 1 was 468,1 ng/ml (362,4-573,9 ng/ml) with a median LIC of 2.6 mg/g liver dry weight (dw) measured by liver MRI using the Gandon protocol. In group 2 the median serum ferritin level was 748,9 ng/ml (18,9-1379 ng/ml) with a median LIC of 3,7mg/g dw. The median serum ferritin level in group 3 was 1.912,5 ng/ml (844-2606 ng/ml) with a median LIC of 15,8 mg/g dw. Iron chelation therapy with deferasirox is being performed in 5 of these patients. Influencing factors of the serum ferritin like inflammation, infection or liver damage were excluded before testing.

**Conclusions:** As a conclusion, liver iron measurement by MRI is a simple and validated procedure and can be routinely performed when serum ferritin exceeds 800 ng/ml. Lower serum ferritin levels were not correlated with elevated liver iron and were not followed by chelation therapy nor further diagnostics.

**Disclosure:** No conflict of interest disclosed.

**P510**

**The risk profile for thrombotic events in early prefibrotic PMF – Leukocytosis is a risk factor for arterial thrombosis in early prefibrotic PMF but not in ET**


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**Background:** There is strong evidence indicating that the clear-cut separation of prefibrotic primary myelofibrosis (pPMF) from essential thrombocythaemia (ET) by consequent application of the WHO 2008 criteria is reflected in different, well defined clinical pictures and divergent prognoses. All published data on vascular events in ET and primary myelofibrosis (PMF) so far were exclusively based on the outdated criteria of the Polycythemia Vera Study Group. Consequently risk profiles for vascular events within the sub-entities of Bcr-Abl negative myeloproliferative neoplasm ask for re-assessment. We aimed to evaluate whether patients with pPMF have a distinct risk profile for vascular events.

**Methods:** Risk and risk profiles for vascular complications were determined in 87 patients from our database with a valid diagnosis of pPMF according to the WHO 2008 criteria and compared to a cohort of 127 patients diagnosed with WHO-defined ET.

**Results:** Leukocytosis and a JAK2V617F mutated genotype emerged as significant risk factors for arterial thrombosis after diagnosis in pPMF, whereas in WHO-diagnosed ET generic vascular risk factors such as arterial hypertension and diabetes mellitus enhanced the risk for arterial thrombosis.

**Conclusions:** Our results challenge the current knowledge of established and suspected risk factors for thrombosis and certainly need to be confirmed in larger studies. If validated, the finding of a different relevance of certain risk factors within the increasing variety of sub-entities will certainly change the current treatment strategies and help to better allocate patients to the appropriate treatment.

**Disclosure:** No conflict of interest disclosed.

**P511**

**Primary myelofibrosis in a Jak2 negative patient with a balanced t(5;12) translocation – Complete response after treatment with a second generation tyrosine kinase inhibitor and allogeneic transplantation**

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Up to 30% of patients with primary myelofibrosis (PMF) have cytogenetic abnormalities which have been considered to predict a shorter survival. In these patients chromosomal abnormalities are more frequent whereas balanced translocations are rare.

Here, we report about a 39 year old patient with a Jak2 negative PMF with a t(5;12) translocation who was first diagnosed in January 2009. FISH analysis
revealed an involvement of etv6 and pdgfrb genes. He was treated with hydroxyurea 1g twice daily for 10 months. Because of progressive pancytopenia therapy was changed to interferon.

In July 2010, the patient was referred for transplantation from an unrelated donor with a Lille Score of 2. Follow up examination of bone marrow revealed a MPN with 40% eosinophiles and myelofibrosis (MF-2) without increased blasts. To improve disease status before transplant a treatment with a tyrosine kinase inhibitor (TKI) nilotinib was initiated which targets the fusion genes. The patient achieved a normalized blood cell count within 2 months. However, bone marrow status did not change.

In October 2010, the patient underwent stem cell transplantation from an unrelated mismatched donor after myeloablative conditioning (TBI, endoxan). GvHD prophylaxis consisted of cyclosporine A (CSA), methotrexate and ATG. Sustained neutrophil and platelet engraftment was achieved on day +15 and day+14 respectively. The patient had no severe complications and could be discharged on day +28. Donor hemopoetic cell engraftment demonstrated a 100% donor chimerism on day +80. Only a mild myelofibrosis with 15% eosinophiles could be observed in bone marrow analysis. FISH analysis of the etv6-pdgfrb rearrangement was negative.

3 months after transplant the patient presented with a normochromic normocytic anemia and normal leukocytes and platelets. He had no signs of GvHD and CSA could be discontinued. However, bone marrow evaluation showed no signs of fibrosis, no eosinophilia and normal red cell aplasia. Q-PCR for etv6-pdgfrb was positive. We reintroduced nilotinib with a dosis of 400 mg twice daily with a rapid normalization of hb levels after two weeks. 12 weeks after reinitiating of TKI treatment hb level was 11,7g/dl. Etv6-pdgfrb expression level significantly decreased.

In conclusion, detection of rare balanced translocation in patients with PMF by cytogenetic or FISH analysis offers the possibility for therapeutically approches and allows further exploration of additional pathogenetic pathways.

Disclosure: No conflict of interest disclosed.

P512
A case of inv(12)(p13q24) in a patient with myelodysplastic syndrome and a course of fulminant pneumonia

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Introduction: inv(12) belongs to the well known regions rearranged in human hematologic malignancies. ETv6 is the main gene in this region. Only few aberrations in 12p13 without ETv6-involvement are described. MDS commonly show unbalanced karyotypes with deletions in 5q,7q,20q but rarely translocations or inversions. We report the case of a 73 year old male diagnosed with MDS RAEB 1 cytogenetically specified as inv(12)(p13q24) who had a very aggressive disease course. After initial presentation with subdural bleeding and pancytopenia the patient rapidly developed severe pneumonia and septic multiple organ failure. He died soon after despite a promptly initiated intensive antibiotic and antifungal management.

Methods: We performed conventional G-banding on metaphase spreads from bone marrow direct preparations and from 3-day PHA-stimulated peripheral blood cultures and described the karyotype according to ISCN-nomenclature. For FISH-analysis we used the commercial probe sets ETV6-BA, MLL-BA and EGR1/D5S23 (VYSIS) and counted at least 200 nuclei and 10 metaphases. Bone marrow and peripheral blood smear were evaluated.

Results: In bone marrow cytology signs of dysplasia were present in all cell lineages. A pathologic left shift with excess blasts was seen, but no blasts were found in peripheral blood smear. G-banding from bone marrow cells showed a simple aberrant karyotype with a structurally abnormal chromosome 12. FISH analysis confirmed an inv(12)(p13q24). Normal signal distribution in the probes MLL-BA and EGR1/D5S23 ruled out cells with trisomy 11, translocation in 11g23/MLL or deletion in 5q31/EGR1. ETV6-BA showed no split-signals, only the position from 12p13 moved to 12q in all 17 metaphases analysed. Thus the nuclei showed normal disomic signal distribution. Therefore it is not possible to quote the percentage of abnormal cells among the non-dividing cells.

Conclusion: We identified a new chromosomal aberration in MDS otherwise not seen in acute leukemia. The rapid death of the patient could not be fully explained by clinicopathologic findings. So we suggest that the inv(12)(p13q24) and the aggressive disease course may not be coincidental. It has been shown that aberrations in 12p13 are often associated with complex karyotypes and can predict a poor prognosis. Further studies will be necessary to clarify the full impact of the observed inv(12)(p13q24).

Disclosure: No conflict of interest disclosed.

P513
Always felt, never shown: MDS patients in private practices differ significantly from MDS patients treated in university hospitals

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Introduction: Myelodysplastic syndromes (MDS) are mainly a disease of the elderly. Commonly, MDS patients are treated in an outpatient setting making hematological/oncological private practices (PP) an important backbone in the management of MDS patients. To gain more insights into the characteristics of patients with MDS treated in hematological/oncological PP and to evaluate the daily diagnostic routines we performed questionnaire-based analyses. Moreover, we compared this cohort to a patient cohort treated in specialized MDS centers in university hospitals (UH).

Methods: Data were collected after written informed consent. Between January 2008 and June 2009 questionnaires were sent to 8 PP in the area of Berlin/Brandenburg, Germany. Clinical and laboratory data from patients in MDS centers at two university hospitals were collected during routine visits. Statistical analyses were done by using the GraphPad Prism version 5 software and SAS System software. For continuous parameters a Mann-Whitney test was used and for non-continuous parameters a chi-square test was performed. Differences were considered significant if p < 0.05.

Results: A total of 197 and 165 patients were enrolled in PP and UH, respectively. Distribution between gender was similar in the two groups. Patients in PP were significantly older with a median age of 71 years as compared to UH (65 years) (p<0.001). Distribution of IPSS risk groups in UH versus PP were as follows: low risk 24% versus 45%; intermediate-1 risk 55% versus 40%, intermediate-2 risk 22% versus 7%, high risk 19% versus 7% (p=0.008). Patients in UH had significantly lower median blood counts compared to PP: hemoglobin 9.3 g/dl versus 10.3 g/dl (p=0.0002); absolute neutrophil counts 1720/μl versus 2423/μl (p=0.0002). Only for a minority of patients conventional cytogenetic analyses were performed in PP (32%) as compared to 84% of patients in UH.

Conclusion: PP play a central role in the management of older MDS patients particularly patients with early stage MDS. Younger patients with advanced stage MDS and higher IPSS risks and/or patients that show cytogenetic alterations that have therapeutic implications such as deletions on chromosome 5q and chromosome 7 aberrations are treated at UH. In our opinion, these findings should be taken into consideration when clinical trials particularly on early stage MDS are planned in the future.

Disclosure: No conflict of interest disclosed.

P514
Update of a German retrospective multicentre analysis on MDS diagnosis and management in clinical routine

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Introduction: The aim of this analysis was to describe the current pattern as well as changes in the diagnosis and management of MDS patients (pts) in Germany.
P516

A complex deletion/insertion mutation in MPL exon 10 in a patient with essential thrombocythaemia (ET)

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Introduction: The 2008 WHO criteria for ET require inter alia the demonstration of JAK2-V617F or other clonal markers. In up to 9% of JAK2-V617F-negative ET, mutations at codon 515 of the MPL gene can be detected. The two most common MPL mutations are W515L and W515K. Other rare MPL mutations (e.g. MPL W515R, W515S, W515G, W515A) have been reported, representing gain-of-function mutations leading to a constitutive, cytokine-independent activation of MPL. A MPL exon 10 deletion/insertion mutation, W515P-S18delinsKT, has been reported as well. We can show a more complex mutation than the previously described W515-P518delinsC mutation.

Methods: A 78-year-old woman has had increasing thrombocythemia since 2004. The medical history included hypertension for a few years lasting need for medication. There was no history of thromboembolic occurrences. A cytoreductive therapy with Hydroxyurea was initiated and is well tolerated since then. A BCR/ABL fusion gene and a JAK2-V617F mutation were excluded and a mutation analysis for exon 10 of the MPL gene was done by PCR and direct bidirectional sequencing. Two allele-specific PCRs with a forward primer complementary to mutation c.1543_1552delinsAAA and a reverse primer complementary to mutation c.1557_1559delinsC were performed followed by direct sequencing.

Results: A c.1543_1552delinsAAA and a c.1557_1559delinsC were found. The mutation-specific PCR followed by direct bidirectional sequencing demonstrated that both deletions/insertions were present on the same allele, leading to deletion of amino acids 515 to 518 (WQFP) with insertion of amino acids KT and substitution of histidin by threonin at position 520.

Conclusion: Since 2008 azacitidine (AZA) is approved in Europe for the treatment of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) with less than 30% blasts. Compared to conventional care regimens AZA has been shown to improve median survival, to prolong average time from diagnosis to AML transformation and increase number of transfusion independent patients.

Methods: In this retrospective analysis we report the results of ten patients with MDS and AML treated with AZA between October 2008 and April 2011. In these 10 patients median age was 73.5 years (range 62-90), 80 % were male (8/10). In this analysis we enrolled 7 patients with WHO-defined RAEB-1 or RAEB-2 and IPSS Int-2 or High risk and one patient with AML (blasts < 30%). One patient had persistent MDS (WHO-defined RAEB, IPSS Int-1) as a result after therapy of secondary AML. The last patient received AZA because of distinctive pancytopenia in chronic myelomonocytic leukemia (CMML) with fewer than 10% blasts.

Results: The cohort was analysed using the modified International Working Group response criteria: None of the patient achieved complete remission, there were 3 partial remissions, and 5 hematologic improvements. Two patients achieved transfusion independence. In three patients (30%) we observed transformation to AML after onset of azacitidine treatment, two of them died. Our patient with persistent MDS after secondary AML died because of thrombocytopenia-related bleeding complication. Severe sepsis was cause of death in the patient with AML.

Conclusion: This group of patients represent a small and heterogeneous cohort with variable clinical features and outcome. In this analysis the rate of patients with partial remission and hematological improvement is higher or comparable to trials like AZA-001 and CALGB 9221.

Disclosure: No conflict of interest disclosed.
**PS17**  
**Rapid and simple detection of pseudohyperkalemia in patients with myeloproliferative neoplasia**  
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**Introduction:** Essential thrombocythemia mostly presents in the elderly and is attended by pseudohyperkalemia in many cases. As these patients are often multimorbid the elevation of serum potassium asks for further diagnostics. A quick answer can be made by blood gas analysis (BGA). In case of pseudohyperkalemia the potassium concentration here reveals normally while potassium-sparing drugs could be excluded. The measurement of potassium was repeated quickly by a blood gas analysis and demonstrated a normal concentration of 4.4 mmol/l. This phenomenon could be confirmed by more patients with essential thrombocythemia.

**Moleculargenetic findings revealed a positive V617F-mutation in the JAK2-gene, bcr-abl was negative. As other myeloproliferative diseases were excluded, the diagnosis of an essential thrombocythemia was made. After a follow-up of three months the platelet count presented stable with 1418 G/l, a cytotoxic therapy with hydroxyurea (Litalir®) was started at dose of 1.5 g per week. Further blood analyses showed a decrease of the platelet count and serum potassium (5.6 mmol/l). Six months after starting the chemotherapy the dose of hydroxyurea was increased due to rising thrombocytes. Currently, the patient remains asymptomatic, and the platelet count is < 900 G/l. Meanwhile the serum potassium concentration is lowered to 5.4 mmol/l.

**Conclusion:** Blood gas analysis is an immediately available method for potassium measurement and assessment in MPN patients with pseudohyperkalemia in emergency setting.

**Disclosure:** No conflict of interest disclosed.

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**PS18**  
**CMML – a heterogenous disease: first analysis of the Austrian CMML registry**  
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**Introduction:** Chronic myelomonocytic leukemia (CMML) is a clonal disorder of the hematopoietic stem cell characterized by the presence of absolute monocytosis in peripheral blood. In the past the dilemma was to integrate a disorder with myelodysplastic and myeloproliferative features within the myelodysplastic syndromes. This issue was resolved by a new category of myelodysplastic syndromes/myeloproliferative neoplasms (MDS/MPN). Survival of patients with CMML is highly variable and ranges between 1 and more than 100 months. Median survival is between 20 and 40 months in most series.

**Methods:** All patients from two different centres (KH Elisabethinen, Klinikum Wels) with newly diagnosed CMM were included. The aim of this retrospective/prospective registry is the evaluation of frequency, course and therapeutic management of the disease.

**Results:** Thirty patients with a median age of 72 years from two centres (KH Elisabethinen 14 patients, Klinikum Wels 16 patients) were available for this analysis. Cytogenetic analysis was done for all patients. Six patients (20%) showed an abnormal karyotype – two patients with t(1:8), one patient with del(7), one patient with t(1:14), one patient with +8 and another patient with del(14). One patient was tested positive for a JAK2 V617F mutation.

**Conclusion:** This analysis of the first participating centres of the Austrian CMML registry shows preliminary data of CMML patients. Inclusion of patients from other centres is planned.

**Disclosure:** No conflict of interest disclosed.

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**PS19**  
**JAK2 mutations in chronic myeloproliferative neoplasm patients in Saudi Arabia**  
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The chronic myeloproliferative neoplasms (CMPN) are a group of clinically related clonal hematopoietic stem cell disorders in which large numbers of red blood cells, white blood cells, or platelets grow and spread excess in the bone marrow and the peripheral blood. Cytogenetic analysis of the t (9;22) and molecular detection of BCR/ABL is the main diagnostic criteria in Philadelphia positive CMPN (CM). The identification of non-receptor tyrosine kinase JAK2 mutations (JAK2V617F and exon 12) have significantly contributed to our understanding of the molecular mechanisms in the pathogenesis of Philadelphia negative CMPN such as polycythemia vera (PV), essential thrombocytemia (ET) and myelofibrosis (MF) patients. According to the revised WHO classification, JAK2 mutation is considered as a major diagnostic and clonal marker in CMPN. However, the genotyping studies for JAK2 in CMPN patients from the Saudi Arabia have not been reported thus far, which has resulted in a lack of an established diagnostic criterion in these neoplasms. We therefore, examined the prevalence of JAK2 mutations in CMPN cases in the western region of Saudi Arabia by direct PCR and sequencing of genomic DNA. The diagnosis of CML was confirmed by cytogenetic and/or molecular studies. The current study included 79 CMPN patients diagnosed with which comprises of 45 CML cases (57%), 10 ET cases (13%), 12 MF cases (15%) and 11 PV cases (14%). Our results revealed JAK2 V617F mutation in 10 PV cases (91%), 4 ET cases (40 %), 3 MF cases (25 %) and 0 CML cases (0%). We report V617F mutations in CMPN cases for the first time from Saudi Arabia. In conclusion, we recommend V617F JAK2 mutation to be included in the routine clinical diagnosis of PV at all cancer centres in Saudi Arabia.

**Disclosure:** No conflict of interest disclosed.

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**Posterdiskussion**  
**Bronchuskarzinom**  
**PS20**  
**Influence of Heparin on metastatic processes in small cell lung cancer**  
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**Introduction:** Treatment of cancer patients with Heparin has shown positive effects exceeding the antithrombotic effect. It has been hypothesized that heparins might have antimetastatic properties due to interferences with the function of CXCR4 or adhesion receptors as shown e.g. in colon or breast cancer cell lines. We have previously demonstrated that the interaction of CXCR4, which is expressed on small cell lung cancer (SCLC) cells, and its ligand SDF-1 (stromal cell derived factor-1) plays a crucial role in metastasis related processes. Therefore we analyzed the influence of unfractionated heparin (UFH) and low-molecular-weight heparin (LMWH) on metastatic processes in SCLC cell lines.
PS21
Toxic epidermal necrolysis related to Cisplatin and Pemetrexed for metastatic non-small cell lung cancer

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Background: Toxic epidermal necrolysis (TEN), also known as Lyell’s syndrome, is an uncommon but life-threatening drug reaction. Pemetrexed is a multitargeted antifolat. It was first used in combination with cisplatin as front-line therapy for metastatic non-small cell lung cancer (NSCLC).

Case report: We report the case of a 50-year-old man treated for metastatic NSCLC. Within 5 days after administration of the second cycle of cisplatin and pemetrexed, he developed large blisters that secondarily became hemorrhagic, and mucosal lesions. The characteristic appearance and the clinical course were decisive for diagnosis. Treatment with systemic steroids and intravenous antibiotics as well as topical wound treatment led to resolution and improvement of his general condition.

Conclusion: To the best of our knowledge, this is the third case of TEN due to pemetrexed in a patient with NSCLC. There is an accelerated use of pemetrexed now for the first and second line as well as maintenance setting in NSCLC. Clinicians should be aware of TEN as a rare but potentially fatal disorder requiring hospitalisation and multidisciplinary management.

Disclosure: Katrin Scheinpflug: Financing of Scientific Research: Referentenhoronorar Lilly; Expert Testimony: Studientätigkeit Lilly (65 PLUS / FRAME); Other Financial Relationships: travel grant DHG 2010


PS22
Improved lymph node staging of lung cancer using EBUS-TBNA and liquid-based cytology: an alternative to mediastinoscopy?

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Introduction: Endobronchial ultrasound with real-time-guided transbronchial fine needle aspiration (EBUS-TBNA) is now considered the gold standard procedure for staging of lung cancer that allows access to a wider range of mediastinal lymph node stations than mediastinoscopy. When combined with endoscopic esophageal ultrasound fine-needle aspiration (EUS-FNA), both methods together offer an almost complete evaluation of all mediastinal lymph node stations. We report our initial experience with EBUS-TBNA and discuss to what extent this new technique may become an alternative to surgical staging procedures in the future.

Methods: EBUS-TBNA was performed in 301 patients with suspected lung cancer or unexplained lymphadenopathy. In order to optimize the diagnostic yield, at least four aspirations per lymph node were taken. With more experience, rapid on-site evaluation (ROSE) was provided. The probes were investigated using liquid-based cytology (LBC). In 288 cases, also histological specimens of subsequent surgical staging procedures were available.

Results: Using EBUS-TBNA, lung cancer was found in 297 of the patients. There were 48 cases with lymph node metastases of non-pulmonary malignancies and 34 probes showing reactive changes like sarcoid granulomas. There was a good correlation between EBUS-TBNA and surgical staging. During the initial phase, bronchoscopists faced a learning curve with EBUS-TBNA. The use of LBC reduced the number of slides and the screening time required for diagnosis. With the preparation of cell blocks, additional histological and immunohistochemical staining and molecular testing (as for instance EGFR mutations) are feasible and give further diagnostic impact.

Conclusion: EBUS-TBNA is an accurate, inexpensive, save and minimally invasive method for staging lung cancer or unexplained lymphadenopathy. In the hands of experienced bronchoscopists, this new method may be a substantial alternative to surgical staging and will be able to reduce the number of mediastinoscopies. In cases with high probability of malignancy, negative results of EBUS-TBNA should be validated with mediastinoscopy. The use of liquid-based cytology is recommended.

Disclosure: No conflict of interest disclosed.

PS23
Pegfilgrastim prophylaxis for reduction of febrile neutropenia (FN) – interim analysis of the lung cancer subgroup of a German prospective multicentric observational study (PROTECT)

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Introduction: FN is a dose-limiting haematological toxicity in chemotherapy treated patients. FN can interfere with the delivery of optimal treatment, adversely affecting patient outcomes. Reducing the incidence of FN should be considered a clinical priority. EORTC guidelines recommend primary prophylactic use of a granulocyte colony-stimulating factor (G-CSF) when the risk of FN is high (≥20%) and recommend consideration of G-CSF prophylaxis with intermediate risk (10% - 20%).

Methods: This prospective non-interventional study evaluated the efficacy of pegfilgrastim primary (PPP) vs. secondary prophylaxis (PSP) in cancer patients with a FN risk ≥20% / 10-20 % as estimated by the treating physician.
Key inclusion criteria for the reported subgroup were diagnosis of lung cancer, FN risk of >10% according to EORTC guidelines and pegfilgrastim primary prophylaxis (PPP) or secondary prophylaxis (PSP) according to SMPC.

Results: In March 2011, data from 83 (out of 150) planned lung cancer patients were available (median age 63 y, 63% male). 24% were diagnosed at T4 stage, 7% were nodal-negative, 82% had metastatic disease. Most frequent planned chemotherapy regimens were etoposide/carboplatin (28%), etoposide/cisplatin (14%) and topotecan (8%). For 51% of patients physicians rated the risk of FN 20%, in 43% this risk was estimated to be 10-20%, in 6% FN risk was rated as < 10%. PPP was planned in 86% of patients, PSP in 14%. Based on cycles, pegfilgrastim was administered in 88% of all cycles. Neutropenia grade IV occurred in 28% of patients, FN was reported in 8% of patients. 12% of cycles were delayed, in 9% of cycles the dose of administered chemotheraphy was reduced. FN was reported in 9 cycles (2%) affecting 7 patients (8%), 5 pts with PPP and 2 pts with PSP FN led to cycle delays or dose reductions in 4 (5%) of all patients, and was less frequent with PPP than with PSP (1 vs. 3 pts.). 3 pts were hospitalized due to FN or infections, with a median stay of 8 d, 29 pts died, with no deaths due to FN.

Conclusions: In this descriptive study, the FN risk of lung cancer patients was rated intermediate to high or high in almost all patients. Adequate administratio of pegfilgrastim prophylaxis resulted in a low rate of FN, adequately supporting treatment for lung cancer patients.

Disclosure: Stefan Fruehauf: Advisory Role: Amgen; Expert Testimony: Amgen
Martina Schaffrik: Employment or Leadership Position: Amgen; Stock Ownership: Amgen.

P524 Paraneoplastic hyponatremia (SIADH) in course of small cellular lung cancer (SCLC) – new therapeutic option with V2 – receptor antagonists (Vaptans)
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Background: Hyponatremia is a common finding in hospitalized patients. They are found in nearly 15% of the patients in emergency units. Hyponatremia is associated with an increased mortality especially in patients suffering from oncologic and cardiovascular diseases. The possible causes are manifold. One of the most common causes is the small lung cancer in terms of an inadequate high excretion of adipocytokine (SIADH). Since September 2009 a new therapeutic option with V2-receptor antagonists (vaptans) has been available. We report two cases of SIADH conditioned by SCLC that was treated with Tolvaptan.

Case report: In the first case a 67-years-old patient presented with the strong suspicion of a lung cancer in a ct-scan of the chest. The symptoms involved cephalgia, vomit and cough. Rapidly we could verify the suspicion, in this case a SCLC, through histology. In laboratory tests we found a hyponatremia of 112 mmol/l. According to the diagnostic criteria of a SIADH we found a reduced serum-osmolality of 230mOsm/kg, an inadequate urine excretion of 120 mmol/l with an urine-osmolality of 435 mOsm/kg. Parallel to chemotherapy we started a treatment with the oral V2-receptor antagonist Tolvaptan in different doses ranging from 15mg to 30mg/day. In both cases a normal level of serum-sodium could be reached very fast without occurring side effects.

Discussion: The diagnosis of the inadequate excretion of adiuretin (SIADH) as a paraneoplastic side effect of an SCLC could be rapidly verified through easy diagnostic methods including laboratory tests and urine chemistry. The efficacy of Tolvaptan was rapid and sustained. This reconfirmed the good results of the run-up studies. However, additional reports on the tolerance of Vaptans in different indications in wide use are necessary.

Disclosure: No conflict of interest disclosed.

P525 The chemokine receptor CXCR4 in Small Cell Lung Cancer (SCLC) and its role in metastasis
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Introduction: SCLC is a highly aggressive and metastatic neuroendocrine carcinoma. There is growing evidence that the chemokine receptor CXCR4 and its ligand CXCL12 are involved in migration and metastasis of SCLC. As STAT3 has been shown to be potentially involved in the CXCR4 signaling pathway, we are investigating the therapeutic use of inhibitors of CXCR4 and/or STAT3 pathway components in SCLC metastasis formation.

Methods: STAT3 activation in SCLC cell lines and its activation in Rho GTPasses mediated signalling cascade were analyzed using western blot and pulldown-assays. Further functional investigations (e.g. Chemotaxis, Pseudoepheropilosis, cell viability assessment) will reveal the involvement of STAT3 and Rho GTPases in CXCR4 mediated metastatic processes.

Results: Analysing the CXCR4/CXCL12 axis we could demonstrate different STAT3 activation types. STAT3 was constitutively phosphorylated on both tyrosine and serine residues. Tyrosine phosphorylation induces the dimerization and translocation of STAT3 to the nucleus. STAT3 phosphorylation on serine residue is required for enhanced transcriptional activity. The constitutive phosphorylation on both residues could be further increased upon CXCL12 stimulation and was completely inhibited by TN14003, a CXCR4 antagonist and/or by CDDO-Me, a synthetic triterpenoid which inhibits STAT3 directly. We could show an increased STAT3 acetylation specific of the nuclear fraction upon CXCL12 stimulation. STAT3 may also be involved in Rho GTPases mediated signalling. We could show a strong CXCR4 mediated RhoA activation as potential upstream signaling of STAT3 activation. To investigate the role of CXCR4 functional inhibitors and inhibitors of downstream pathways on metastasis formation in vivo, an orthotopic tumor mouse model is being established. Human SCLC cells were injected intrathoracically into the pleural space of NMRI nude mice. Tumor engraftment could be observed using BL-Imaging. The growth of the primary tumor could be increased by using matrigel and by using tumor cells from donor mice.

Conclusions: We have shown constitutive STAT3 Phosphorylation and CXCL12 induced regulation. CXCL12 also induces activation of RhoA. These results are clarifying the CXCR4 downstream cascade in small cell lung cancer and underline the interest of characterizing inhibitors for this receptor and its targets in this pathway.

Disclosure: No conflict of interest disclosed.

P526 In patients with metastatic lung cancer several novel and known immunogenic tumor-associated antigens (TAA) induce specific T-cell responses and are therefore candidates for targeted immunotherapies
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In the last years, new therapeutic strategies of lung cancer with encouraging results have been investigated including immunotherapeutic approaches, e.g. against the antigens MAGE-A3 and H1ERT. Thus, tumor vaccination seems to be a promising strategy especially in situations of reduced tumor load, i.e. in maintenance therapy. Here we address the question, whether there are further interesting candidates besides the antigens MAGE-A3 and H1ERT that induce intensive immune reactions in a high frequency in lung cancer and are therefore eligible for immunotherapeutic approaches.

Immune reactions of CD8+ T-cells were measured in ELISPOT assays for H1ERT, MAGE-A3 and glycosylase B. Moreover, tetramer assays and chromium release assays were performed. Epitopes were tested derived from the lung cancer associated antigens MAGE-A3 and hTERT, and from the antigens CUBN, Survivin, WT-1, PRAINE, HER2 and G250 known from other tumor entities as well as novel antigens, like Auroarkinase A and B. For these novel antigens more than 10 HLA-A2-binding peptides were predicted and tested in 20 healthy volunteers and 15 lung cancer patients.
Specific T-cell responses could be detected against at least one peptide in all patients. Most frequent responses were detected against PRAME (67%), hTERT (60%), G250 (60%) and RHAMM (40%). Lower frequency was measured for Survivin- (27%), WT-1- (27%), the two MAGE-A3- (27 and 20%) and Her-2- (15%) derived peptides. Specific T-cell responses could be also detected against Aurora kinase A and B. The novel peptides Aurora_A_01 and Aurora_A_03 showed specific T-cell responses in 33% and 40% of patients respectively. Serological immune responses are under investigation.

Specific T-cell responses against several TAA could be detected for the antigens hTERT, PRAME, G250 and RHAMM in a high frequency of patients with lung cancer, but also in a lower frequency against several other antigen peptides. Moreover, novel immunogenic targets like Aurora kinase A were identified. Therefore, further antigen structures are appropriate for immuno-targeted approaches in lung cancer.

Disclosure: No conflict of interest disclosed.

P527
LEF1 identifies a prognostically unfavourable subgroup of lung adenocarcinoma brain metastases

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A previous in vivo study on lung adenocarcinoma cells demonstrated a functional relevance of the transcription factor 4 (TCF4) and Lymphoid Enhancer 1 (LEF1) for the later onset of metastasis. Additionally a TCF4 gene signature in primary lung tumors of patients with stage I-III adenocarcinoma was predictive for metastasis whereas a β-catenin signature failed.

To address the expression and distribution of TCF4, β-Catenin and LEF1 in metastatic tissues, we analyzed 14 brain metastases of lung adenocarcinomas by immunohistochemistry. Furthermore, an external gene expression dataset of 19 brain metastases of lung adenocarcinomas was statistically analyzed. TCF4 was expressed in all samples with variable intensities, 42.9% of the samples showed nuclear LEF1 and 42.9% nuclear β-Catenin staining with only minimal overlap. Nuclear LEF1 correlated with the intensity of TCF4 (p=0.02), had a tendency to shorter survival (p=0.14) but no correlation with nuclear β-Catenin expression (p=0.64). The external gene expression dataset yielded a strong correlation between TCF4 and LEF1 as well as VEGF (p=0.05).

Conclusion: We identified a subgroup of LEF1 expressing brain metastases of lung adenocarcinomas with potential prognostic impact. The expression pattern suggests a role for LEF1 independent of β-Catenin-signaling. Further studies have to be performed to validate these results and evaluate LEF1 as a potential therapeutic target.

Disclosure: No conflict of interest disclosed.

P528
Whole blood transcriptomics analysis of 24h-responses to bevacizumab/erlotinib in non-squamous non-small cell lung cancer: A multicenter phase II trial SAKK19/05

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24-h effect of the combined BE therapy on these patients using whole blood transcriptomics. MMP-9 showed a significant down-regulation after 24h (p=0.02). The 24h-response varied among patients, 72% of patients showed a down-regulation of MMP-9.

Conclusions: The 24h-effect of BE, as measured in the blood, induced the regulation of a limited, yet consistent number of genes among which MMP-9. This gene, which is involved in the pathogenesis of metastatization, may be a potential early marker of clinical response to BE therapy. After unblinding for clinical outcome it is planned to identify potential early markers of 12-week disease stabilization, tumor response and overall survival with BE.

Disclosure: No conflict of interest disclosed.

P529
Influences of quality indicators in a NSCLC in a group of medical practices specialized in oncology (PIO)

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Subject and aims: The data of the treatment of NSCLC patients have been recorded and evaluated in a group of medical practices specialized in oncology (PIO) since January 2003 to April 2011. The aim is to document the general treatment, quality and indicators in Germany outside the framework of studies.

We have analyzed particular quality indicators in NSCLC – course of weight, age, ECOG, histology, smoker or non-smoker.

Methods: Out of 1550 registered patients, 1212 have been documented and evaluated so far. 79 medical practices specialized in oncology from Germany are involved.

Results: The median overall survival from pts < 70 y (733pts) was 12.1 months and from pts > 70 y (479pts) was 11.9 month. Median survival analysis show:

- age + histology: pts < 70 y with AC 12.2 mo, SCC 11.5 mo, BAC 6.5 mo; pts > 70 y with AC 12.9 mo, SCC 11.8 mo, BAC 21.6 mo.
- age + ECOG pts < 70 y with ECOG 0 16.9 mo, ECOG 1 13.4 mo, ECOG 2 8.5 mo, ECOG 3 10.5 mo; pts > 70 y with ECOG 0 14.2 mo, ECOG 1 12.9 mo, ECOG 2 9.5 mo, ECOG 3 8.5 mo.

survival dependence course of weight: lost of weight 7,4 mo, increase of weight 15,4 mo, no change of weight 11,5 mo.

Smoker or non-smoker we have evaluated with histology. Median survival analysis show for smokers with AC 10.5 mo, SCC 9.5 mo, BAC 6.5 mo; non-smoker with AC 18.9 mo, SCC 14.7 mo, BAC 5.6 mo.

Conclusions: In our group of medical practices specialized in oncology (PIO) with 1212 pts we could show that the strong quality indicator of the overall survival is ECOG status, smokers history and the change of weight. Histology and age was not an indicator of survival.

Disclosure: No conflict of interest disclosed.
Outcome of non small cell lung cancer (NSCLC) patients with mutations of the epidermal growth factor receptor (EGFR) gene- one center experience

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Introduction: EGFR has become an important therapeutic target for the treatment of lung cancer. Small-molecule inhibitors that target the tyrosin-kinase domain (TKD) of EGFR, the so-called TKIs are especially effective in patients whose tumors harbor activating mutations in this TKD. More recent randomized trials have demonstrated that for advanced NSCLC patients with EGFR mutant tumors, initial therapy with a TKI instead of chemotherapy is the best choice of treatment.

Methods: Genomic DNA was isolated from paraffin-embedded tumor specimens and amplified for EGFR (exons 19 and 21) by nested PCR and sequenced in both sense and antisense direction.

Results: Somatic mutations of the EGFR gene were detected in 47 patients (12%). Charts of 34 patients were reviewed. Patient characteristics were as follows: male/female: 23:11; median age at diagnosis: 67.5 yr (40-83yrs); histology adeno/squamous/large cell: 31/2/1; smoking status never/former/current: 24/6/4. Mutations in exon 19 were found in 22 pts, exon 21, L858R in 11 pts. In one patient two mutations were detected, one in exon 19 and one in 21.

21 pts were treated with specific EGFR tyrosine kinase inhibitors (TKIs), 12pts with gefitinib, 5 with erlotinib, in 3 pts there was a TKIs switch (gefitinib/erlotinib). Median duration of the treatment was 7.3 mos (0.5-21). 12 other pts did not receive any TKIs. The median overall survival for patients who did not receive TKIs was 22 months, while it has not been reached in patients treated with any TKI.

Conclusions: Even though preliminary, our results demonstrate a striking improvement in overall survival in patients treated with TKI, compared to reported survival rates with chemotherapy in NSCLC.

Mutation testing is mandatory to identify patients with EGFR mutant tumors, given that selection based only on clinico-pathologic characteristics is inadequate. The data strongly suggest that EGFR-TKI therapy should be considered in patients with EGFR mutations.

Disclosure: No conflict of interest disclosed.

Progestereon and estrogene prevent cisplatin-induced apoptosis of lung cancer cells

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Introduction: Lung cancer is the most common cause of tumor mortality worldwide. A growing body of evidence shows involvement of the female sex steroid estrogen in formation, growth and dissemination of lung cancer in both smokers and non-smokers. However the role of progesterone and the contribution of both sex steroids on development of therapy resistance remain unclear.

We investigated the influence of estrogen and progesterone on in vitro chemoresistance of the non small cell lung cancer (NSCLC) cell line A549 against cisplatin and on expression of the copper transporter 1 (CTR1), which is responsible for the intracellular accumulation of cisplatin.

Methods: A549 cells were pretreated with estrogen and progesterone before exposure to cisplatin. Cell growth, cell viability, metabolic activity and levels of apoptosis rates were measured via the xCelligence systems, LDH/MTS assays and Annexin apoptosis assays. The influence of estrogen and progesterone on expression levels of CTR1 was investigated using semi-quantitative RT-PCR. Activation levels of caspsases 3-7, 8 and 9 after cisplatin exposure with or without estrogen and progesterone pretreatment were determined using luminescent caspase assays.

Results: A549 cells express estrogen receptor (ER) alpha, beta, and progesterone receptor (PR). Pre-treatment with both estrogen and progesterone pretreatment resulted in a significant attenuation of cisplatin induced apoptosis. These effects could not be antagonized by the classical ER or PR antagonists ICI 182,780 and RU486 (mifepristone). Cisplatin induced activation of caspsases 8, 9, 3 and 7 was attenuated by estrogen and progesterone. In contrast to these findings expression of CTR1 was not modulated by estrogen or progesterone.

Conclusions: Estrogen and progesterone give rise to cisplatin resistance in vitro and cisplatin induced activation of caspases is counterbalanced by estrogen and progesterone pretreatment. CTR1 expression levels were not altered by estrogen and progesterone. Decline of intracellular cisplatin levels is therefore not responsible for the described effect. Attenuation of cisplatin induced apoptosis could not be antagonized by ICI and mifepristone. Therefore we conclude that membranous sex steroid receptors and the consecutive activation of non-classical signaling pathways play a key role in this context.

Disclosure: No conflict of interest disclosed.

Carboplatin and Paclitaxel plus ASA404 as first line chemotherapy for extensive-stage small-cell lung cancer (ES-SCLC): A multicenter single arm phase II trial (SAKK 15/08)

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Introduction: ASA404 (DMXXX, vadimezan) is a novel flavonoid, non-tubulin-binding tumor-vascular disrupting agent (tumor-VDA) leading to hemorrhagic tumor necrosis. In preclinical studies a synergistic anti-tumor effect in combination with taxanes has been reported. Carboplatin/paclitaxel (CP) is an active combination in first line ES-SCLC. SCLC is a highly vascularised tumor and adding a vessel targeting agent to chemotherapy may potentially improve treatment outcome.

Methods: Patients with previously untreated histologically or cytologically confirmed metastatic SCLC and a performance status of 0-2 were registered to receive paclitaxel (200 mg/m²) + carboplatin (AUC 6) + ASA404 (1800 mg/m²) every 21 days for up to 6 cycles. Patients with brain metastases, uncontrolled hypertension, significant arthritiasms and haemoptysis were excluded. The primary endpoint was progression free survival at 6 months, secondary endpoints included objective response rate, time to tumor progression and overall survival.

Results: 17 out of 56 planned patients received CP plus ASA404. The treatment was generally well tolerated. In one patient, trial treatment had to be stopped due to a serotonin syndrome with agitation and muscle rigors lasting several hours after the infusion of ASA404. The trial was closed prematurely following the company’s decision to stop drug supply due to lack of efficacy of ASA404 in non-small cell lung cancer, prostate and ovarian cancer clinical trials.

Conclusions: Vasculature targeting agents are of interest in improving outcomes in SCLC. This is the first trial reporting on the use of ASA404, a novel tumor-VDA, in patients with SCLC. Results on efficacy and toxicity of the combination of CP+ASA404 will be available and presented at the meeting.

Acknowledgement: Prof. L. Bubendorf, Brian Mezeh

Disclosure: Martin Früh: Advisory Role: advisory functions for Novartis (on one occasion)

Miklos Pless: No conflict of interest disclosed.
The treatment of lung cancer in German outpatient centres. Data from a clinical registry – TLK Registry

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Introduction: In clinical registries the actual treatment of patients can be studied. In daily practice – unlike the selected population in clinical trials – patients of all ages, with various concomitant diseases and risks need optimal treatment. We present data regarding the first-line palliative treatment of lung cancer patients in outpatient centres in Germany.

Methods: iOMEDICO conducts in collaboration with the Arbeitskreis Klinische Studien (AKS) a clinical registry (TLK Registry) with a target population of 2000 patients with Non-Small Cell Lung Carcinoma (NSCLC) and 500 with Small Cell Lung Carcinoma (SCLC). Beside demographic data and the course of treatment the registry collects data on tumour characteristics, comorbidity, surgery and metastases among other relevant variables.

Conclusion: Of 537 patients receiving (neo-)adjuvant and/or palliative treatment were available for analyses. Thus far, a total of 330 NSCLC and 103 SCLC palliative first-line treatments are documented. At treatment start NSCLC patients were 67.7 years of age (65% male) and SCLC patients were 66.0 years of age (66% male). Within the main histological subtypes, 62% of the NSCLC tumours are adenocarcinoma, 35% are squamous cell carcinoma, and 3% are large cell carcinoma. Palliative first-line, NSCLC is most frequently treated with Carboplatin + Paclitaxel (13%), Cisplatin + Pemetrexed (10%), Carboplatin + Gemcitabine (9%) and Cisplatin + Vinorelbine (9%). The treatment of SCLC is dominated by Carboplatin + Etoposide (57%) and Cisplatin + Etoposide (29%).

Results: The TLK registry started in Jan 2010. By Jan 2011 data of 537 patients receiving (neo-)adjuvant and/or palliative treatment were available for analyses. Thus far, a total of 330 NSCLC and 103 SCLC palliative first-line treatments are documented. At treatment start NSCLC patients were 67.7 years of age (65% male) and SCLC patients were 66.0 years of age (66% male).

Disclosure: No conflict of interest disclosed.

Dasatinib reduces the ability of cancer-associated fibroblasts (CAFs) to promote tumor proliferation in vitro

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Introduction: Fibroblasts (CAFs) are known to associate with tumours and support their growth and metastasis. There is a new interest in the role of CAFs in tumour progression. We therefore present our observations of Dasatinib’s impact on human CAFs.

Methods: Human CAFs were isolated from perifused lung tissue. Dasatinib was added to CM from CAFs and the effects on HGF and IGFBP4 secretion were assessed. In a second step, HGF and IGFBP4 were secreted in CM from Dasatinib treated CAFs, and the effects on proliferation of H1299 lung cancer cells were measured. In a third step, these CAFs were used in a co-culture system with H1299 cells.

Conclusion: Dasatinib reduces the ability of CAFs to promote cancer cell proliferation. The role of these factors for the observed phenotype will be further investigated.

Disclosure: No conflict of interest disclosed.
Jürgen Wolf: Advisory Role: Roche; Financing of Scientific Research: Roche; Expert Testimony: Roche.

P536 Monitoring therapy with erlotinib and afatinib in a patient with advanced non-small cell lung cancer and activating EGFR-mutation by sequential FLT-PET

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Introduction: [18F]fluorothymidine (FLT)-PET is a noninvasive tool for the visualization of tumor proliferation. In patients with advanced non-small cell lung cancer (NSCLC) harboring sensitizing EGFR mutations, quantification of standard uptake values (SUVs) can be used to determine GI-arrest under treatment with EGFR-targeting tyrosine kinase inhibitors (TKIs) thereby acting as a pharmacodynamic marker.

Methods: FLT was synthesized as described. PET images were obtained by using an ECAT EXACT 47 scanner (CTI/Siemens, Munich, Germany) after 6 hours of fasting. Acquisition of PET scans started 60 minutes after injection of 300 MBq FLT. For EGFR mutational diagnostics, we performed massively parallel sequencing by using GS FLX Standard or GS FLX Titanium chemistry according to standard protocols (Roche Diagnostics, Penzberg, Germany).

Clinical Course and PET

Results: A 66 year-old female patient without smoking history presented in January 2009 with cerebral ischaemia. CT scans revealed pulmonary tumors with osteolytic lesions and mediastinal lymph node enlargement. Biopsies led to the diagnosis of NSCLC with adenocarcinoma histology and EGFR del19 mutation. Erlotinib treatment (150 mg/d) was initiated in February, 2009. FLT-PET after one week of therapy with erlotinib demonstrated a dramatic decrease in tumor activity, followed by a partial response in CT scans.

Sequential FLT-PET scans confirmed these results during the following year, in which erlotinib was stepwise reduced to doses of 25 mg/d due to adverse events and eventually discontinued. After 2 months without therapy, SUVs increased again, and erlotinib treatment was reinitiated. However, SUVs continued to increase under therapy suggesting EGFR-TKI resistance. Treatment was changed to the irreversible EGFR TKI afatinib. After one week of therapy, SUVs decreased. The next scan was performed due to paraneoplastic cerebral ischaemias indicating tumor activity, and demonstrated an increase of SUV in one lesion. A few weeks later progress of the disease occurred and worsening of the paraneoplastic neurological symptoms lead to death of the patient in October 2010.

Conclusions: FLT-PET can be used to visualize EGFR-TKI induced GI arrest under treatment, allowing to visualize drug efficacy, resistance and early pharmacodynamics effects.

Disclosure: Matthias Scheffler: Advisory Role: Boehringer Ingelheim; Financing of Scientific Research: Boehringer Ingelheim; Jürgen Wolf: Advisory Role: Boehringer Ingelheim, Roche; Financing of Scientific Research: Boehringer Ingelheim, Roche; Expert Testimony: Boehringer Ingelheim, Roche.

P537 Randomized round robin test to evaluate the reproducibility of an immunohistochemical score with therapeutic relevance that dichotomizes non-small cell lung cancer into tumors with high and low epidermal growth factor receptor expression

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Background: The randomized phase III FLEX study demonstrated that the addition of cetuximab to chemotherapy significantly improved overall survival compared with chemotherapy alone in the first-line treatment of advanced non-small cell lung cancer (NSCLC). A positive association between high tumor epidermal growth factor receptor (EGFR) expression (score ≥200) as measured by immunohistochemistry (IHC) on a continuous scale of 0-300) and clinical outcome in patients receiving chemotherapy plus cetuximab has been reported. This international round robin test (RRT) evaluated the inter-observer reproducibility of the EGFR IHC score.

Methods: A feasibility study was undertaken that identified factors impacting on reproducibility. Subsequently, in a reference laboratory, serial sections of a tissue microarray (TMA) comprising NSCLC tumor cores were stained using the DAKO EGFR pharmDx™ kit/autostainer. An EGFR IHC score was calculated for tumors based on membrane staining intensity (graded 0-3+) and the percentage of cells demonstrating each staining intensity. After training, 10 expert lung cancer pathologists then evaluated EGFR expression for 30 selected TMA cores, which were categorized after reference evaluation, as clearly high (n=10), clearly low (n=11) or equivocal (n=9), relative to the threshold EGFR IHC score of 200. Analysis of between-rater agreement was based on the allocation of tumors into low (<200) and high (≥200) EGFR expression groups. The overall concordance rate was defined as the mean of the per-rater concordance rates with respect to the reference evaluation. Kappa coefficients were calculated for the comparison of each rater with the reference evaluation.

Results: The RRT showed a high inter-observer agreement in EGFR IHC scoring among study participants, with an overall concordance rate of 91% and a mean kappa coefficient of 0.81. Tumors with a reference EGFR IHC score clearly below or above the cut-off (<150 or ≥250) were each categorized with an almost perfect mean concordance rate of 98%. Samples with a reference EGFR IHC score around the cut-off (150 < ≤250) showed a high mean concordance rate of 74%.

Conclusions: The RRT demonstrated that after appropriate training, assessing EGFR expression by this IHC scoring method allowed a highly reproducible allocation of NSCLCs into high or low EGFR expression groups, based on a cut-off score of 200. The study indicates that this evaluation of the EGFR IHC score may be a clinically reliable method to facilitate the selection of patients with NSCLC expressing high levels of EGFR for first-line chemotherapy plus cetuximab who are those most likely to derive a substantial treatment benefit.

Disclosure: No conflict of interest disclosed.

Posterdiskussion Multiples Myelom

P538 Natural human IgM antibodies targeting primary multiple myeloma

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The existence of humoral tumor immunity in cancer patients is established and have opened the avenue of isolating tumor reactive antibodies that can be used
for cancer immunotherapy. We therefore analysed a panel of tumor reactive IgM antibodies if they can specifically bind and induce lysis of primary multiple myeloma (MM) cells. Two fully human IgM antibodies - Mab 1 and Mab 2 – were able to specifically target several myeloma cell lines as well as primary MM cells, which were freshly obtained from MM patients. Immunochemical staining on bone marrow sections revealed a binding of Mab 1 in 10 of 10 myeloma patients at primary diagnosis as well as in 10 of 10 patients at stage of relapse. A homogeneous binding pattern was observed in 15 of 20 samples. In the others binding ranged from 30 to 70%. In contrast no binding was detected on primary healthy hematopoietic tissue.

In general antibodies can mediate cytotoxicity by intrinsic activity (e.g. by induction of apoptosis), complement activation or antibody dependent cellular cytotoxicity (ADCC). Antibody treatment of both MM cell lines and primary MM cells caused 90% cell death. For primary MM cells the mean amount of propidium iodide positive cells was increased from 25% in the controls to 57% in the Mab 1 treated cells (p= 0.0033). In two primary MM samples Mab 1 killed over 90% of the MM cells by intrinsic activity. Further flow cytometry based analysis revealed the induction of apoptosis as main mode of action. In addition, cell death was increased by adding complement to the cell cultures resulting in significant complement dependent cytotoxicity (CDC). This CDC interaction was observed in primary MM samples (n=9) independent from myeloma subtype and stage of disease.

In summary, patient derived IgM antibodies induce cytotoxicity by intrinsic induction of apoptosis and CDC and therefore provide a promising approach for immune therapy of multiple myeloma.

Disclosure: No conflict of interest disclosed.

P539
Analysis of combined PI3K/Akt and MEK/MAPK blockade in Akt-dependent and Akt-independent multiple myeloma cells

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Introduction: Targeted therapies have significantly improved the survival of multiple myeloma (MM) patients, but the problems of relapse and drug resistance persist and may be a consequence of dysregulated growth and survival pathways. We have shown that about 50% of primary MM samples display an Akt-dependent phenotype, whereas the MAPK pathway appears to play a less prominent role. However, both pathways may crosstalk and confer functionally redundant signaling. Therefore, the effects of combined PI3K/Akt and MEK/MAPK blockade by pharmacological inhibition in MM cell lines (n=6) and primary MM samples (n=23) and by shRNA-mediated knockdown in MM cell lines were analyzed.

Methods: MM cell survival was determined after treatment with Akt-inhibitor Akti-1,2 and MEK-inhibitors PD184352 or PD325901 alone or in combination. In MM cell lines, survival was assessed after transient transfection with shRNA expression constructs against Akt1, Erk1 and Erk2 alone or in combination. The rate of apoptotic cells was analyzed using flow cytometry with Annexin V-FITC/PI.

Results: Combined inhibition of Akt and MEK led to strongly enhanced apoptosis compared to single treatments in Akt-dependent MM cell lines, whereas cell lines resistant to Akt blockade remained unaffected by the combination treatment. Single or combined blockade via shRNA-mediated knockdown of Akt1 and Erk1/2 produced similar results. In Akt-dependent primary MM samples, pharmacological inhibition of Akt and MEK again reflected the enhanced cell apoptosis effects. Primary MM cells resistant to Akt blockade showed a more heterogeneous pattern and responded to combination blockade in half of the cases.

Conclusion: Combination treatment with PI3K/Akt and MEK/MAPK inhibitors could improve the anti-MM effects in Akt-dependent MM and in a subset of Akt-independent MM cases.

Disclosure: No conflict of interest disclosed.

P540
Novel agents have a significant impact on survival of patients with multiple myeloma

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Background: In addition to conventional chemotherapeutic regimens and autologous transplantation, novel agents (thalidomide, bortezomib, lenalidomide) are now part of the treatment armamentarium against multiple myeloma (MM). To evaluate the presumed benefit of novel agents, we performed an analysis of patients with MM at our institution before and after the availability of novel agents (thalidomide since August of 1999).

Patients and Methods: 200 consecutive patients with newly diagnosed MM (male n=119; female n=81; median age: 61.5 years) treated at our institution with newly diagnosed MM between June 1993 and December 2008 were included in this retrospective analysis. Patient cohorts were defined according to date of diagnosis (before and after 01 JAN 2000, respectively), treatment received (chemotherapy only versus therapy including novel agents), risk profile (ISS-stage) and cytogenetic features (chromosome 13q status by FISH). Primary focus of the analysis was overall survival (OS).

Results: Median OS for MM patients after initial chemotherapy was 45.2 months (36.6 – 54.1 95% CI) and for patients who received novel agents 74.6 months (59.8 – 89.3 95% CI; P < 0.01). OS for those patients who relapsed after autotransplantation before 2000 was 35.2 months (18.1 – 52.9 95% CI), but 72.7 months (49.7 – 95.7 95% CI; P < 0.01) for those patients with a later relapse. Prolongation of survival for patients receiving novel agents was most evident for patients with ISS stage III (median OS 68.4 months vs. 11.2 months for patients with chemotherapy only; P< 0.01). MM patients with an intermediate risk (chromosome 13q-deletion by FISH; 39% of patients) also had longer median OS when receiving novel agents (47.2 months versus 32.8 months). No significant difference of OS was observed between patients receiving bortezomib (87.6 months) or immunomodulatory agents (72.5 months).

Conclusion: Treatment with novel agents in MM resulted in a significant prolongation of OS. Benefit of therapy with novel agents was particularly evident for transplant-eligible patients and MM patients with unfavourable prognosis (ISS stage III).

Disclosure: No conflict of interest disclosed.

P541
Atypical manifestation of primary systemic amyloidosis: recurrent liver hemorrhage and hemoperitoneum

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Introduction: Primary systemic amyloidosis is a protein misfolding disorder characterized by deposition of insoluble fibrils, derived from monoclonal light chains, in the extracellular matrix. Although the liver is a common site of amyloid deposition, clinically significant deteriorations of hepatic functions are rare and reports on spontaneous liver bleeding are exceptional. Herein we describe the case of a female, born 1950, repeatedly admitted to hospital with recurrent spontaneous liver bleeding due to amyloidosis.

Case report: Dyspeptic symptoms and subfebrile temperatures were the first manifestations appearing in our patient in 2008. After finding a liver infarction suspected of hemangioma, an embolization was performed in June 2009. This procedure was complicated by a liver rupture and the development of a chronic subhepatal and retroperitoneal hematoma. However, the diagnosis of recurrent liver hemorrhage and hemoperitoneum was only made later. A liver biopsy revealed atypical amyloidosis which was confirmed by immunohistochemical staining. An extensive search for monoclonal paraproteins in serum and urine was negative. The patient was treated with interferon and died in September 2009. The histological examination of the liver showed characteristic amyloid deposits in the liver parenchyma.

Disclosure: No conflict of interest disclosed.
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P543

Association of multiple myeloma (MM) with different neoplasms (DN), including second primary malignancies (SPM): systematic analysis in consecutive patients (Freiburg experience) and future suggestions of screening assessments

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Introduction: MM has envisioned numerous innovative treatment approaches, this resulting in prolonged overall survival (OS). Current attempts in MM focus on further improving survival, nevertheless, one challenge is that second primary malignancies (SPM) may occur. Recently, SPM after novel agent treatment in first-line- (MM-015) and maintenance-trials (IFM/CALGB) have demonstrated that p/s DN are more frequent than s DN, but the prognosis specifically impaired with s DN. Age ≥ 70 years was 7.8%, 10.3% and 11.6%, respectively. MM patients with p/s DN showed a hazard ratio (HR) for impaired OS of 1.231 (95% CI: 0.76-1.993), whereas with s DN of 2.523 (95% CI: 1.447-4.399). Our initial study demonstrated that p/s DN are more frequent than s DN, but the prognosis specifically impaired with s DN. Age ≥ 70 years was a confounding covariable (HR 2.021; 95% CI: 1.581-2.583). In our subsequent investigation of 681 patients between 1/1997-3/2011. Patients’ medical histories were reviewed due to specific therapies or other risk factors. Of note, SEER and Swedish cancer registry data on SPM have observed overall cumulative incidences in MM of 5.1-5.5%. Until now, only few prior reports have evaluated definite rates of SPM/DN and none have reported survival data or specific risk factors in MM.

Methods: In an initial analysis, we assessed consecutive MM patients treated at our institution between 1/1997-7/2008; and in a second analysis, all MM patients between 1/1997-3/2011. Patients’ medical histories were reviewed based on their records and each case analyzed according to the onset of the first and subsequent malignancy.

Results: Of 589 within our initial analysis, 59 (10%) had DN; in 78% solid tumors (ST) and in 22% hematological neoplasms (HN). DN were separated in those emerging prior or synchronously (p/s DN; n=41; 69%) vs. subsequently (s) after the MM (s DN; n=18; 31%). The estimated DN-rates at 2, 5 and 10 years were 7.8%, 10.3% and 11.6%, respectively. MM patients with p/s DN showed a hazard ratio (HR) for impaired OS of 1.231 (95% CI: 0.76-1.993), whereas with a DN of 2.523 (95% CI: 1.447-4.399). Our initial study demonstrated that p/s DN are more frequent than s DN, but the prognosis specifically impaired with s DN. Age ≥ 70 years was a confounding covariable (HR 2.021; 95% CI: 1.581-2.583). In our subsequent investigation of 681 consecutive MM patients, we observed 86 (13%) DN, again predominantly ST (n=67; 78%) and lesser HN (n=19; 22%). p/s and s DN were found in 62 (72%) and 24 (28%) patients, respectively.

Conclusions: Our findings support specific risks and shared genetic and/or environmental susceptibility that predispose to MM and SPM/DN. Significant

P542

Free light chains in urine – an additional diagnostic tool to detect cast nephropathy?

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Background: The examination of free light chains (FLC) in serum is a very useful diagnostic tool in patients with monoclonal light chain (MCL) disease. About 30% of patients with multiple myeloma have renal involvement at the time of diagnosis increasing to 50% during the course of disease. But different kinds of renal involvement have different prognostic values. Cast nephropathy usually progresses very rapidly to end stage renal disease, whereas nephrocalcinosis can disappear completely with treatment. Currently the type of renal involvement can be diagnosed correctly only by kidney biopsy. We investi-
subgroup scrutiny and risk factors (e.g., age, gender, treatment and intensity, bone marrow function, cytogenetics, predisposition, occupation, smoking status, immunologic alterations) are currently evaluated in our cohort and will be presented at the meeting. These results are crucial to implement into future trials and prospective analyses.

Disclosure: No conflict of interest disclosed.

P544
CD117 (c-kit) expression in monoclonal gammopathies is associated with an altered expression of the myeloid and lymphoid hematopoietic cell compartments and favorable disease features

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Introduction: CD117 (c-kit) expression by clonal plasma cells (cPC) confers a favorable prognosis in multiple myeloma (MM) and in vitro studies showed that it is efficiently coupled to downstream pathways (e.g., the PI3K/Akt pathway), suggesting ckit is functional in cPC.

Methods: We analyzed 106 patients with symptomatic MM (n=50), smoldering MM (n=38) and monoclonal gammopathy of undetermined significance (MGUS, n=18) by multiparameter flow cytometry and fluorescence in situ hybridization (FISH) to elucidate biological features of CD117+ vs. CD117 monoclonal gammopathies.

Results: CD117+ cPC were detected in 30% of symptomatic MM, 45% of smoldering MM and 72% of MGUS patients. CD117 expression was associated with higher percentages of normal bone marrow PC, CD117+ myeloid precursors and CD38+ B lymphocytes in all groups of monoclonal gammopathies. Conversely, the number of bone marrow CD34+ myeloid cells and peripheral blood neutrophils was reduced among CD117+ MM but not MGUS patients.

Conclusions: CD117 expression by cPC is associated with favorable disease features and uniquely altered patterns of production of hematopoietic bone marrow cells with decreased peripheral blood neutrophil counts and persistence of normal residual bone marrow PC. Hereby, CD117 expression by cPC may alter their homing in the bone marrow and could redirect them into neutrophil precursor niches, where CD117 might act as an anchor molecule.

Disclosure: No conflict of interest disclosed.

P545
The combination of Lenalidomide, Bortezomib, Liposomal Doxorubicin and Dexamethasone (LBlipDD) may overcome resistance to prior treatments in patients with plasma cell disorders

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Introduction: The era of novel agents in multiple myeloma started with the introduction of thalidomide by Barlogie et al. in 1999. Since then four additional drugs – bortezomib, lenalidomide, dexamethasone and bendamustine – have gained regulatory approval for patients with multiple myeloma. Furthermore these substances work by different modes of action which creates new possibilities for meaningful combination therapies.

Methods: Single center case series of four relapsed and/or refractory patients with plasma cell disorders were treated with LBlipDD (lenalidomide 15-25 mg day 1-21, bortezomib 1mg/m^2 day 1+4+8+11, ldp 50 mg/m^2 day 4, dex-amethasone 20 mg day 1, 2, 4, 5, 8, 9, 11, 12, q28 days). Descriptive statistics was applied.

Results: Our cohort included one female and three male patients with a median age of 61.5 years, multiple myeloma was diagnosed in three patients, extramedullary plasmacytoma incl. amyloidosis of the mesenterium was detected in one patient. These patients received a mean of 3.5 prior lines of therapy (range: 1 – 7) before they were switched to LBlipDD. In total a mean of 4.75 cycles (range 2 – 9) were applied and one patient was still receiving therapy, a second patient might continue after BM biopsy re-evaluation. One patient died after four cycles of LBlipDD (his 5th line of treatment), his overall survival with the disease was 6.5 years. The patient with extramedullary plasmacytoma is currently receiving local radiotherapy.

Four out of four patients achieved a partial response (IMWG Resp. Crit.) resulting in an overall response rate (RR) of 100%. Most impressive are the results for one patient, who responded initially to three cycles of LBlipDD resulting in a treatment-free remission period of more than 3 years. Upon relapse the patient and was re-exposed to six additional cycles of LBlipDD and again tumour reduction could be achieved.

Conclusions: In our case series all four patients responded to treatment with the four drug regimen LBlipDD (100% RR). This regimen is safe and highly effective leading to tumour response in heavily pre-treated patients with recurrent and/or refractory disease.

Disclosure: No conflict of interest disclosed.

P546
Clinical usefulness of the Hevylite™ assay in patients with monoclonal gammopathy

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Introduction: By now, quantitative analysis of immunoglobulin (Ig) A/G/M concentrations included both kappa and lambda isotypes. The Hevylite™ assay enables separate quantitative determination of kappa and lambda immunoglobulin pairs, in contrast to the qualitative discrimination by immunofixation (IFIX). By that the proportion of monoclonal versus non-involved immunoglobulins can be measured. We studied the clinical use of this assay with respect to secondary antibody deficiencies in patients with paraproteinemias.

Methods: Serum samples from patients (pts) with known monoclonal gammapathies and from patients with polyclonal hyperimmunoglobulinemia were analyzed by nephelometry using polyclonal sheep antibodies to kappa and lambda isotypes (Hevylite™ assay, The Binding Site Ltd.). Ranges of normal ratios were 0.98 – 2.75 for IgG, 0.80 – 2.04 for IgA and 0.96 – 2.30 for IgM. Quantitative determination of residual polyclonal IgG was done using a specific formula.

Results: 131 samples from 104 pts were analyzed. Results included 54 IgG studies from 43 pts, 63 IgA studies from 47 pts and 14 IgM studies. 76 pts had known multiple myeloma or Waldenström’s macroglobulinemia, 10 had MGUS and 18 no monoclonal gammapathy. 21 samples showed a normal kappa/lambda ratio, while in 110 samples we found a pathological ratio. Ratio and IFIX were applied.

Conclusion: Determination of Ig isotypes with the Hevylite™ assay appears to be a useful tool for initial work-up of monoclonal gammapathy as well as for prognostication and response assessment under treatment. Discrimination of pathological from residual polyclonal immunoglobulins allows to identify

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Onkologie 2011;34(suppl 6):1-305

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pts with secondary antibody deficiency requiring immunoglobulin substitution in order to prevent severe infectious complications.

Disclosure: No conflict of interest disclosed.

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Plasma cell leukemia following primary systemic AL-amyloidosis in a patient with kappa light-chain MGUS

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AL-Amyloidosis is rare and may either be a stand-alone disease or secondary to multiple myeloma in up to 10-15% of all cases. Commonly affected organs are kidneys (up to 70%) and heart (up to 60%). Untreated it may lead to organ failure. Therapeutic options include bortezomib, lenalidomide or melphalan. We present a 60 year-old patient who was initially referred with acute renal failure (unselected proteinuria 10g/d). Immuneelectrophoresis uncovered a MGUS, furthermore bone marrow biopsy revealed a 25% plasma cell infiltration both of kappa light chain type. Renal and skin biopsies lead to diagnosis of primary systemic AL-Amyloidosis (Congo red positive protein). The patient underwent induction chemotherapy with etoposide/ cyclophosphamide followed by stem cell separation and tandem high dose melphalan with autologous stem cell support. The patient remained in partial hematologic remission (significant reduction of the M-gradient, < 5% bone marrow plasma cells) and, in addition, achieved organ response (proteinuria 1g/d) for 11 months until the plasma cell dyscrasia progressed into overt myeloma with progression of the free kappa light chains up to 570mg/l while renal function was still improving. We started a salvage therapy with lenalidomide/ dexamethasone/ doxorubicin, which lead to stable disease (hematologic response criteria) after 4 cycles. Soon the disease progressed to a multiple myeloma with 80% bone marrow infiltration and significant pancytopenia despite another cycle of bortezomib/ cyclophosphamide followed within one month. No signs of hypercalcemia or osteolytic bone lesions could be found. We performed a third high dose melphalan therapy with autologous stem cell support. Three months later it came to a transformation into a plasma cell leukaemia with >70% plasma cells in the peripheral blood stream. The treatment according to the B-ALL-protocol for aggressive lymphomas (block C1) including rituximab (due to the plasma cell immunophenotypical change: coexpression of CD20 antigen) remained unsuccessful. Two years after initial diagnosis of AL-Amyloidosis the patient died due to secondary complications of plasma cell leukemia.

According to our knowledge this case reports for the first time on both hematologic as well as organ remission in AL amyloidosis and a concomitant secondary progression into a highly aggressive plasma cell leukaemia while classic “CRAB” myelomatous end organ damage was never encountered throughout the course of the disease.

Disclosure: No conflict of interest disclosed.

P549

Remineralization of osteolyases in myeloma bone disease in the era of novel therapeutic drugs

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Introduction: Osteolytic bone disease is present in about 90% of multiple myeloma patients. In the majority of myeloma patients it causes pain, fractures and instability leading to myeloma diagnosis. During the entire course of the disease, bone disease impairs mobilization and life quality of the patients and thus is of clinical relevance. With novel therapeutic drugs allowing rapid tumor responses and high rates of complete and very good partial remissions, the probability of remineralization and gain in stability is of high interest. Non-enhanced, whole-body, low-dose multidetector CT (WBLD-MDCT) permits sensitive detection and monitoring of osteolytic bone destruction and of medullary and extramedullary myeloma disease. Due to its higher sensitivity, WBLD-MDCT is better suited for detection and monitoring of osteolytases than conventional X-ray. In contrast to MRI it enables documentation of de- and recalcification processes.

Methods: We performed a retrospective analysis of 58 multiple myeloma patients who underwent WBLD-MDCT between 12/2003 and 01/2010. All patients were treated for myeloma disease with bortezomib and/or immunomodulatory drugs (IMiDs). Analysis was performed at baseline either at primary diagnosis or before initiation of relapse treatment and during follow-up. A median of 5 WBLD-MDCT per patient was performed. 225 osteolyases were analyzed in 58 patients. Size and degree of recalcification under therapy were related to clinical response.

Results: All but one patient showed at least minimal response. Significant recalcification of preexisting osteolytic bone lesions was detected in 40 of 58 patients under therapy with bortezomib and/or IMiDs. Recalcification was associated with prolonged response duration and a less aggressive course of disease. No correlation was found with type of paraprotein, quality of response or concomitant bisphosphonate therapy.

Conclusion: Effective myeloma treatment in the era of novel agents leads to significant recalcification of osteolyases in myeloma bone disease. WBLD-MDCT is the radiographic assessment of choice for detection and follow-up analysis. Recalcification of osteotasis is of high clinical impact for myeloma
patients. It is associated with prolonged response to treatment and reduces pain and fracture incidence, one of the key factors for life-quality improvement.

Disclosure: No conflict of interest disclosed.

P550
The HIV protease inhibitor Nelfinavir has proteasome-inhibiting activity in vivo distinct from Bortezomib
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Rationale: HIV protease inhibitors (HIV-PI: Ritonavir, Lopinavir, Saquinavir, Nelfinavir, Amprenavir, Indinavir, Atazanavir, Tipranavir and Darunavir) are oral drugs for HIV treatment. Although designed to inhibit the HIV protease, HIV-PI may also target the proteasome implicating therapeutic potential especially in hematologic malignancies.

Objective: We compared the effects of HIV-PI on proteasome activity, cytotoxicity, ER-stress induction and AKT-phosphorylation in myeloma and AML cells.

Results: Lopinavir, Nelfinavir, Ritonavir and Saquinavir showed biological and molecular activity at concentrations within or near therapeutic drug levels (10-20 μM). In this dose range, they triggered ER stress-induced apoptosis, inhibited AKT-phosphorylation and showed synergistic cytotoxicity with Bortezomib. Nelfinavir stood out as the only HIV-PI with proteasome-inhibiting activity at near-therapeutic drug levels. Nelfinavir induced not only the Bortezomib-sensitive proteasome β1/β5 subunits, but to a similar extent also the Bortezomib-insensitive β2 subunits. It induced significant additional proteasome inhibition and synergistic cytotoxicity in Bortezomib-pretreated cells and Bortezomib-resistant primary myeloma cells. Significant inhibition of the proteasomal β1, β2 and β5 active subunits was observed in PBMCs from patients treated with Nelfinavir.


Disclosure: No conflict of interest disclosed.

P551
Lenalidomide as salvage therapy before autologous blood stem cell transplantation
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Lenalidomide (len) is an important module in the concept of multiple myeloma (MM) treatment in relapsed MM patients. Nevertheless there is still heterogeneity in response and survival duration from months to years among patients treated with lenalidomide/dex/etanercept (len/dex).

The aim of the retrospective study is to analyse the clinical outcome of patients with relapsed or refractory MM treated in median with 3 cycles len/dex (range 2-5 cycles) followed by high-dose therapy with melphalan 200mg/m² and autologous blood stem cell transplantation (HDT/ASCT) at our institution. We evaluated 48 patients who received ASCT between December 2007 and November 2010. Maintenance therapy (thalidomide n=10, lenalidomide n=1) was administered to 11 patients.

The retrospective analysis included 30 males and 18 females with a median age of 61 years at start of len/dex treatment (range 46-70 years). Median time between first chemotherapy and start of len/dex treatment was 44 months (range 21-155 months). 20 of 48 patients were pretreated with novel agents: thalidomide (n=10), bortezomib (n=7) and both substances (n=3). Four patients received allogeneic SCT after HDT/ASCT. These four patients were censored for event-free survival (EFS) at time of allogeneic SCT.

Overall survival (OS) and EFS were calculated from the date of ASCT (n=48) and 100 days after ASCT (n=45 and 42, respectively). The median EFS were 18.5 and 21.8 months [14.3 months-not reached (n.r.) and 17.6 months-n.r.], respectively. For OS 82% and 92% of patients were alive 36 months after above-mentioned time points, respectively.

Log-rank test and Cox PH regression as well as landmark analyses were utilized to assess the impact of response at time of ASCT and 100 days after ASCT, respectively. The results show, achieving PR or better is not statistically significant for EFS and OS. However, time between first chemotherapy and start of len/dex treatment shows trend to an impact of EFS (p=0.07).

Further, we analysed the impact of applying novel agents before salvage therapy of EFS and OS. Patients with and without thalidomide and/or bortezomib in prior treatment received median EFS from 17.5 (range 13 months-n.r.) and 23.7 months (range 21.7 months-n.r.), respectively. 94% of patients with novel agents in prior treatment and all patients without novel agents were alive 18 months after start of salvage therapy.

Further results in this subject are expected from the clinical trial “RelApSe” of the GMMG study group.

Disclosure: Christiane Heiss: Expert Testimony: Celgene
Hartmut Goldschmidt: Advisory Role: Celgene; Financing of Scientific Research: Celgene; Expert Testimony: Celgene.

P552
Mutations of IDH1 and IDH2 are not frequent in Multiple Myeloma
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Multiple myeloma (MM) is characterized by frequent and complex genomic abnormalities. However, most genetic abnormalities are already present in the precursor state of MM, the monoclonal gamopathy of undetermined significance (MGUS). Therefore, it is likely that secondary genetic events might contribute to the development from MGUS to symptomatic MM. Mutations in isocitrate dehydrogenase 1 (IDH1) and 2 (IDH2) genes have recently been described as relatively frequent molecular lesions in gliomas and in acute myeloid leukemia (AML). However no larger study has so far examined the frequency of IDH1 and IDH2 gene mutations in patients (pts) with MM.

Methods: In 188 pts with MM genomic DNA from CD138 sorted plasma cells was used for analyses. Exon 4 of both IDH1 and IDH2 were amplified by PCR and the amplicons were analyzed using a combination of denaturing high-performance liquid chromatography and DNA sequencing. All patients were also characterized by a comprehensive set of FISH probes for the presence of recurring cytogenetic abnormalities.

Results: 185 out of 188 samples were evaluable for analyses. One missense mutation in the IDH2 gene (c.G419A) was identified in the cohort of 185 MM pts (0.5%). This mutation was described as the most frequent IDH2 mutation in AML and is predicted to cause an amino acid change from arginine to glutamin at position 140 (p.R140Q). On cytogenetic analysis this patient harbored a translocation t(11;14) resulting in aberrant expression of CCND1. Additionally, in 15 pts (8%) the recently described single nucleotide polymorphism (SNP) in the IDH1 gene (rs11554137) was detected that has been reported as an adverse prognostic factor in cytogenetically normal AML.

Summary: Mutations in the IDH1/2 genes are a rare event in MM (0.5%). Further studies are warranted to address the issue if IDH1/2 mutations are restricted to distinct genetic subgroups as for example the group of MM pts with translocation t(11;14).

Disclosure: No conflict of interest disclosed.
Preclinical analysis of the novel Hsp90 inhibitor NVP-HSP900 in multiple myeloma cells

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Introduction: Pharmacological blockade of the molecular chaperone Heat shock protein of 90kD (Hsp90) is currently being pursued in various malignancies. Although single agent activities in multiple myeloma (MM) have been modest, it is hoped that approaches that combine Hsp90 inhibition with other therapies will yield better effects. Here we analyse the effects of a novel Hsp90 inhibitor with improved oral bioavailability (NVP-HSP900) in MM cells.

Methods: Dose-effect relationships were analysed with annexin V-FITC/PI staining or the alamarBlue colorimetric method. Western blotting was used to assess drug effects on Hsp90 client proteins. Drug combination effects were based on constant ratio designs.

Results: Based on constant ratio designs.

Conclusions: NVP-HSP900 blocks Hsp90 in MM cells, and has preferential cytotoxicity against malignant cells. Combination with chemotherapeutics generally enhances the cytotoxic effects. Addition of PI3-Kinase inhibitors might be a feasible approach to subdue the upregulation of Hsp72 seen after Hsp90 inhibition, possibly opening a way to extend the clinical efficacy of Hsp inhibition in MM.

Disclosure: No conflict of interest disclosed.

Phase I study of lenalidomide with pioglitazone, dexamethasone and low-dose treosulfan: Combined anti-inflammatory, immunomodulatory and angiostatic treatment as third-line therapy for patients (pts) suffering from multiple myeloma (MM)

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Introduction: Therapeutic options for pts with repetitively progressive MM are still limited. Continuous production and release of pro-inflammatory cytokines may account for its resistance towards cytotoxic drugs. Therefore, a phase I study was implemented as third-line therapy for pts with MM to assess maximal tolerable dose of lenalidomide in combination with other biomodulatory agents.

Methods: Six pts with progressive MM were enrolled in one German study center. In the phase I part (core study, lasting one cycle, 4 weeks) and thereafter, pts were treated continuously with daily doses of lenalidomide (cohort 1, 3pts with 10 mg; cohort 2, 3 pts with 15 mg), pioglitazone, low-dose treosulfan and dexamethasone until disease progression. During the study, respective paraprotein levels in serum and urine, ECOG performance status and QoL, besides routine laboratory parameters were continuously assessed. Pts responsive to study medication were allowed to enter the extension phase until complete remission, disease progression or intolerable toxicity occurs.

Results: The core phase of this study was finished in March 2011. Six pts are currently under treatment in the extension phase, cycle 4 to 19. All pts were intensively pretreated, and have received at least two conventional chemotherapy regimens, 5 of 6 pts double HD-Melphan with autologous transplantation and/or Vel/Dex, one pt lenalidomide. Patients experienced no dose-limiting toxicity at both dose levels of lenalidomide. Hemoglobin levels generally remained by phosphorylation of its catalytic subunit. Inactivation of PP2A is responsible for the hyperphosphorylation of autoantigenic targets. We conclude that the genetic defect underlying the dominantly inherited hyperphosphorylation of autoantigenic paraprotein targets is not in the PPP2A itself, but in genes or proteins controlling PPP2A activity by phosphorylation of its catalytic subunit.

Disclosure: Klaus-Dieter Preuss: Honoraria: Patentantrag eingerichtet Sandra Grass: No conflict of interest disclosed.

Hyperphosphorylation of autoantigenic targets of paraproteins is due to inactivation of PP2A

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Paratarg-7, a frequent autoantigenic target, and all other autoantigenic targets of human paraproteins molecularly defined to date are hyperphosphorylated in the respective patients compared to healthy controls suggesting that hyperphosphorylation of autoantigenic paraprotein targets is a general mechanism underlying the pathogenesis of these paraproteins. We now show that hyperphosphorylation of paratarg-7 is due to an additional phosphorylation of Ser17, which is located within the paraprotein-binding epitope. Co-immunoprecipitation identified PKCzeta as the kinase responsible for the phosphorylation of most and PP2A as the phosphatase responsible for the dephosphorylation of all hyperphosphorylated autoantigenic targets of paraproteins. SNPs or mutations of PKCzeta and PP2A were excluded. However, PP2A was inactivated by phosphorylation of its catalytic subunit at Y307. Stimulation of T cells from healthy carriers of wild-type paratarg-7 induced a partial and transient hyperphosphorylation between days 4 and 18, which was maintained by incubation with inhibitors of PP2A, again indicating that an inactivation of PP2A is responsible for the hyperphosphorylation of autoantigenic paraprotein targets. We conclude that the genetic defect underlying the dominantly inherited hyperphosphorylation of autoantigenic paraprotein targets is not in the PPP2A itself, but in genes or proteins controlling PPP2A activity by phosphorylation of its catalytic subunit.

Disclosure: No conflict of interest disclosed.

Onkologie 2011;34(suppl 6):1–305

Abstracts
Disclosure: Lenalidomide may thus be a new and effective treatment for long-term use even in patients with severe polyneuropathy associated with POEMS syndrome. The lack of neurotoxicity may allow further use in patients with NXG and severe cutaneous involvement failing dermatological treatment. We observed that pro-inflammatory CD4+/IFNγ+ T cells were rapidly increased 1 week after Len treatment (0.5% vs. 6.5%, p < 0.03), which then limiting toxicity. Further we found a significant increase in activated T cells 2 weeks after len treatment (13% vs. 41%, p < 0.05), this effect was mainly due to the KIR receptors (31% vs. 14%, p< 0.05). Non responder expressed more inhibitory receptors (2.8%%, p< 0.05), while non responder expressed more inhibitory receptors (2.8%%, p< 0.05), while had a marked by NKp44 expression (1% vs. 4.3%, p< 0.05).

Results: We observed GvHD in 8 out of 24 patients, which was the dose-limiting toxicity. Further we found a significant increase in activated T cells 2 weeks after len treatment (13% vs. 41%, p < 0.05), this effect was mainly based on the activation of CD8+ cells (29% vs. 60%, p < 0.05). Interestingly we observed that pro-inflammatory CD4+/IFNγ+ T cells were rapidly increased 1 week after Len treatment (0.5% vs. 6.5%, p < 0.03), which then resulted in an increase of CD8+/IFNγ+ cells after 1 month of treatment (1.8% vs. 6.7%, p < 0.05). Additionally we found an increase of activated NK cells, marked by Nkp44 expression (1% vs. 4.3%, p< 0.05). In our cohort, 3 patients progressed during len treatment. Those patients showed significant less activated T cells (13% vs. 28%, p< 0.05), while had a 3 fold increase in their Treg level. Furthermore patients who benefit from len treatment showed a significant increase in activated NK cells (0.8% vs. 2.8%, p< 0.05), while non responder expressed more inhibitory receptors like the KIR receptors (31% vs. 14%, p< 0.05).

Conclusions: This data suggest that Lenalidomide has immune stimulatory properties and may contribute to the induction of GvHD as well as to response when used early after allo SCT. Disclosure: No conflict of interest disclosed.

Successful treatment of rare plasma cell dyscrasias with lenalidomide
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Introduction: Lenalidomide is a highly effective drug for treatment of malignant plasma cells. Besides its immunomodulatory effects, it reduces the production of proinflammatory and proangiogenic cytokines. The POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal protein and skin changes) syndrome and the necrobiotic xanthogranuloma (NXG) are rare paraneoplastic syndromes associated with monoclonal gammopathy. Both syndromes are driven by inflammatory and angiogenic cytokines. However, there exist only anecdotal reports for successful treatment strategies.

Patients and Results: Here we report two patients with POEMS syndrome and two patients with NXG who were treated with lenalidomide and dexamethasone (Rd). One patient with POEMS syndrome who suffered from severe edema and polyneuropathy received primary induction treatment with Rd. After significant clinical improvement the patient could be treated with high dose melphalan: A hematological complete remission was achieved which was sustained with lenalidomide maintenance treatment after an immunofixation-positive relapse. In the second patient, the POEMS syndrome was complicated by extensive hemangiomas with cutaneous, subcutaneous, bone and central nervous system involvement. Rd was initiated as treatment for relapse, which lead to rapid regression of hemangiomas and resulted in hematological remission. In addition, two patients with NXG and severe cutaneous involvement failing dermatological treatment developed a durable clinical remission of cutaneous lesions after initiating treatment with Rd.

Conclusion: The successful use of lenalidomide in POEMS syndrome and NXG supports the notion of these two rare entities as cytokine-driven diseases, since such mechanisms can be directly suppressed by lenalidomide. Furthermore, lenalidomide is able to suppress the malignant plasma cell clone since such mechanisms can be directly suppressed by lenalidomide.

P556

Disclosure: No conflict of interest disclosed.

In patients with relapsed multiple myeloma long-term treatment of bortezomib and pegylated liposomal doxorubicin is a feasible and safe treatment option
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In patients with relapsed or refractory multiple myeloma (MM) the combination of bortezomib and pegylated liposomal doxorubicin (PLD) is superior to bortezomib monotherapy. Although this combination therapy is well tolerated, properties and may contribute to the induction of GvHD as well as to response when used early after allo SCT. Disclosure: No conflict of interest disclosed.

Activation of NK and T-cells after lenalidomide treatment post allo SCT correlates with response
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Background: Multiple Myeloma (MM) is a malignant, clonal plasma cell disease. Allogeneic stem cell transplantation after reduced intensity conditionings (allo SCT) offers a curative approach for MM, but the high incidence of relapse highlighted the need for post transplant strategies to improve outcome. Lenalidomide (len) has shown high efficacy in treatment of MM, but it’s immunomodulatory effects are poorly understood and myelosuppression is of major concern for len’s use after allo SCT. We therefore investigated len in dose finding study early (day 100-180) after allo SCT as well as immunomodulatory effects.

Methods: A total of 24 patients were treated in a dose finding study of lenalidomide after allo SCT. Toxic side effects were recorded according to CTC criteria. MM response was measured by immunofixation and other biochemical tests. For immunological analysis blood was taken in weeks and monthly intervals and analysed by FACS for the activated T-cells, activated NK cells as well as regulatory T (Treg) cells and KIR expression.

Results: We observed GvHD in 8 out of 24 patients, which was the dose-limiting toxicity. Further we found a significant increase in activated T cells 2 weeks after len treatment (13% vs. 41%, p < 0.05), this effect was mainly observed that pro inflammatory CD4+/IFNγ+ T cells were rapidly increased 1 week after Len treatment (0.5% vs. 6.5%, p < 0.03), which then increased in an increase of CD8+/IFNγ+ cells after 1 month of treatment (1.8% vs. 6.7%, p < 0.05). Additionally we found an increase of activated NK cells, marked by Nkp44 expression (1% vs. 4.3%, p< 0.05).

In our cohort, 3 patients progressed during len treatment. Those patients showed significant less activated T cells (13% vs. 28%, p< 0.05), while had a 3 fold increase in their Treg level. Furthermore patients who benefit from len treatment showed a significant increase in activated NK cells (0.8% vs. 2.8%, p< 0.05), while non responder expressed more inhibitory receptors like the KIR receptors (31% vs. 14%, p< 0.05).

Conclusions: This data suggest that Lenalidomide has immune stimulatory properties and may contribute to the induction of GvHD as well as to response when used early after allo SCT. Disclosure: No conflict of interest disclosed.
P559
Neoplastic meningitis – a rare but devastating manifestation of Multiple Myeloma

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Introduction: Involvement of the central nervous system (CNS) in patients with Multiple Myeloma is a rare event that occurs mostly during late stage of disease. However, there are reports about an increasing incidence of extramedullary myeloma manifestations since the introduction of new agents such as bortezomib,thalidomide and lenalidomide. We therefore tried to optimize the diagnostic workup in patients with suspected neoplastic meningitis of Multiple Myeloma – so called leptomeningeal myelomatosis (LMM) – by combining different techniques.

Methods: Between 04/2005 and 4/2011 we identified 9 cases with LMM. The involvement was confirmed by magnetic resonance imaging (MRI), cerebrospinal fluid (CSF) cytology as well as by flow cytometry. Additionally, clg-FISH and DNA probes mapping to chromosome bands 1q21.2, 9q34, 13q14, 1q432, 17p13, and 22q11 were applied to 4 of the 9 cases.

Results: The median time from initial diagnosis until the occurrence of LMM was 410 days. Only two patients presented with CNS manifestation within 180 days after initial diagnosis. Seven patients were diagnosed at late stage of disease e.g. after high dose melphalan treatment. At diagnosis of LMM, the median age was 59 years. The median cell count in the cerebrospinal fluid was 18/µl (Range 1/µl – 1333/µl). All CSF samples showed malignant pleocytosis, confirmed by flow cytometry in 89 patients. Clg-FISH presented cytogenetically defined high risk features in all samples tested: 3 of 4 patients showed a translocation t(4;14), one patient had a 17p13 deletion. Treatment for LMM consisted of intrathecal chemotherapy (7 of 9 cases) and radiation therapy (4 of 9 cases). Despite treatment, the outcome of patients with confirmed LMM was dismal with a median overall survival after diagnosis of LMM of 69 days. Only one patient survived longer than 2 years after diagnosis of CNS involvement.

Conclusion: By combining several technical procedures (MRI, cytology, flow cytometry and clg-FISH) it is possible to identify the vast majority of patients with LMM. However, management of affected patients is challenging and the survival generally only short after diagnosis of LMM.

Disclosure: No conflict of interest disclosed.

Fig. 1. Overall survival of patients with LMM

P560
Synergistic activity of mToR and PI3K inhibitors in blocking growth and survival of malignant plasma cells

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The phosphomositide-3-kinase (PI3K)-AKT pathway and its nutrient-dependant downstream target, the mToR (mammalian target of rapamycin) kinase, are essential for the growth and survival of malignant plasma cells. mToR inhibitors show moderate activity in myeloma patients, although the clinical activity may be limited by the fact that after inhibition of the rapamycin-sensitive Raptor complex, AKT is activated in tumor cells by feedback loops. Selective PI3K inhibitors (Ly294002, NVP-BKM120) as well as dual PI3K-mToR inhibitors (NVP-BEZ235) are available for in vitro studies and in clinical development. In five myeloma cell lines, rapamycin, everolimus, Ly294002, NVP-BKM120 and NVP-BEZ235 (Novartis) guided a dose-dependent growth inhibition. Despite the observed strong anti-myeloma activity of the mToR inhibitors, the AKT pathway was activated in vitro as well as in explanted tumors of INA-6 xenografted SCID mice upon treatment with rapamycin. Therefore, combining mToR and PI3K inhibitors could be an effective strategy to overcome rapamycin induced AKT activation. Rapamycin together with Ly294002 or NVP-BKM120 led to synergistic growth inhibition in plasma cell lines, accompanied by the abrogation of AKT activation. Combined treatment enhanced apoptosis in cell lines and in patient myeloma cells. Interestingly, the activity of the dual inhibitor NVP-BEZ235 was enforced by rapamycin. Our data suggest that a combination of mToR inhibitors with PI3K targeting compounds may lead to additive therapeutic chances and should be further explored.

Disclosure: Andreas Günther: Advisory Role: Novartis, Celgene; Financing of Scientific Research: Novartis, Celgene; Expert Testimony: Novartis
Martin Gramatzki: Advisory Role: Novartis; Financing of Scientific Research: Novartis; Expert Testimony: Novartis
Andreas Günther: Advisory Role: Novartis, Celgene; Financing of Scientific Research: Novartis; Expert Testimony: Novartis

P561
Ferritin- and iron status in association to disease characteristics, organ function, comorbiditys and progression free- (PFS) and overall survival (OS) in multiple myeloma (MM) patients (pts)

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Introduction: Anemia is a relevant and well acknowledged risk factor, that if induced by MM-progression – leads to red blood cell (RBC) transfusions and anti-MM-treatment. Similarily to the negative impact of iron-overload in MDS or allogeneic transplants, iron-overload vs. -deficiency is currently discussed to assess which predominantly exists in MM and whether these constitute potential risks.

Methods: We analysed a cohort of 91 MM pts treated at our department between 1997-2009, assessing pt characteristics, treatment and known risk factors (cytogenetics, ß2-MG, LDH, creatinine-, eGFR, CRP) and other factors (cytogenetics, ß2-MG, LDH, creatinine-, eGFR, CRP) and other parameters (proBNP, in association with iron-, ferritin-, transferrin-, transferrin saturation- levels and transfusion requirements (RBC- + platelet-transfusions [PTT]).

Results: Median iron-, ferritin-, transferrin- and transferrin saturation (TS)- levels were 73µg/dl (range 20-242), 590µg/dl (range 7-15779), 189µg/dl (range 100-354), and 27% (range 7-91), respectively. The distribution of ferritin levels < 400, >400 – < 1000 and >1000µg/dl in our MM cohort was 34%, 34% and 33%, respectively; suggesting either iron overload or ferritin elevation due to the myeloma itself. Our median elevated ferritin- (590µg/dl), low iron- (<73µg/dl), low transferrin- (189µg/dl) and low TS (27%)- levels, however, suggested that iron deficiency is the largely underlying phenomenon in MM. The distribution of TS-levels < 400, 20-45% and >45% were observed in 32%, 46% and 21% of pts, respectively. These results reflected two MM-subgroups: 1.
those with iron deficiency (TS ≤20%; n=30 [32.1%]) and 2. those with iron overload (ferritin>1000 µg/mL; n=15 [16.0%]). Of interest, median RBC and PT in our cohort were quite substantial with 16 and 5, respectively. With increased RBC transfusions, median iron, transferrin- and TS-levels remained unchanged, whereas ferritin levels increased expectedly. Median PFS and OS in our cohort was 31 (range 27-69) and 79 months (56-116), respectively. No significant survival differences were observed in pts with various ferritin-cut-offs, nor in subgroups of iron-overload, -deficiency, their occurrence, frequency and influence on clinical practice in this MM cohort is ongoing and will be presented at the meeting.

Conclusion: Further analysis on pt characteristics within defined subgroups (iron-overload vs. -deficiency), their occurrence, frequency and influence on clinical practice in this MM cohort is ongoing and will be presented at the meeting.

Disclosure: Christine König: No conflict of interest disclosed.
Monika Engelhardt: Other Financial Relationships: Educational Grant Novartis.

P562
Incidence of venous thromboembolism (VTE) in patients with multiple myeloma (MM) and monoclonal gammapathy of unknown significance (MGUS) before the introduction of IMiDS

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Introduction: Immunomodulatory drugs such as thalidomide and lenalidomide are associated with a high risk of venous thromboembolism (VTE) and have led to increased clinical awareness of this complication in patients with MM. Population based studies from Sweden have shown that patients with MM as well as MGUS have a 2-7 fold increased risk of VTE and the incidence of VTE was 2.6% in approximately 200 patients treated with MP or Dexamethasone. We decided to conduct this prospective single center study to analyse the incidence and type of VTE as well as possibly associated comorbidities.

Patients and Methods: All patients with MM and MGUS treated at Magdeburg University Hospital between 1997 and 2007 were retrospectively analysed. VTE had to be confirmed by ultrasound, angiography or CT-scan. Patients had been treated with a variety of protocols including stem cell transplantation, but excluding thalidomide or lenalidomide.

Results: A total of 216 patients were included in this study, 200 had MM, 16 had MGUS. The majority had stage III disease (37%). 127 were male (59%) and 89 were female (41%), median age was 59 years. A total of 29 patients (13%) had a VTE – mostly DVT – that occurred before the initiation of chemotherapy in the majority. Interestingly a high rate of arterial thrombosis of 10% – mostly myocardial infarction – was also observed in male patients. 2 VTE (7%) occurred in 27 patients with either MGUS or MM stage I. Hypertension was a common comorbidity (42%) as well as renal failure (29%) and diabetes (17%). 17 patients (8%) had secondary malignancies.

Discussion: Our findings confirm the high incidence of VTE in patients with MM and MGUS even if not treated with IMiDS. The VTE rate of 10% compares to that observed in patients with solid tumors or other hematologic malignancies. A high rate of comorbidities was also observed with arterial thrombosis being of particular importance. These findings are surprising, especially since the patient cohort analysed was rather young with a median age 59 years only.

Conclusion: VTE are common in patients with MM and MGUS, thus physicians need to be alert and should employ prophylaxis in high risk situation such as immobilization or surgery. The high rate of comorbid conditions may contribute to thrombosis risk and possibly adversely influence prognosis of MM.

Disclosure: No conflict of interest disclosed.

P563
Inhibition of mTOR with everolimus (RAD001) and silencing by vascular endothelial cell growth factor specific siRNA induces additive antitumor activity in multiple myeloma cells

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Background: Angiogenesis plays an important role in the pathogenesis and progression in multiple myeloma (MM). MM cells secrete vascular endothelial growth factor (VEGF), which further promotes proliferation of the tumor cells. Several RNA interference (RNAs) methodologies are rapidly being established and hold promise to specifically inhibit gene expression in mammalian cells. RNAs is the sequence-specific, posttranscription gene silencing mechanisms initiated by double-stranded RNAs, which are homologous to the gene being suppressed. Mammalian target of rapamycin (mTOR) is an essential part of tumour growth being capable of integrating proliferative, antiapoptotic and angiogenic signalling by connecting VEGF, hypoxia-inducible factor 1 (HIF-1) and HER family receptors.

Aims: Therefore, we evaluated the anti-myeloma effect of VEGF siRNA silencing in MM cells and whether it can be augmented by the additional application of everolimus.

Methods and Results: After transfection with VEGF siRNA we observed a reduction of VEGF expression in all studied cell lines: OPM-2, RPMI-8226, INA-6, Jurkat, Raji and Karpas-299, as well as in cells of MM- and lymphoma patients. Next, using the MM cell line OPM-2 we studied the time courses of VEGF siRNA transfection in order to investigate the knock-down efficiency of VEGF expression. The efficiency of VEGF siRNA transfection in treated OPM-2 cells showed a reduction of 75.5% VEGF protein levels after 24 h compared to the untreated OPM-2 cells (<0.001). Further, VEGF siRNA both significantly induced apoptosis and inhibited proliferation in OPM-2 cells (<0.001), RPMI-8226 (p<0.001), and in INA-6 (p<0.01) versus controls. To assess whether everolimus treatment of OPM-2 and RPMI-8226 affects the viability of these cells, cells were treated with various doses (1-20 nM) of everolimus for 24 hours, harvested, and analyzed for cell viability by MTT assay. Everolimus significantly decreased the viability of OPM-2 cells (IC50 = 1.9 nM) and of RPMI-8226 cells (IC50 = 2.1 nM), respectively. Everolimus and siRNA both together might to reduce the VEGF gene expression up to 33% (p<0.001) compared to siRNA VEGF (61%) alone or everolimus alone (39%), that demonstrated additive effects of everolimus and siRNA.

Conclusions: These findings suggest that mTOR inhibition and silencing by VEGF specific siRNA may be associated with an additive antitumor activity and might be a suitable target for new therapeutic strategies using RNA interference in MM.

Disclosure: No conflict of interest disclosed.

P564
SOX2-specific autoantibodies occur in patients with multiple myeloma after allogeneic stem cell transplantation

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Background: SRY-related HMG box-2 (SOX2) is a transcription factor involved in embryonic and cancer development. Recently, the occurrence of

Disclosures: No conflicts of interest identified.
SOX2-specific autoantibodies have been reported to correlate with prognosis and a reduced risk of progression to multiple myeloma (MM) in patients with monoclonal gammopathy of undetermined significance. However, it has remained unclear if and at which point during the course of the disease SOX2 specific antibodies also occur in symptomatic MM.

Methods: 1094 peripheral blood (PB) samples from 196 MM patients and 100 PB samples from healthy donors were screened for SOX2-specific antibodies by ELISA. Expression of SOX2 in bone marrow (BM) samples of 25 MM patients and 10 healthy donors, respectively, as well as in a collection of various healthy tissues and myeloma cell lines was assessed by flow cytometry, RT-PCR, and Western Blot.

Results: SOX2 was detectable in myeloma cell lines and the malignant plasma cells of myeloma patients but it was also expressed at varying levels in the majority of healthy tissues analyzed. Expression of SOX2 was found to be comparable in the BM of MM patients and healthy donors and, accordingly, SOX2 expression did not correlate with the number of BM-resident plasma cells. We detected SOX2-specific autoantibodies in 7.7% (15/196) of MM patients and 2.0% (2/100) of healthy donors. The presence of anti-SOX2 immunity was not related to SOX2 expression levels or the tumor burden in the patients' BM. The only clinical factor predicting the development of anti-SOX2 immunity was the application of allogeneic stem cell transplantation (alloSCT). Anti-SOX2 antibodies occurred more frequently (p<0.0001) in the group of patients (N=74) who had received alloSCT. Moreover, 80% of the SOX2 antibody-positive patients had only become positive after being treated with alloSCT.

Conclusions: We describe for the first time a correlation between anti-SOX2 immunity and alloSCT, indicating that alloSCT is able to break tolerance towards this commonly expressed antigen. The question whether the appearance of SOX2-specific antibodies merely represents an epiphenomenon, is related to graft-versus-host effects or whether it plays a role in the immune control of myeloma needs to be answered in prospective studies.

Disclosure: No conflict of interest disclosed.

P566 Multikinase inhibitor sorafenib induces apoptosis, CD138-downregulation, actin depolymerisation and inhibition of M210B4-triggered chemotaxis in multiple myeloma (MM) cell lines and synergizes with proteasome inhibitor bortezomib

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Introduction: As the development of MM therapies is pursued to improve survival, various innovative agents are eagerly tested. The Ras/Raf/MEK/ERK pathway is critical for proliferation of MM cells and its blockade may also influence cell adhesion and migration. We sought to elucidate the effects of sorafenib on proliferation, phenotype, chemotaxis, signalling pathways, and cytotoxic interactions when combined with other MM agents.

Methods: Cell viability and cytotoxicity were assessed via trypan blue and PI-staining. MM-cells co-expressing cytochrome c (cyc c)-GFP and histone 2B (H2B)-mCherry allowed detection of early vs. late apoptosis. CD138-expression was evaluated via immunocytochemistry, chemokine receptors by flow cytometry. Chemotaxis to diverse chemotactic agents was assessed and signalling pathways by western blot. The sorafenib and bortezomib effect was analyzed via Calcusyn software, a combination index < 1 indicating synergism.

Results: With 10 and 100μM sorafenib, PI+ cells significantly increased, and viable- and CD138+-cells decreased in a dose- and time-dependent manner. CD138-downregulation, morphologic changes and actin depolymerization were observed with already 1μM sorafenib via confocal microscopy. Both chemokine receptors CCR4 and CCR5 were detected on L363 cells that underwent chemotaxis to their common ligand CCL5 and markedly to stroma M210B4-supernatant. M210B4-triggered chemotaxis was substantially inhibited with sorafenib, ostensibly as a consequence of actin depolymerization, verified via confocal microscopy. Sorafenib-induced downregulation of phospho-ERK with 10 and 100μM appeared responsible for the actin depolymerisation and reduced M210B4-triggered chemotaxis. Combined bortezomib and sorafenib-supernatant revealed clear synergism. Confocal images of RPMI8226 cells co-expressing cyc c-GFP and H2B-mCherry after bortezomib or sorafenib showed cyc c-GFP release and chromatin fragmentation for early and late apoptosis, respectively. In line with these results, sorafenib maintenance therapy in MM-AL-amyloidosis patients induced disease stabilization.

Conclusions: We observed potent single and combined bortezomib and sorafenib cytotoxicity. Sorafenib induced decreased phospho-ERK, actin depolymerization and consequently reduced M210B4-triggered chemotaxis. This is to the best of our knowledge the first analysis on phenotype, morphology and migration of sorafenib in MM-cells. Our results should be valuable for future clinical trials.

Disclosure: No conflict of interest disclosed.
Response to a novel pomalidomide-based therapy in a patient with relapsed multiple myeloma

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Multiple Myeloma (MM) is characterized by high and cumulative levels of genomic instability that correlate with disease progression. Recently, the central role of the ubiquitin proteasome system (UPS) in the cellular DNA damage response machinery has been appreciated, thus suggesting potential roles for the UPS in both MM development and as a relevant target structure. Indeed, proteasomal inhibition has been successfully introduced into the therapy of MM, indicating the presence of disease-specific deregulated ubiquitylation events. Their identity has however remained largely elusive. F-box proteins are substrate recognition subunits of SCF (Skp1-Cullin-F-box protein) ubiquitin ligases, which mediate the timely proteolysis of key regulatory proteins. The human genome encodes approximately 70 F-box proteins, but only few have established functions.

Recently, genome wide CHIP array analyses identified activation of the evolutionary conserved orphan Fbxo9 gene in MM cell lines and patient samples. To identify biologically relevant substrates of this F-box protein, we conducted an unbiased mass spectrometry based screen that revealed Tel2 (telomere maintenance 2) and Tti1 (Tel2 interacting protein 1) as potential substrates. Tel2 and Tti1 are highly conserved proteins previously shown to form the TTT-complex together with Tel2. This complex interacts with all six mammalian PIKKs (ATM, ATR, DNA-PKcs, SMG-1 TRRAP and mTOR) and profoundly affects their abundance and function. Subsequently, we found that SCFTel2 mutant targets Tel2/Titi1 proteins for degradation in a cell context dependent manner to regulate ATM/ATR dependent DNA damage response, and mTOR dependent response to mitogenic signaling. Specific ubiquitylation of Tel2/ Titi1 by the SCFTel2 ligase was ascertained in ubiquitylation studies using reconstituted proteins. Finally, using FISH analyses, we found primary multiple myeloma tumor samples to be enriched for Fbxo9 amplifications, thus further underscoring a role for Fbxo9 in myelomagenesis. Together, our data identify the Fbxo9-TTT-mTOR axis as a central PIKK regulatory hub, whose deregulation may contribute to the development and progression of MM.

Disclosure: No conflict of interest disclosed.

Response to a novel pomalidomide-based therapy in a patient with relapsed multiple myeloma

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The patient favourably responded despite intensive pretreatment until late course of therapy had to be interrupted after 6 well tolerated courses due to improvement (177/nl platelets; WBC 7,9/nl; Hb 11,3g/dl) was observed. In view of this and without any further therapeutic options we decided to initiate a regimen based on pomalidomide 4mg days 1-21 (followed by 2mg from 2th cycle), doxorubicin 4mg + dexamethasone 40mg (20mg) every 4 weeks. After 3 cycles a partial serologic remission (IgG 1.8 g/dl) and hematologic improvement (177/ml platelets; WBC 7,9/ml; Hb 11,3g/dl) was observed. The course of therapy had to be interrupted after 6 well tolerated courses due to reactivation of a hepatitis B virus infection (6.7x10^6 copies), which the patient supposedly acquired in 1945 and which has never prevailed during therapy before. Antiviral treatment with entecavir was initiated and led to a decline of virus copies (9.7x10^4). 4 months after stop of therapy the good haematological response was still apparent and the patient still reported good life quality.

To the best of our knowledge, this is the first report on the combination of the next-generation IMID, pomalidomide, with adriamycin and dexamethasone. The patient favourably responded despite intensive pretreatment until late reaction of hepatitis B prompted interruption of therapy.

Disclosure: No conflict of interest disclosed.

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Background: Cancer-testis antigens (CTA) are attractive targets for cancer immunotherapy due to their tumor-restricted expression and immunogenicity. Some CTA, including MAGE-A3, are already under clinical investigation and induce strong immunity in cancer patients receiving active immunotherapy. However, little is known about the fine specificity and the function of vaccine-induced antibody (Ab) responses and their relation to spontaneous anti-CTA immune responses.

Methods: We analyzed spontaneously occurring Ab responses against MAGE-A3 in sera (N=1347) from patients with multiple myeloma (N=225) over 6 years. Ab titers and IgG subtypes were analyzed by ELISA and numbers of MAGEA3-specific B cells in the peripheral blood (PB) were determined in an ELISPOT. Fine specificity of Ab responses was examined using overlapping 20mer peptides spanning the whole MAGE-A3 sequence. The quality of MAGE-A3-specific Abs was analyzed by western blot and affinity assays. Results were compared to those of patients with non-small cell lung cancer (NSCLC; N=57) vaccinated with full-length MAGE-A3 protein and adjuvant AS15.

Results: Of 225 myeloma patients 3 (1.3%) showed spontaneous Ab responses against MAGE-A3. Spontaneously occurring anti-MAGE-A3 Abs were of low titer and short-lasting. In contrast, all vaccinated patients showed high-titered and persisting Ab responses appearing around week 6 after treatment initiation and underwent affinity maturation with ongoing vaccination. We found high numbers of vaccine-induced MAGE-A3-specific memory B cells in the PB of NSCLC patients while they remained undetectable in most myeloma patients. MAGE-A3-specific Abs consisted of IgG1 and IgG2b/IgG3/IgG4 subtypes in vaccinated patients while spontaneously occurring Abs were mainly of the IgG3 subtype. Spontaneous and vaccine-induced Abs both recognized the natural protein. Interestingly, spontaneous and vaccine-induced Ab responses most frequently recognized a specific region within the MAGE-A3 protein corresponding to amino acids 51-70.

Conclusions: We demonstrate important qualitative differences between spontaneously occurring and vaccine-induced Ab responses against MAGE-A3 in cancer patients. While the potential of both types of Ab responses to promote antigen uptake and induction of T cell responses might differ, they both recognized the same restricted region within the MAGE-A3 protein. This find-
ing might be important for the design of future immunotherapies targeting MAGE-A3.

Disclosure: No conflict of interest disclosed.

P570
Is ‘Hevytite’ an useful test for the quantitative monitoring of monoclonal gammapathy?

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Introduction: For the quantitative analysis of paraprotein in monoclonal gammapathy today the common methods are capillary zone electrophoresis (CZE), total Ig and serum free light chain analysis (sFLC). In the present study we investigated the new, commercially available test, HevytiteM (HLC) which allows analysis of IgG and IgA, IgMk and IgMλ, IgAx and IgAα concentrations by pairs. Comparable to the use of the sFLC-ratio the HLC-ratio of e.g. IgG/IgM could be a measure of clonality.

Methods: sIFE was performed on a Hydrasys by Sebia, M-spike was evaluated by CZE (Capillary, Sebia), total immunoglobulins by immunonephelometry and HLC concentrations were quantitated on a Siemens BNII Analyzer.

Results: We investigated more than 300 patients with monoclonal gammapathy and pathological (but in part very slightly) sIFE (53 IgA-MM, 38 IgM-MG, 188 IgG-MM, 25 LCMM). In patients with IgA or IgM paraproteinemia the percentage of patients with pathological HLC-ratio (IgA 89%; IgM 89%) is significantly higher than in the CZE or the analysis of total IgA/M (CZE: IgA 62%, IgM 65%; total IgA 55%, IgM 65%). The percentage of pathological HLC-R in patients with IgG paraproteinemia is with 67% only minimally higher than in CZE (65%), but more than twice as much than total IgG analysis (30%).

Suppression of the uninvolved subtype (e.g. IgG in IgM myeloma) is a new phenomenon called HL-pair suppression. We find HL-pair suppression in 96% of the patients with pathological HL-IgG ratio, as well as in 36% of patients with pathological HL-IgA and in 35% of patients with pathological HL-IgM ratio.

Conclusions: We conclude that the new test HevytiteTM is an interesting tool for disease monitoring in patients with paraproteinemia. Most notably in patients with pathological HL-IgA or IgM. In patients with IgG paraprotein the suppression of the uninvolved IgG subtype might be an indication of immunosuppression in myeloma patients.

Disclosure: No conflict of interest disclosed.

P571
A novel recurrent chromosomal aberration, duplication of chromosomal region 1q22-q25, identified in human multiple myeloma cell lines

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Duplications and other structural abnormalities of the long arm of chromosome 1 represent one of the most frequent chromosomal aberrations in multiple myeloma (MM) and seem to correlate with poor outcome. However, the exact regions and genes involved in these abnormalities are not well known yet. In the present study we performed a molecular cytogenetic investigation of 8 MM cell lines in order to identify and characterize novel recurrent breakpoints, deletions and duplications targeting chromosome 1q using multicolor fluorescence in situ hybridization (M-FISH). Additionally, we performed metaphase FISH with 30 region-specific overlapping probes on all cell lines spanning the whole long arm of chromosome 1q. M-FISH analyses revealed complex karyotypes with numerical and structural aberrations in all cell lines used in this study, RPMI-8226, AMO1, KMS-12-BM, EJM, LP-1, MOLP-2, U266 and NCi-H929. The modal chromosome number was triploid in all cell lines but one, U266, which presented a hypo-diploid karyotype. Using M-FISH and FISH, we were able to identify 1q aberrations in 7 cell lines (all except U266). Moreover FISH helped to redefine breakpoints in all cases.

Chromosome 1q breakpoints were distributed heterogeneously but clustered in 1q21.1 (4 cell lines). Gain of 1q21 is described in about 30-37% of patients, and is already known to be associated with a more proliferative disease. Four breakpoints were located at centromeric/pericentromeric regions, of which one was located at the region of constitutive heterochromatin 1q12. The most common 1q rearrangement found in this work was duplication of 1q22-q25 in 5 cell lines (RPMI-8226, AM01, MOLP-2, LP-1, and NCi-H929). In addition we found gain of 1q31.1, 1q44 chromosomal region, which was described to show gain in 54% of patients with MM. Furthermore, jumping translocations were identified in two cell lines. Chromosome region 1q jumped to the non-homologous chromosomes 1 and 14 in cell line RPMI-8226, and to 11, 12, and 14 in cell line AM01.

In summary, in our study we found a novel recurrent chromosomal aberration, duplication of 1q22-q25, which has not been described before, neither in cell lines nor in patient materials. This region might also play a role in MM pathogenesis. The possible target genes located at this chromosomal region still remain to be elucidated and are currently under investigation.

This work was supported by the Erich und Gertrud Roggenbuck-Stiftung.

Disclosure: No conflict of interest disclosed.
P573
Renal impairment in patients with monoclonal light chain disease – histological findings and outcome
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Introduction: Renal involvement is common in monoclonal light chain diseases (mLCD). Interestingly the type of the renal impairment and the related outcome substantially differ from each other.

Methods: We analysed retrospective data of patients with mLCD who had a renal biopsy performed at our hospital. We documented renal function, the degree of proteinuria, the type of renal disease and the renal outcome. The patients with mLCD were divided as follows: Multiple Myeloma (MM), AL-Amyloidosis (ALA), Monoclonal Gammapathy of unknown Significance (MGUS) and B-Non Hodgkin Lymphoma (B-NHL). The different kidney diseases were: Cast Nephropathy (CN), Light Chain Deposition Disease (LCDD), AL-Amyloidosis (ALA) and other renal diseases (ORD).

Results: 94 patients were analysed. The underlying diseases were: MM n=52, ALA n=24, MGUS n=22, B-NHL n=5. Among the 52 MM-patients we found 26 patients with CN, 5 with LCDD, 4 with ALA. 17 with ORD. All patients with MGUS had ORD, all except one with ALA had as well renal amyloidosis. B-NHL patients showed all types of renal disease. The mean observation time was 21,9±25,4 months. Renal function was significantly worse in patients with CN at the time of biopsy. Patients with ALA had the highest levels of proteinuria. 1,9 months after renal biopsy 50% of the CN patients depended on dialysis. After 12 mos 60% of the CN patients but only 20% of patients with ALA, 20% with LCDD and 7% with ORD needed dialysis (p=0,0004). In addition patients with mLCD who depended on dialysis had a significantly shorter survival (50% survival: 8,9 vs. 42, months, p<0,005).

Discussion: Our data show that not all patients with mLCD have directly associated renal diseases. The outcome of the patient strongly depends on the type of renal impairment. Thus patients with CN have the worst outcome, followed by ALA. Especially patients on dialysis show shorter survival.

Therefore we recommend renal biopsy in patients with mLCD and proteinuria and/or renal insufficiency in order to clarify the type of renal involvement.

Disclosure: No conflict of interest disclosed.

Posterdiskussion
Immuntherapie

P574
Catumaxomab observational study to investigate efficacy and safety profile in clinical practice – the CARMA study
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Introduction: The trifunctional anti-EpCAM / anti-CD3 antibody catumaxomab is approved in the EU for intraperitoneal (i.p.) treatment of malignant ascites in patients with EpCAM-positive carcinomas. Clinical data for catumaxomab are based on the randomized, pivotal trial and several phase III trials. So far, the routine use of catumaxomab in clinical practice has not been evaluated systematically. Therefore, a large prospective observational study was started in 2010, investigating the administration of catumaxomab in patients with malignant ascites under routine conditions in daily clinical practice.

Methods: In this study, a total of 160 patients with malignant ascites due to EpCAM-positive carcinomas (e.g. ovarian, breast, gastrointestinal cancer) treated with i.p. catumaxomab under routine conditions in clinical practice will be evaluated. Participating centres are hospitals and practices of oncologists in Germany and Austria. The following data are being collected (amongst others): demographic factors, cancer disease, distant metastasis, chemotherapy regimen, surgery, laboratory parameters, ascites signs and symptoms, management of catumaxomab therapy, number of punctures, tumor response, survival, quality of life, safety results. The data will be analyzed by descriptive statistical methods.

Results: The results of the first interim analysis are reported. The analysis includes 50 patients with malignant ascites and catumaxomab treatment. Data for therapy management, clinical efficacy, quality of life and safety are presented. The collection of data is ongoing.

Conclusion: We report here the data of an interim analysis with catumaxomab in malignant ascites. This is the first systematic report including a large patient number for the routine use of catumaxomab in clinical practice.


M. Essing: Employment or Leadership Position: Fresenius Biotech.
P576
EpCAM+ tumor cells are detected in malignant ascites fluid with high prevalence: results from a randomized phase IIb study

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Introduction: The tumor-associated epithelial cell adhesion molecule (EpCAM) is a marker frequently expressed on various carcinoma tissues. The trifunctional monoclonal antibody (AB) catumaxomab (anti-EpCAM x anti-CD3) has been approved for the i.p. treatment of patients with malignant ascites due to EpCAM+ carcinomas. Catumaxomab effectively controls malignant ascites (MA) by extinction of EpCAM+ tumor cells in the peritoneal cavity.

The aim of the reported investigation was to assess the frequency of EpCAM expression on tumor cells in malignant ascites.

Methods: The clinical phase IIb study CASIMAS investigated a 3 hour i.p. infusion of catumaxomab with and without prednisolone premedication in MA patients. From 193 patients ascites samples were collected for detection of EpCAM+ tumor cells before treatment. Cells were harvested, spun onto slides and labeled with the EpCAM-specific AB Ber-EP4. Cell-bound Ber-EP4 was detected with a biotinylated horse anti-mouse IgG and visualized with an avidin/biotinylated horseradish peroxidase complex and 3-amino-9-ethylcarbazole as substrate. Mayer’s hemalaun was used as counterstaining. EpCAM+ cells were evaluated by light microscopy. The cytological data were related to the histology of the primary tumor.

Results: The primary tumors of 193 patients evaluated were mainly gastric, colon, pancreatic, breast, ovarian, lung and endometrial carcinomas. In the majority of evaluable patient samples (n=183, 95%) EpCAM+ tumor cells were detected (n=170, 93%). In 2 patients tumor cells were EpCAM-negative (1%) and in 11 patient samples tumor cells were not detectable (6%). 5% of the test samples were not evaluable.

With regards to the primary tumor, in 100% of evaluable ascites samples from gastric, colon and endometrial, 99% from ovarian, 86% from breast, 80% from pancreatic and 85% of other carcinomas EpCAM+ tumor cells were detected.

Conclusions: The presented method for detection of EpCAM+ tumor cells in MA can be performed with standard equipment. In the MA samples of the vast majority of patients, over all primary tumors investigated EpCAM+ tumor cells were detected at a high frequency. Based on these data it is concluded that the vast majority of patients with MA may be indicated for an EpCAM-targeted treatment with catumaxomab.

**P568**

**Relevance of receptor affinity for the adoptive CD8+ T cell therapy of HLA-A2/NY-ESO-1 positive Multiple Myeloma**

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**Introduction:** Despite remarkable progress in the treatment of Multiple Myeloma, it still remains for most patients an incurable disease. *Ex vivo* generated and adoptively transferred T-cells endowed with chimeric antigen receptors (T-bodies) specific for a tumor specific antigen represent an alternative and promising therapeutic approach: *in vitro* expansion allows for the generation of a sufficiently large number of CD8+ lymphocytes with anti tumor specificity enabling recognition and destruction of cancer cells. As target we chose the Cancer-testis (CT) antigen NY-ESO-1, that is expressed in a variety of cancers but not in normal adult tissue except for the immunoprivileged tests.

**Methods:** Chimeric antigen receptors were expressed on human CD8+ T-cells via retroviral mediated gene transfer. These receptors are composed of single chain variable fragments of distinct affinities binding to the NY-ESO-1-peptide presented in the HLA-A2 complex fused to an intracellular signal domain of CD28 and CD3 zeta. T-bodies were specifically enriched in vitro by using Tap deficient minigene transfected T2-cells presenting the respective peptide or previously selected anti-idiotypic Fab antibodies.

**Results:** Our results show specific expansion and activation of CD8+ T bodies as well as cytotoxicity and Interferon-γ production upon antigen encounter. The differences in Interferon-γ production and cytotoxicity are dependent on the different binding affinities of the constructs. T bodies can be used to effectively kill Multiple Myeloma cells.

**Conclusion:** Adoptive T cell therapy with T bodies specific for the Cancer-Testis-Antigen NY-ESO-1 potentially represents a new approach to treat Multiple Myeloma. Further therapeutic effects of these T-bodies are currently being analyzed in a xenograft mouse model.

**Disclosure:** No conflict of interest disclosed.

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**P579**

**Immunosuppressive drugs upregulate the immune inhibitory receptor osteoactivin in monocyte-derived dendritic cells**

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**Introduction:** Osteoactivin, also known as transmembrane glycoprotein NMB (GPNMB) and dendritic cell-associated transmembrane protein (DC-HIL), is being analyzed in a xenograft mouse model.

**Disclosures:** No conflict of interest disclosed.

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**P580**

**Follow-up results from a phase II/III study of catumaxomab in patients with malignant ascites: evaluating relative lymphocyte count as a potential biomarker for overall survival**

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**Introduction:** Catumaxomab (anti-EpCAM x anti-CD3) is currently approved in the EU for the intraperitoneal treatment of malignant ascites in patients with EpCAM-positive carcinomas. Here we report follow-up results from a pivotal phase II/III randomized study in patients with malignant ascites due to different epithelial cancers. In addition to measuring long-term overall survival (OS), we evaluated the impact of relative lymphocyte count (RLC) as a potential biomarker for the efficacy of catumaxomab on the basis of a separate small hypothesis-generating study.

**Methods:** Survival analyses were performed for the safety population enrolled in the pivotal trial (paracentesis + catumaxomab [catumaxomab]: n=157; paracentesis alone [control]: n=88). We evaluated the impact of treatment and RLC using a Cox model. The Kaplan-Meier method and log-rank test were applied for group comparisons in order to identify patients with pronounced OS benefit.

**Results:** We observed a statistically significant benefit in OS in catumaxomab-treated patients compared with controls (p=0.0219, HR=0.649). The

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**Results:** Screening of different NP formulations showed that amino-functionalized NP were best suited for efficient T-cell labeling. A NP concentration of 500 µg/mL and incubation over 16 h led to strong cellular uptake without detectable cytotoxicity. The second day after antigen-specific stimulation resulted in strongest NP uptake. cLSM and TEM studies indicated that NP were localized in endosomal structures. Preliminary assays showed a moderate co-localisation between NP and the small GTPase Rab11, characteristic of a part of the endosomal recycling pathway. In contrast, co-localisations of NP with early or late endosomes and lysosomes could not be demonstrated. Functional testing of NP-labeled T cells showed that IFN-γ production and specific cytolyis toward target cells were not adversely affected by NP incorporation. Uptake kinetics indicated that endocytosis and exocytosis of NP were dynamic and primarily temperature (energy)-dependent processes. Particle uptake could not be blocked by pharmaceutical inhibitors of major endocytic pathways revealed that NP were incorporated by non-clathrin-non-caveolin-mediated endocytosis.

**Conclusions:** We have developed a protocol using amino-functionalized polymeric NP that allows the labeling of human T lymphocytes without a detectable negative impact on cellular function. We will use this experiences to design custom-made NP that are composed of biodegradable envelope material and carry a freight (e.g. MRI tracking molecules, RNA) on demand for application in adoptive immunotherapy in vivo.

**Disclosure:** No conflict of interest disclosed.
6-month OS rate in these patients was 28.9% compared to 6.7% for the control group. Increase of RLC had a positive impact on OS (p<0.0001): in the subgroup of patients with a RLC >13% (n=159; 100 catumaxomab; 59 control), catumaxomab treatment was associated with a pronounced benefit on OS vs controls (p=0.0072, HR=0.518); median/mean OS benefit was 109 vs 68/209 vs 78 days with catumaxomab; and the 6-month OS rate was 37.0% with catumaxomab vs 5.2% in the control group. The time to first paracentesis was significantly prolonged by catumaxomab treatment – independent from RLC (p<0.0001 for both subgroups, HR=0.159 / 0.182) for patients below/above the threshold of 13%.

Conclusion: Intraperitoneal treatment with catumaxomab significantly prolonged OS in patients with malignant ascites due to EpCAM-positive carcinomas. A pronounced OS benefit following catumaxomab therapy was observed in patients with a RLC >13% at start of treatment, which might be of predictive relevance. Further investigation of the immunological background of this clinical observation is warranted.

Disclosure: M. Heiss: Advisory Role: Trion, Fresenius Biotech; Expert Testimony: yes
M. Hennig: Employment or Leadership Position: Fresenius Biotech; Stock Ownership: yes, <5000€

P583
Chemotherapeutic drugs applied in combination with Rituximab differentially affect antibody-dependent cellular cytotoxicity (ADCC) of NK cells

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Introduction: The CD20 antibody Rituximab is an essential component of most treatment strategies for B cell lymphoma. Its success has been attributed to several particularities including its ability to induce antibody-dependent cellular cytotoxicity (ADCC) of Fc receptor-bearing immune effector cells like NK cells. However, conventional chemotherapeutic agents may impair NK cell reactivity (Markas 2007). As several different chemotherapeutic regimes are clinically applied with Rituximab, we studied here the influence of frequently used combinations of chemotherapeutic drugs on the Rituximab-induced immune responses.

Methods: Peripheral blood mononuclear cells (PBMC) and NK cells were exposed to various concentrations of the components (in combination and as single agents) of the following chemotherapeutic regimes: (i) CHOP: Cyclophosphamide (C), Doxorubicine (H), Vincristine (O) and Prednisone (P); (ii) FC, Fludarabine (F) and Cyclophosphamide (C); (iii) Bendamustine.

Rituximab-induced ADCC and cytokine production of NK cells in cultures with lymphoma cells after was determined by cytotoxicity assays and ELISA. Cellular integrity was determined by WST-1 assays.

Results: Rituximab-induced ADCC and IFN-γ production by PBMC and NK cells was dramatically and significantly reduced upon exposure of effector cells to plasma peak levels (PPL) of the CHOP combination, and an inhibitory effect was already observed at 10-fold lower drug levels. This was not due to the effects of a single drug but rather caused by an additive effect of Doxorubicine and Prednisone and, to a lesser extent, Vincristine. In contrast, no relevant effect was observed with PPL of Bendamustine. Unphysiologically high Bendamustine levels reduced NK reactivity starting at 2-fold PPL. Exposure to FC did not reduce NK reactivity up to concentrations corresponding to 5-fold PPL. Inhibition of NK reactivity by PPL of CHOP and high concentrations of Bendamustine and FC could be attributed to a corresponding reduction of NK cell integrity, which in case of CHOP occurred at concentrations low as 1/20 PPL.

Conclusion: Chemotherapeutic drugs applied for treatment of lymphoma in combination with Rituximab differentially influence ADCC of NK cells. Our data indicate that choice, dosing and likely also the sequence of application of chemotherapeutic drugs applied in combination with Rituximab should be carefully considered to assure optimal efficacy of Rituximab’s immunostimulatory effects.

Disclosure: No conflict of interest disclosed.

P584
A multicenter observational study on the efficacy and tolerability of Privigen® (intravenous immunoglobulin preparation) – Interim analysis with focus on immune thrombocytopenia (ITP)

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Introduction: Privigen® is a 10% liquid preparation of polyclonal human IgG for intravenous administration. The use of a novel stabiliser, L-proline, fully preserves IgG functional activity without refrigeration, making Privigen® ready-to-use. Privigen® is licenced as a maintenance therapy in primary or secondary immunodeficiencies and as an immunomodulatory therapy in autoimmune or inflammatory diseases.

Methods: This is an interim analysis of an ongoing multicenter observational study started in 2008 to evaluate the efficacy and tolerability of Privigen®. In April 2009, a revision of the case report form (CRF) was implemented, capturing additional efficacy data in most of the registered indications including ITP. Only treatment courses documented with the revised CRF were included in this interim analysis. The cut-off date was Dec 13, 2010.
Results: 313 patients (143 m, 170 f, mean age 60 years) received a total of 1586 Privigen® infusions with a mean dose of 15 g. The indications were primary immunodeficiency (N=36), secondary immunodeficiency (N=175), ITP (N=26), multiple sclerosis (N=43), chronic inflammatory demyelinating polyneuropathy (N=10), other polyneuropathies (N=10), other autoimmune diseases (N=10), and others (N=5). Across all indications, the efficacy of Privigen® was judged as very good or good in 91%, as moderate in 2% and as insufficient in 1% of the cases; 5% of the patients were judged as not evaluable for efficacy; in 1%, data are missing. 26 patients with ITP received a total of 128 Privigen® infusions with a mean dose of 22 g; the efficacy was judged as very good or good in 22 cases, as moderate in 1 case and as insufficient in 3 cases. Over all patients, the tolerability of Privigen® was judged by the physicians as very good or good in 92%, as moderate in 3%, and as insufficient in 3% of the cases; in 2%, data are missing. Adverse events possibly or probably related to Privigen® were reported for only 42 of the 1586 infusions (2.6%); 2 of them (0.1%) were serious (both patients recovered completely). Among the 128 infusions in ITP patients, only 3 (2%) were associated with non-serious adverse reactions.

Conclusion: Privigen® demonstrated very good or good efficacy and tolerability in >90% of 313 patients receiving 1586 infusions.


P568

Anti-idiotypic Fab-antibody-fragments and their potential usage for specific activation and expansion of chimeric antigen receptor grafted T cells
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Introduction: Cytotoxic T-lymphocytes are known to play an important role in controlling tumor growth. Previous studies have shown that tumors can be targeted by adoptive transfer of tumor specific T-cells or specific chimeric antigen receptor grafted T-cells (T-bodies). However, one limitation of the adoptive T-cell therapy is the appropriate T-body-expansion for the transfer. Tumor specific T-bodies can be specifically activated by either antigen presenting cells pulsed with specific peptide, artificial antigen presenting cells or anti-idiotypic antibodies specific for the chimeric antigen receptor in contrast to unspecific stimulation by lectins or receptor crosslinking antibodies. For adoptive T cell therapy in humans, T-body-expansion without any other stimulation cell system is desired.

Methods: In the present study we selected anti-idiotypic Fab fragments specific for the 3M4E5 chimeric antigen receptor, that is binding the cancer testis antigen NY-ESO-1157-165 /HLA-A2 complex. They were compared to HLA-A2 complexes pulsed with the specific peptide and were used to activate the T-bodies and specifically increase their number.

Results: No conflict of interest disclosed.

Conclusion: Anti idiotypic Fab-antibody-fragments are able to specifically activate T-bodies. They represent a valuable tool for specific T-body-expansion for adoptive therapy without the usage of further antigen presenting cells or unspecific receptor crosslinking antibodies.

Disclosure: No conflict of interest disclosed.

P567

Suppression of tumor antigen-specific human T lymphocytes by arginine depletion
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Introduction: One of the main obstacles to an effective immunotherapy of tumors is the phenomenon of tumor immune escape. Tumor-induced evasion of host anti-tumor immune effector pathways is often mediated by infiltrating myeloid-derived suppressor cells expressing the arginine-metabolizing enzyme arginase. We therefore analyzed the influence of arginase on the activation of expanded MART-1 antigen-specific human T cells.
Methods: T cells of 17 healthy donors were expanded with in vitro generated dendritic cells. Subsequently, T cell activation was analyzed upon tumor antigen-specific restimulation by peptide-loaded T2 cells in the presence/absence of arginine. Antigen-specific IFN-γ secretion by CD8+ T cells was analyzed by ELISpot, ELISA and intracellular staining of IFN-γ.

Results: In all ELISpot assays IFN-γ secretion by MART-1 specific T cells was significantly reduced in the absence of arginine. Summarized, IFN-γ specific spots were reduced to 37% ± 13% (p< 0.001) compared to stimulation in the presence of arginine. Furthermore we quantified IFN-γ secretion by ELISA in the supernatant of T cell activation cultures of 3 healthy donors. In all experiments, IFN-γ secretion was significantly reduced in the absence of arginine. Summarizing the 3 experiments, we found a 61 % reduction (p< 0.001) of IFN-γ secretion. Finally, we analyzed MART-1 specific T lymphocyte IFN-γ synthesis by intracellular staining and flow cytometry analysis. In all 5 healthy donors, the absence of arginine decreased the amounts of IFN-γ producing CD8+ T cells. T cell activation in the absence of arginine reduced the fraction of IFN-γ-expressing T cells to 45 ± 18% (p< 0.001) compared to activation in presence of arginine. Furthermore IFN-γ producing T lymphocytes showed a CD8+ CD28+ CD45RA−CCR7+ memory T cell phenotype, representing antigen-experienced T cells after expansion and activation by MART-1 pulsed DC and MART-1 pulsed T2 cells.

Conclusions: Our data clearly demonstrate that antigen-experienced human tumor peptide-specific CD8+ T lymphocytes are significantly impaired regarding their effector function in the context of arginine deprivation. This emphasizes the need for counterregulatory immunotherapeutic strategies to enhance the endogenous or therapeutically-induced anti-tumor immune response.

Disclosure: No conflict of interest disclosed.

P588

Pre-treatment of HLA-E expressing target cells with dasatinib induces inhibition of early but not late natural killer-cell activation events against these target cells

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Introduction: As natural killer (NK) cell immunotherapy against malignancies is still poorly successful, pharmacological approaches seem warranted to enhance NK cell activity. For the SRC/BCR-ABL inhibitor dasatinib an inhibition of NK cells has been demonstrated (Blake et al. 2008, Salih et al. 2010). However, after 24 h pre-treatment of NK cells we found an enhancement of major effector functions against selected target cell lines by dasatinib (Hassold et al. 2009). To understand the mechanisms of this enhancement, we analysed the CD94/NKG2-HLA-E system in early and late NK cell activation events applying the HLA-E+ B lymphoma cell line EBVCC.

Methods: Analysis of drug effects on ex vivo expanded NK cells from healthy human blood donors included: Conjugate formation (0, 20, 60 min), degranulation (CD107a/b), cytokine secretion (IFNγ, TNFα) and cytotoxicity against EBVCC with or without applying a blocking CD94 antibody.

Results: Conjugate formation between NK cells and EBVCC was dose dependently inhibited when dasatinib was present in the assay, whereas 24 h pre-treatment of NK cells, followed by drug wash out, did not reduce conjugate formation. In contrast to K562 and Daudi 24 h pre-treatment of EBVCC led to a significant inhibition of conjugate formation. An enhancement of degranulation and cytokine secretion against EBVCC at 10 nM but an inhibition at 50 nM dasatinib was observed applying the drug in the assay. 24 h NK cell pre-treatment showed the same effect of dose dependent enhancement of degranulation and cytokine secretion as it was observed with K562 and Daudi. Pre-treatment of EBVCC did not alter CD107a/b or cytokine response of NK cells. Interestingly, NK cell cytotoxicity against EBVCC remained unchanged by 24 h pre-treatment of NK or target cells or by drug application in the assay. The same results were obtained when the CD94 receptor was blocked in the kill assay a dasatinib.

Conclusions: Our data show that HLA-E+ targets are sensitive for 24 h pre-treatment with dasatinib regarding early but not late NK cell activation events. For the observed drug effects on late NK activation events the CD94/NKG2-HLA-E system seems not to play a crucial role. As EBVCC are FasR+ expressing cell lines, this argues for a careful application of dasatinib in therapy of lymphoma and leukemia with different NK cell ligand expression features.

Abstracts

P590

Differentiation dependent dendritic cell functions and application in a clinical setting

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Introduction: Dendritic cells (DC) act on the notion of danger coming in the guise of microbial molecules, inflammatory cytokines, or T-cell derived signals. Specialised DC subtypes execute immune stimulatory or suppressive functions. Preliminary evidence suggests that superimposed on DC subtype immune regulation is an additional layer of time kinetic immune regulation: ex vivo monocyte-derived DCs continue to differentiate from an early immune stimulatory into a late suppressive mode of action. This creates problems for using such DCs in cancer immune therapy. Ways to induce suppressive feedback loops may be revealed by an improved understanding of DC differentiation.

Methods: In a first set of experiments we split DC/T-cell co-cultures after 6 hours and re-cultivated the T-cells alone. This allowed us to study T-cells that only received immune stimulatory but no suppressive DC signals. Using genomics methodologies in a second series of experiments, we identified MAPKAP kinase 2 (MK2) as a mediator for switching DCs from the immune stimulatory into the suppressive mode. MK2’s function in DCs was investigated using RNAi to target MK2 in human DCs and a MK2 knockout model. Results: Limiting the DC/T-cell contact to the immune stimulatory DC mode, as well as blocking MK2 in human and murine DCs has significant effects: the proliferative capacity of T-cells is enhanced, the T-cells show a robust type 1 cytokine profile, and mice immunised with MK2 knockout compared to wild type DCs could more effectively kill target cells in vivo.

Conclusion: We confirmed and extended to notion that DCs upon encountering a danger signal execute a differentiation programme that enables them to initially assume an immune stimulatory mode. After one day the DCs switch into an immune suppressive mode. MK2 appears to be critically involved in mediating this transition. When designing DC based cancer vaccine strategies, this differentiation programme needs to be taken into consideration. In an ongoing randomised phase II efficacy trial for the treatment of brain cancer, we expose the DCs for only a few hours to the danger signal lipopolysaccharide enabling DC/T-cell interaction during the immune stimulatory time window of the DC differentiation programme. Clinical observations will deliver information regarding the utility of this concept.

Disclosure: Thomas Felzmann: Employment or Leadership Position: CEO, Trimed Biotech GmbH; Stock Ownership: Trimed Biotech GmbH; Honoraria: IL-12 secreting DCs, US05994126; Genetically Engineered DCs, WO2009074341A1; Expert Testimony: GBM-Vax trial is financed by Trimed Biotech GmbH

Johanna Buchroithner: No conflict of interest disclosed.

P592

The optimisation of a human melanoma-specific single chain T-cell receptor for adoptive T-cell transfer

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Introduction: Adoptive transfer of tumor antigen-specific T-cells is a strategy to induce tumor regression in cancer patients. The transfer of double chain (dc) TCRR into human T-cells contains the risk of forming hybrid dimers with the endogenous TCR chains potentially leading to autoimmunity. To prevent this, a single chain (sc)TCR specific for the Melanoma-derived tumor antigens gp100 and gp100 was constructed. Former analysis showed that functionality of T-cells transduced with the human scTCR/Cα was strongly reduced compared to T-cells equipped with a gp100-specific dcTCR. Functionality was restored when the human constant(c)-domains of the scTCR/Cα construct were replaced by murine c-domains. Since murine elements could lead to inhibition of TCR mediated T-cell activation, we aimed at altering only single amino acids.

Methods: The TCR constructs were transferred into human T-cells or Jurkat cells by retroviral transduction. Expression was determined by β2- and tetramer staining. Cytokine production of transduced T-cells was determined by IFNγ-ELISA, lysis by 51Cr-Release-ASSay.

Results: We and others determined the lysine at position 18 in murine Cβ as a pivotal residue for function. In two recent publications, few murine amino acids being responsible for expression and functionality when introduced into human dcTCRs were identified (Sommermeyer et al., JI 2010; Bialer et al., JI 2010). These amino acids were introduced into the human scTCR/Cα. Compared to the published data, we could only detect a moderate enhancement in expression, cytotoxicity or cytokine production of T-cells bearing the minimal murinised (mm) scTCR/Cα. To improve pairing of the mm scTCR/Cα, cysteine residues resulting in the formation of two disulfide bonds were introduced. One of the disulfide bonds was described by Kuball et al. in Blood 2007. When disulfide bonds were expressed in the mm scTCR/Cα, TCR expression approached the level of the dcTCR. IFNγ-production and lysis of target cells by T cells transduced with the mutant mm scTCR/Cα was comparable.

Disclosure: No conflict of interest disclosed.

P591

Extracorporeal photopheresis constitutes a promising therapy for bronchiolitis obliterans syndrome after lung transplantation

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Introduction: The long-term outcome of patients with end-stage lung disease receiving lung transplantation is unfavourable. A severe and life threatening complication is the manifestation of chronic allograft rejection as bronchiolitis obliterans syndrome (BOS). BOS leads to a progressive decline in pulmonary function with most patients dying of respiratory failure within 5 years of onset. Modern immunosuppressive regimens slow the disease progression down, but the long-term outcome of patients with end-stage lung disease remains poor. ECP therapy is a promising therapeutic option in patients with BOS after lung transplantation.

Methods: Here we report on a 33-year-old male patient with severe hereditary pulmonary fibrosis developing a BOS 42 months after double-lung transplantation. Next to a triple drug therapy with steroids, a calcineurin inhibitor and a cell-cycle inhibitor he received two steroid bolii with progressive decline in lung function. Therefore he was transferred to our unit for photopheresis therapy with BOS grade 3 with a vital capacity (VC) of 5,100 ml (BOS grade 1: VC of 5,940 ml) and a forced expiratory volume in one second (FEV1) of 2,120 ml (BOS grade 1: FEV1 of 4,930 ml). After Vortex™ port implantation he was treated with ECP twice weekly for 8 weeks using the THERAKOS™ UNARIP®XTS™ Photopheresis System. The intensive ECP treatment schedule was changed to twice weekly every second week for 10 weeks, thereafter twice weekly every third week for 9 weeks and then extended to twice weekly every month.

Results: The intensive ECP treatment was well tolerated by the patient. Furthermore, the spirometry 12 weeks after begin of ECP treatment showed no further decline of lung function with a VC of 5,070 ml and a FEV1 of 2,160 ml. Moreover, the lung function fortunately improved to a VC of 5,360 ml and to a FEV1 of 2,410 ml 27 weeks after begin of the ECP treatment. No severe infectious disease complication occurred during the ECP therapy.

Conclusion: ECP is well tolerated by the patient. Furthermore, due to ECP therapy the immunosuppressive regimen associated with toxicity and an increased risk of infectious diseases can be reduced. Moreover, intensive ECP therapy might not only reduce the rate of decline in lung function but even more might improve the lung function. EPC therapy is a promising therapeutic option in patients with BOS after lung transplantation.

Disclosure: No conflict of interest disclosed.
to the dTCR even when only one disulfide bond was combined with minimal
mismatch.

Conclusion: We created a human mutant scTCR/Cα with eminent functional-
ity through introduction of defined murine amino acids and disulfide bonds.
This construct might be a promising candidate for a safe and efficient adoptive
T-cell transfer due to minimising the risk of mispairing with endogenous TCR
chains and providing a substantial antitumor effect.

Disclosure: No conflict of interest disclosed.

P594
Inhibition of tumor angiogenesis by antibody-directed prodrug activation

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Introduction: Angiogenesis is a prerequisite for the continuous progression
of solid tumors due to the indispensable energy requirement of cancer cells.
The extra domain B (ED-B) of fibronectin is a well-known marker for angi-
genesis since it is abundantly expressed around novel blood vessels. Therefore,
ED-B is an appropriate target for tumor therapy as – with the exception of
uterus and ovaries – is not expressed in adult humans.

Antibody-directed enzyme prodrug therapy (ADEPT) is based on a fusion
protein containing a prodrug-converting enzyme and a targeting molecule.
The fusion protein is capable of binding to specific target structures on the cell
surface of cancer cells and converting a non-toxic prodrug into a cytotoxic
drug selectively inside the tumor.

Methods and Results: We developed a fusion protein of the single chain Fv
L19 with yeast cytosine deaminase (CDy) referred to as L19CDy, which
yielded a potential targeting protein for ADEPT. L19 – as the targeting com-
ponent – specifically binds to both purified ED-B expressed in E. coli and
ED-B localized in the extracellular matrix of fibroinectin-expressing F9 terato-
carcinoma cells as demonstrated by enzyme-linked immunosorbent assays.
The catalytic activity of CDy in the fusion protein was confirmed by conver-
sion of the non-toxic prodrug 5-fluorocytosine (5-FC) into 5-fluorouracil
(5-FU) by quantifying the change in absorbance at 255 nm.

Conclusion: 5-FU is an approved cytostatic drug used in the therapy of a wide
range of solid tumors, including breast, gastric and colorectal cancer. Applying
the ADEPT approach in vitro, we could demonstrate that the combined use of
L19CDy and 5-FC reduces the growth of F9 teratocarcinoma cells in a dose-
dependent manner similar to directly applied 5-FU. Our results show that
ED-B is a potential target for tumor therapy, and L19CDy a promising fusion
protein to achieve this.

Disclosure: No conflict of interest disclosed.
TCRs. In contrast, mice receiving opt.cys.sc-p53TCR-transduced T cells survived without developing any sign of GvHD. Our data show that p53TCR gene transfer-induced off-target autoimmunity observed in humanized p53-deficient mouse models could be prevented by the use of an engineered optimized single chain p53-specific TCR and may represent a new and safe approach for TCR-based gene therapy of p53-associated malignancies.

Disclosure: No conflict of interest disclosed.

P596
Recombinant dimeric IgA antibodies recruit different effector cell populations for effective future tumor cell killing

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Introduction: Dimeric IgA antibodies contribute significantly to the humoral part of the mucosal immune system by maintaining the homeostasis of epithelia in different modes of action. Dimeric IgA antibodies are tetravalent molecules concerning antigen and Fc-receptor binding properties and therefore excellent in neutralizing pathogens. However, their potential as immunotherapeutic agent has hardly been explored. Here, we describe the production, purification and functional evaluation of recombinant dimeric IgA, exemplarily directed against the epidermal growth factor receptor (EGFR).

Methods: Human J-chain-containing IgA was produced under serum-free conditions by transfecting non-adherent CHO-K1 cells expressing EGFR-specific antibody (anti-human-EGFR) and anti-His-tag (and one size exclusion chromatography were combined, resulting in a homogenous preparation of highly pure IgA dimers, as determined by gel electrophoresis under denaturing and native conditions.

Results: The functional studies demonstrated dIgA to be at least as effective as mIgA in triggering antibody-dependent cellular cytotoxicity of A431 tumor cells by isolated monocytes, monocyte-derived macrophages, PMN and in human whole blood. Chromium-release-assays. Importantly, dimeric IgA was more effective in F(ab)-mediated mechanisms: inhibition of binding of FITC-labelled EGF to A431 cells by dIgA was achieved at significantly lower concentrations than by mIgA. In addition, growth of EGFR-expressing DiFi colon carcinoma cells was inhibited at significantly lower concentrations by dimeric than by monomeric IgA. Both IgA isoforms were similarly effective in triggering apoptosis of DiFi cells. Furthermore, only dimeric but not monomeric IgA or IgG was directionally transported by the polymeric immunoglobulin receptor (pIgR) through an epithelial cell monolayer by an experimental transcytosis assay with polarized human Calu3 cells.

Conclusions: Together, these studies demonstrate that recombinant dimeric IgA antibodies recruit a distinct repertoire of effector functions compared to monomeric IgA with a vector coding for the His-tagged human J-chain.

Disclosure: No conflict of interest disclosed.

P597
Isoagglutinin apheresis for ABO-incompatible renal transplantation

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Blood group antigens of the ABO-type are expressed with broad tissue distribution, including endothelial, epithelial and red blood cells. This has traditionally restricted solid organ transplantation to major ABO compatibility. We therefore asked, if this barrier could be overcome by isoagglutinin apheresis before transplantation.

In four patients on dialysis with ABO incompatible living related donors, apheresis was performed with Cobe Spectra and Aasorub using Glycoscorb columns. In addition, patients received Rituximab (Anti-CD20) for B cell depletion before transplantation. Isoagglutinin titer were determined at baseline, before and after apheresis, and after transplantation. The isoagglutinin titers at baseline were 8 to 128 (IgG), and 1 to 32 (IgM). Apheresis resulted in a median reduction of 3 titer steps, and 5 (median) procedures were required prior to transplantation. In all patients titers could be reduced to 4 or less before transplantation, and all grafts could be transplanted successfully with excellent function. Isoagglutinin titers remained low (4 or less) after transplantation. ABO-incompatible living donor renal transplantation may be facilitated by prior isoagglutinin apheresis.

Disclosure: No conflict of interest disclosed.

Posterdiskussion
Tumor-Zellbiologie

P598
A microcapillary flow cytometry based assay to assess antibody dependent complement activation

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The mediation of complement dependent cytotoxicity (CDC) is a crucial effector mechanism for several therapeutic antibodies like rituximab or ofatumumab and the assessment of CDC is of high interest for immunological research and drug discovery. Established CDC assays like chromium release or alamar blue are lacking specificity because the results are abstract and always related to artificial killing controls. Here we describe a microcapillary flow cytometry based CDC assay which is easy to perform with low hands on. Due to single cell analysis the exact number of complement destroyed cells can be determined. It can be applied to several haematological cancer cell lines as well as primary B-cells which are freshly obtained from healthy volunteers. Experiments have been validated with the commercially available antibody rituximab. Compared to other CDC assays results were consistent. In summary flow cytometry is a promising addition to common CDC assays with enhanced informative value and increased specificity.

Disclosure: No conflict of interest disclosed.

P599
The Src family kinase inhibitors PP2 and PP1 effectively block TGF-beta1-induced cell migration and invasion in both established and primary carcinoma cells

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TGF-beta1 belongs to the TGF-beta superfamily of growth and differentiation factors and regulates a wide array of cellular processes. It can also promote late stage carcinoma progression through enhancing angiogenesis, cell migration and invasion, and metastasis. Hence, inhibiting TGF-beta signalling through the use of small molecule inhibitors that target the TGF-beta type I receptor (TβRI/ALK5) appears to be a feasible therapeutic approach. We have demonstrated previously that, unexpectedly, the common Src family kinase inhibitors PP2 and PP1 effectively inhibited in ductal pancreatic adenocarcinoma (PDAC) cells both ALK5 kinase activity and various TGF-beta1-related signaling pathways in primary and established carcinoma cells. Here we demonstrate that the Src family kinase inhibitors PP1 and PP2 are equally effective in inhibiting TGF-beta1-induced cell migration and invasion in both established and primary carcinoma cells.

Disclosure: No conflict of interest disclosed.
Disclosure: No conflict of interest disclosed.

P600
Controversial discussion on the use of epigenetically active phytochemicals in tumour therapy

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Epigenetics deals with stable changes in the expression of genes without changing the genomic sequence itself but with structural modification of the DNA like histone-acetylation or DNA-methylation. Pathological changes of the epigenome are often found in cancer, e.g. hypermethylation of genes is found more frequently in tumours than genetic mutations. Epigenetically active substances like 5-Azacitidine or Suberoylanilide hydroxamic acid are approved for treatment of haematological malignancies and are currently tested for therapy of solid tumours.

The anticancerogenic properties of phytochemicals like curcumin or epigallocatechin-3-gallate (EGCG) were proved in numerous publications and build the basis of “traditional medicine” in countries like India and China. Although they show a comparable low bioavailability the broad spectra of beneficial effects as well as the low toxicity make them attractive therapeutics.

Phytochemicals show chemopreventive qualities which are mainly based on their anti-inflammatory, antibiotic and epigenetic actions. Presently substances like resveratrol, quercetin, EGCG and curcumin are tested for their epigenetic activities: Curcumin for example inhibits the histone acetyltransferase p300, due to this feature the water-soluble derivative hydrazinocurcumin reduces the growth of oral tumours in an orthotopic mouse model substantially. In a similar model was shown that curcumin sensitizes human colorectal cancer to capcitabine which was more efficient in tumour volume reduction than chemotherapy alone.

In the treatment of breast cancer the restoration of the estrogen receptor plays a crucial role. Coadministration of trichostatin A (TSA) with EGCG results in a 50fold higher expression of estrogen receptor alpha in MDA-MB-231 cells thereby doubling the effect of TSA alone. Are these findings the base for the usage of phytochemicals in tumour therapy? Is a combinational therapy with conventional chemo- and radiation therapy the answer to resistant tumours and drug intolerance? Are there possible contrindications for the therapeutic use of phytochemicals?

Next to these important questions, topical scientific publications on the epigenetic modulation through phytochemicals as well as current clinical studies on the most common substances will be discussed.

Disclosure: No conflict of interest disclosed.

P601
Epithelial-mesenchymal transition of small cell lung carcinoma (SCLC)

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Small cell lung carcinomas (SCLC) are highly aggressive, invasive and early metastasizing tumors. The process of metastasis is going along with phenotypical changes. Cells have to detach from the primary tumor, invade the surrounding stroma, enter the circulation and escape the detection of the immune system until reaching metastatic sites. Epithelial-mesenchymal transition (EMT) processes, initially known from embryonic development, have recently been described to play an important role for these processes. EMT is going along with phenotypical changes and disabling cell-cell connections that result in a transition from a more epithelial phenotype to a more mesenchymal-like appearance. It is described that the reverse process mesenchymal-epithelial transition (MET) happens at the site of metastasis, and it is postulated that these processes are linked to radio- and chemoresistance. There is very little knowledge on EMT processes and its impact on metastasis in SCLC.

SCLC cell lines NCI-H69, NCI-H82, NCI-N592 are usually forming floating cell clusters when cultured in RPMI 10%FCS, only very few cells are growing adherent to tissue culture flask (3-7%). FACS analysis shows different subpopulations of size and density within a cell line. We started to analyze the phenotypical morphology of the cell lines NCI-H69, NCI-H82, NCI-N592. The addition of low concentrations of BrdU to the culture is inducing a phenotypical change to mainly adherent growing cells. These changes are accompanied by changes of typical EMT markers on gene and protein levels.

For the induction of phenotypical changes 10µM BrdU was added to the culture medium for 14-21 days. Medium was changed every second day by centrifuging and resuspension. Once the majority of cells appear adherent BrdU was not added anymore. The cells remain adherent and show normal growth patterns. We show that the phenotypical changes go along with changes in membrane structures like Tight Junctions, Desmosome, Gap Junctions, Vimentin and Cadherins. Also mesenchymal markers like SNAI1 are downregulated in adherent cells, whereas epithelial markers are upregulated. We are postulating that SCLC cell lines are growing in a mesenchymal-like state when cultured in RPMI and treatment with BrdU is inducing mesenchymal-epithelial transition in all three investigated cell lines.

Disclosure: No conflict of interest disclosed.

P602
Peroxisome proliferator-activated receptor (PPAR) δ agonists induce endothelial ICAM-1 expression

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It is a known fact, that endothelial cells isolated from human tumors express much lower levels of adhesion molecules, that are involved in leukocyte vessel wall interactions, such as intercellular adhesion molecule-1 (ICAM-1). These mechanisms seem to be evolved by tumors to escape immunosurveillance. Therefore, the development of angiogenesis inhibitors that can make the tumor additionally more vulnerable for the immune system might be a new approach for the treatment of cancer. Peroxisome proliferator-activated receptor (PPAR) δ agonists display a variety of effects on pro- and antitumor processes.

Recently, we could demonstrate, that PPARδ agonists induce pro-inflammatory cytokines in human endothelial cells and inhibit endothelial cell proliferation and angiogenesis. We now hypothesized that PPARδ agonists might also enhance the expression of ICAM-1 which would be an important prerequisite for tumor specific leukocites to reach the tumor cells through the tumor vasculature.

We found that treatment with PPARδ agonists induced endothelial ICAM-1 protein expression in a time- and concentration-dependent manner. The expression of soluble ICAM-1 was not significantly affected by PPARδ ago-
nrist treatment. We also demonstrated that PPARγ agonists significantly induced accumulation of ICAM-1 mRNA. The treatment considerably induced transcriptional activity of 5′-deleted ICAM-1 promoter gene constructs. PPARγ agonist-mediated induction was conveyed by a GC-rich region, harboring one consensus Sp1 binding site. EMSA analysis demonstrated that constitutive Sp1-dependent DNA binding is increased by PPARγ activation. Hence, the induction of ICAM-1 expression might represent a critical molecular mechanism which might be essential for a pro-immunogenic and therefore anti-tumorigenic effect of PPARγ agonists.

Disclosure: No conflict of interest disclosed.

P603
Epigenetic regulation of cellular adhesion in cancer

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The aim of this presentation is to review and combine recent data about epigenetic modifications in cancer and the influence of changes in cellular adhesion molecules on the behavior of cancer cells to show that epigenetic regulation of adhesion is one of the key factors of carcinogenesis. The increasing knowledge in the field of epigenetics reveals its major role in many physiologic processes but also in some diseases such as cancer. Recent data have shown that the complexity of carcinogenesis cannot longer be referred only to mutations but must involve epigenetic mechanisms. These epigenetic alterations can cause permanent changes in gene expression patterns and thus essentially contribute to characteristics of cancer cells like loss of growth control, altered intercellular communication and enhanced motility. If normal cells loose their constitutive cell-cell-connections or cell-matrix-connections they go into apoptosis. The cadherins are among the most important and best-examined adhesion molecules and there have been described changes in adhesion dependency as a hallmark mechanism which might be essential for a pro-immunogenic and therefore anti-tumorigenic effect of PPARγ agonists.

Disclosure: No conflict of interest disclosed.

P604
High EGFR and Aurora A expression defines a poor risk group in SCCHN patients – efficacy of a combined targeted treatment

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Squamous cell cancer of the head and neck (SCCHN) is the sixth leading cause for cancer death worldwide. Despite extensive knowledge of risk factors and pathogenesis about 50 percent of all patients and essentially every patient with metastatic disease finally succumb to SCCHN. Addition of the monoclonal anti-EGFR antibody cetuximab to the standard first-line regimen cisplatin/5-fluorouracil increased response rate and improved progression free and overall survival in patients with recurrent or metastatic SCCHN. There is however pressing need for better targeted treatment approaches. Next to epidermal growth factor receptor (EGFR), high Aurora kinase A (Aurka) mRNA expression has been identified as adverse prognostic factor in SCCHN. We analyzed clinical data and performed immunohistochemistry for EGFR and Aurka expression in 180 SCCHN patients. Patients characterized by elevated EGFR and elevated Aurka protein expression in tumor tissue represent a risk group with poor survival (Fig. 1, EGFRhi Aurka hi vs EGFRlo Aurka low, p=0.024). Next, SCCHN cell lines were assessed for effects of single and combined Aurk and EGFR inhibition. Small molecule inhibitors were used for Aurk inhibition, and cetuximab was applied for EGFR targeting. Treating SCCHN cell lines with a pan-Aurk inhibitor results in defective cytokinesis, polyploidy and apoptosis, which is effective irrespective of the EGFR status. Combined Aurk-EGFR targeting significantly adds to single EGFR and Aurk inhibition effects. Comparing Pan-Aurk and specific Aurka targeting hints towards a strong and clinically relevant biological effect mediated via Aurk inhibition.

Our findings characterize a new poor risk group in SCCHN patients defined by elevated EGFR and Aurka protein expression. We demonstrate that combined targeting of EGFR and Aurk represents a therapeutic means to activate cell cycle checkpoints and apoptosis in SCCHN.

Disclosure: No conflict of interest disclosed.

Fig. 1.
Abstracts

P605
SIRT3 steps out of the shadow of SIRT1 – functional analyses and its role as novel tumor marker and therapeutic target
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Introduction: Sirtuins are critical players within multiple cellular pathways such as stress response, apoptosis and energy metabolism and are associated with metabolic diseases and cancer pathogenesis. They are key elements in the regulation of cellular life span, strength and form within the 7 human sirtuins. SIRT3, which mimics effects of caloric restriction, is the only one with correlations to longevity. Further more we and others found over expression of SIRT3 in tumors (e.g. AML cells), which correlates with a better prognosis. In addition we identified two non-synonymous SIRT3 SNPs and evaluated their functional impact.

Methods: Genomic DNA from 640 healthy Caucasian donors was extracted from peripheral blood by salting out. In silico analyses were performed with the dbSNP database. Allelic discrimination was performed with allele specific TaqMan genotyping assays. The impact of single over expressed sirtuins on cellular metabolism was monitored subsequent to transient transfection of HeLa cells via real-time in vitro monitoring of glycolysis and respiration with the Biosan biosensor chip system. SIRT3 constructs were immunoprecipitated and used for in vitro dectylation assays. Protein synthesis was stopped with cycloheximide 24 hours after transfection and protein samples were analyzed at different time points by Western blotting.

Results: Our analyses indicate that SIRT3 is a rather stable protein, with a half-life of over 48 h and that SIRT3 has only two non-synonymous SNPs, which however seem to have no functional impact. Furthermore, we show in living cells that SIRT3 has strong activating effects on cellular respiration (80 %) and that SIRT3 is over expressed in all AML subtypes.

Conclusion: Our results further prompt the emerging role of SIRT3 in the context of cell survival, metabolism and cancer and reveal that the impressive functions of SIR2 in model organisms can only be assessed in humans in different situations considering all or at least the most important sirtuins, under which SIRT1 and SIRT3 are the most important candidates. In this context and with regard to promising results of HDAC-inhibitor treatment in leukaemia, the significant SIRT3 over expression in tumors further indicates its role as novel diagnostic marker and therapeutic target in cancer therapy.

Disclosure: No conflict of interest disclosed.

P606
RIG-I is a dual activator of Card9 and inflammasome signaling for IL-1beta production upon RNA virus recognition
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Background: Viruses can cause systemic life-threatening diseases in immunocompetent and immunocompromised individuals and host protection against viruses depends on intact innate and adaptive immune responses. Antiviral immunity is initiated by germine encoded pattern recognition receptors (PRRs) that recognize viral pathogen-associated molecular patterns (PAMPs) such as nucleic acids. For their replication process, viruses require the cytosolic compartment and specific PRRs take advantage of this dependence to initiate signaling pathways that trigger activation of NF-κB and interferon regulatory factor (IRFs) for the induction of proinflammatory cytokines and Type I Interferons (Typ I IFN). In addition, Interleukin 1 beta (IL-1β) is a potent proinflammatory factor that is released during viral infection. Its production is tightly controlled by NF-κB dependent transcription of Il1b and subsequent processing of pro-IL-1β by an inflammasome. However, the sensors and mechanisms that facilitate RNA virus-induced production of IL-1β are not well defined. Here we report a dual role for the RNA helicase RIG-I in RNA virus-induced proinflammatory responses.

Methods: Human PBMCs and bone-marrow-derived dendritic cells (BMDC) were isolated and grown as described and incubated for 6-8 hours with 2 μg/ml-1 of synthetic RNA, 3pRNA or double-stranded DNA (poly(dA:dT)) or the indicated viruses. Cell supernatants were analyzed for cytokine secretion and caspase-1 processing by ELISA and Western blot.

Results: Whereas RIG-I-mediated activation of NF-κB required the signaling adaptor MAVS and a complex of the adaptors CARD9 and Bcl-10, RIG-I also bound to the adaptor ASC to trigger caspase-1-dependent inflammasome activation by a mechanism independent of MAVS, CARD9 and the nod-like receptor protein NLRP3. Our results identify the CARD9-Bcl-10 module as an essential component of the RIG-I-dependent proinflammatory response and establish RIG-I as a sensor able to activate the inflammasome in response to certain RNA viruses.

Conclusions: As a selective activation of inflammatory responses by RIG-I may form a crucial link to the development of adaptive immunity, future research on RIG-I-induced inflammasome activation may have clinical implications for the modulation of IL-1β responses during acute infection as well as for the development of future vaccines.

Disclosure: No conflict of interest disclosed.

P607
Firstline-therapie mit Carboplatin/Vinorelbine/Bevacizumab für metastasiertes adrenocortical carcinoma
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Introduction: Adrenocortical carcinoma (ACC) is a rare tumor entity (Incidence 2 /1.000.000 / year). Surgery is the therapy of choice for primary, localized tumors. The ideal strategy to treat tumor recurrence or metastasized disease remains to be defined. Here we report a case of combined chemotherapy (CTX), and antibody therapy (AB) in a patient with tumor recurrence.

Case-Report: In 02/2009 the previously healthy 50 year female sought medical attendance in a different hospital, because pain in the upper abdomen. Ultrasound revealed a tumor of the right adrenal gland 7 cm in diameter. Further staging (A-CT, T-CT, MRI) showed no evidence of metastasis. Results of routine lab-testing, adrenal hormonal status, blood pressure holter monitoring was unremarkable. Histological classification following complete tumorexstition was pT2N0M0. Thus an adjuvant therapy was not recommended. In 11/2009 the patient was without complaint, and referred to our hospital for restaging. CT-scans of the abdomen and thorax showed a local tumor recurrence, as well as left sided lungmetastasis (segment 2, 3, and 6). Surprisingly extra-anatomic wedge-ressection revealed a primary multifocal NSCLC (adenocarcinoma, pT4pN0pM0,G2, Stadium IIIA). Immunohistochemistry excluded the possibility of an ACC metastasis.

In 12/2009 tumor debulking, partial peritoneal excision, as well as right sided nephrectomy was performed. Histology confirmed the diagnosis of local ACC recurrence. In 11/2009 the patient was without complaint, and referred to our hospital for restaging. CT-scans of the abdomen and thorax showed a local tumor recurrence, as well as left sided lungmetastasis (segment 2, 3, and 6). Surprisingly extra-anatomic wedge-ressection revealed a primary multifocal NSCLC (adenocarcinoma, pT4pN0pM0,G2, Stadium IIIA). Immunohistochemistry excluded the possibility of an ACC metastasis.

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revealed no signs of tumor recurrence (01/2011). No serious side effects have been reported by the patient.

Conclusions: The therapeutic strategy for metastasized ACC should be multi-modal. Following tumor debulking a combined CTX/AB therapy with Carboplatin, Vinorelbine, and Bevacizumab is safe and effective approach. Further randomized controlled trials are indicated. Because of the low incidence a multicentered therapeutic protocol with Carboplatin, Vinorelbine and Bevacizumab should be preferred.


P608 Sorafenib in older patients with advanced renal cell carcinoma: subanalysis by age of an integrated database of 8 company-sponsored clinical trials


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Introduction: Sorafenib (SOR) was effective and well tolerated in a broad range of patients (pts) with renal cell carcinoma (RCC) in 1 phase III trial and 2 expanded-access studies. Senior pts, a large proportion of pts with RCC, are included in 2 expanded-access studies. Age is an important strategy in maintaining patients on sorafenib therapy.

Methods: The SorRCCID was created using individual pt data from 6 clinical trials and 2 expanded-access studies evaluating Sor monotherapy in RCC. National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 terms were used for the selected drug-related adverse events (DRAEs).

Results: 4684 pts (71% male; median age 62 years and 41% ≥ 65 years of age; 87% prior nephrectomy; 52% prior cytokine therapy) were included in SorRCCID. The mean daily dose was 676 mg for the total population, 656 mg for pts who received Sor ≥12 mg, 660 mg for pts ≥65 to < 75 years, and 650 mg for pts >75 years. 0/1/2/3/4 dosage reductions or interruptions due to an AE occurred in 57%/22%/11%/5%/5% of pts in the total population (n=4684), in 41%/21%/13%/10%/15% of pts who received Sor ≥12 mg (n=707), in 53%/24%/13%/4%/6% of pts ≥65 years (n=1941). 1738/4001 pts (38%) had a dosage reduction or interruption from 800 mg/d due to an AE, and of these, 1488/86% resumed or re-escalated the dosage; 907 pts (52%) were re-escalated to the full dosage.

Conclusions: The SorRCCID describes the treatment patterns of the largest number of patients with advanced RCC to date. Sorafenib was well tolerated at a range of doses in this diverse patient population. Most patients in the database received standard doses of sorafenib, and 57% of patients did not have a dose modification. These dosage patterns were similar in patients ≥ 65 years to that observed in the entire study population. Patients who received sorafenib ≥ 12 months tended to have more dose adjustments than the overall population. The dose reescalation by 86% of patients who had a DRAE-associated dose reduction or interruption is notable. Most patients were reescalated in < 2 weeks. Dose modifications due to DRAEs tended to occur in the first 3 months of therapy. DRAEs that occurred after that time did not lead to dose modifications as frequently. These findings indicate dose adjustment is an important strategy in maintaining patients on sorafenib therapy.


P609 Sorafenib (SOR) dosage patterns in >4600 patients (pts) with renal cell carcinoma (RCC), including the elderly and pts treated for >12 months (mo): Results from an integrated database of 8 company-sponsored trials


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Introduction: SOR has been effective and well tolerated in clinical trials and community settings for the treatment of advanced RCC. The Sor-RCC Integrated Database (SorRCCID) was constructed from data from 8 company-sponsored clinical trials with Sor monotherapy for RCC. Here we investigate dosage patterns in the SorRCCID.

Methods: SorRCCID was created using individual pt data from 6 clinical trials and 2 expanded-access studies evaluating Sor monotherapy in RCC. National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 terms were used for the selected drug-related adverse events (DRAEs).

Results: 4684 pts (71% male; median age 62 years and 41% ≥ 65 years of age; 87% prior nephrectomy; 52% prior cytokine therapy) were included in SorRCCID. The mean daily dose was 676 mg for the total population, 656 mg for pts who received Sor ≥12 mg, 660 mg for pts ≥65 to < 75 years, and 650 mg for pts >75 years. 0/1/2/3/4 dosage reductions or interruptions due to an AE occurred in 57%/22%/11%/5%/5% of pts in the total population (n=4684), in 41%/21%/13%/10%/15% of pts who received Sor ≥12 mg (n=707), in 53%/24%/13%/4%/6% of pts ≥65 years (n=1941). 1738/4001 pts (38%) had a dosage reduction or interruption from 800 mg/d due to an AE, and of these, 1488/86% resumed or re-escalated the dosage; 907 pts (52%) were re-escalated to the full dosage.

Conclusions: The SorRCCID describes the treatment patterns of the largest number of patients with advanced RCC to date. Sorafenib was well tolerated at a range of doses in this diverse patient population. Most patients in the database received standard doses of sorafenib, and 57% of patients did not have a dose modification. These dosage patterns were similar in patients ≥ 65 years to that observed in the entire study population. Patients who received sorafenib ≥ 12 months tended to have more dose adjustments than the overall population. The dose reescalation by 86% of patients who had a DRAE-associated dose reduction or interruption is notable. Most patients were reescalated in < 2 weeks. Dose modifications due to DRAEs tended to occur in the first 3 months of therapy. DRAEs that occurred after that time did not lead to dose modifications as frequently. These findings indicate dose adjustment is an important strategy in maintaining patients on sorafenib therapy.

P610
Results of a pre-planned interim analysis of a non-interventional study of everolimus after failure of the first anti-VEGF therapy in metastatic renal cell carcinoma

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Introduction: Everolimus (EVE) is approved and recommended for the treatment of metastatic renal cell carcinoma (mRCC) after failure of the first anti-VEGF therapy. The option to treat mRCC with six targeted agents has fostered the need for data beyond clinical trials to determine optimal sequencing of these agents. Here, we report prospective non-interventional data on EVE in routine use after failure of the first anti-VEGF therapy (one TKI or bevacizumab).

Methods: A prospective, multi-center non-interventional study for patients with mRCC was initiated in Germany in 08/2009 to evaluate EVE after the first anti-VEGF therapy in routine clinical use. Accrual is still ongoing. Safety and efficacy (time from first EVE intake to progression due to any cause [TPP] of EVE were analyzed in a pre-planned interim analysis after 100 patients had been enrolled.

Results: At data cut-off, 113 patients were enrolled at 59 German sites and had been followed for a median time of 116 days. 92% of patients exhibited clear-cell mRCC. The MSKCC risk score of patients assessed before first-line treatment was mostly intermediate (55%), followed by favorable (38%) and poor (9%). Median time to progression on EVE was 9.7 months (95% CI: 6 months; n.d.). The median time of treatment was not reached because the majority of patients was still on treatment at the time of analysis. 230 AEs occurred in 61% of patients and lasted an average of 48 days. These included 18 serious drug reactions in 12 patients (12%). Most common AEs were anemia, pruritus, fatigue, dyspepsia and diarrhea of any grade (>5% incidence each). 80% of patients did not require dose adjustment during the study. 9 patients required a median treatment interruption of 15 days. Median Karnofsky performance score (KPS) at study start was 80% and remained ≥80% across all visits. 80% of treating physicians attested EVE favorable tolerability, coinciding with high therapy compliance of patients as assessed by physicians and patients' diaries (>80%).

Conclusions: This non-interventional study confirms the safety profile of EVE in the treatment of mRCC after prior failure of anti-VEGF therapy originally reported in the pivotal trial. The results furthermore provide promising data on the efficacy of EVE in routine use after failure of the first anti-VEGF therapy.


Abstracts

Onkologie 2011;34(suppl 6):1–305

P611
Compassionate use of Lenalidomide with Pioglitazone, Etoricoxib, Dexamethasone and low-dose Treosulfan: Combined anti-inflammatory, immunomodulatory and angiostatic treatment in patients (pts) with docetaxel-refractory, castration-resistant prostate cancer (CRPC)

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Introduction: Therapeutic options for pts with docetaxel-refractory CRPC are still limited. Continuous production and release of pro-inflammatory cytokines may account for its resistance towards cytotoxic drugs. Therefore, a phase II study was implemented in pts with CRPC to assess tumor response to combined biomodulatory agents. Primary objective was the effect of this treatment approach on PSA-response rate in pts with CRPC.

Methods: 12 pts with histologically confirmed docetaxel-refractory CRPC (criteria according to EAU guidelines) were treated continuously with daily doses of lenalidomide 5 or 10 mg, pioglitazone 60 mg, etoricoxib 60 mg, dexamethasone 0.5 mg and treosulfan 250 mg twice daily until PSA progression. During the study, PSA-values, ECOG performance status were continuously assessed. Pts responsive to study medication were allowed to continue therapy until disease progression or intolerable toxicity occurs.

Results: The last patient started with combined therapy approach in January 2011. Six pts are currently under treatment. At baseline the median PSA value was 202.7 ng/ml (92 – 2413 ng/ml) and all patients doubled PSA during docetaxel therapy. 12 patients (50.0%) were considered as PSA responders with a confirmed PSA decline of at least 50%. During the treatment period PSA decreased from 324.7 (± 853.1) to 10.8 (±17.6) ng/ml. Median time to PSA response and to progression as well as overall survival were not yet achieved. Of the 6 non-responders, 2 pts showed a stable disease ≥ 4 months.

Conclusions: We evaluated a new multi-targeted approach for pts with docetaxel-refractory CRPC. Although the substances show limited efficacy in single therapeutic use, this multi-targeted approach led to an impressive response rate of 50.0%. In addition the comparatively low and manageable toxicity might be an advantage compared to present treatment regimens.

Disclosure: No conflict of interest disclosed.

P612
Quantitative analysis of PTEN-dependent glycoprotein patterns reveals predictive biomarker signature for response of human patients to docetaxel therapy in metastatic castration resistant prostate cancer (mCRPC)

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Background: Since 2004 chemotherapy with docetaxel has been the standard therapy in progressive mCRPC. Unfortunately, only a subgroup of patients responds to this treatment. As rebioxy is rarely done in mCRPC, predictive serum biomarkers for therapy response would be of great value. We recently presented a novel platform for human biomarker discovery and validation based on a large-scale quantitative analysis of N-linked glycoproteins of the Phosphatase and Tensin homolog (PTEN) conditional knockout mouse model for prostate cancer progression. This work delivered biomarker signatures for PTEN-status, Gleason sum and diagnosis in localized prostate cancer (Cima et al., PNAS 2011). This model has also revealed a prognostic biomarker signature in patients with mCRPC (manuscript submitted).

We screened our biomarker set for factors response to treatment with docetaxel in mCRPC patients
seemed a reasonable step towards the vision of a personalized cancer medi-
cine.

**Methods:** In serum samples from 40 patients with mCRPC who underwent chemotherapy with docetaxel we measured 13 proteins with ELISA and 66 different proteins by selected reaction monitoring (SRM) mass spectrometry. Random forest algorithm was applied to establish a multifactor signature pre-
dictive for response. Therapy response was defined as at least stable disease/biochemically (PSA increase < 25% over baseline) and by imaging after three cycles of therapy with docetaxel.

**Results:** Serum samples of 40 patients with mCRPC under chemotherapy with docetaxel were retrospectively analyzed. We identified four factors correlating significantly (p < 0.05) with therapy response in a univariate analysis. Additionally we performed a random forest analysis identifying combined predictive biomarker signatures. Intriguingly the serum concentration of two identified factors in combination significantly predicted whether patients with mCRPC responded to taxane therapy or not with an accuracy of 85% in a confusion matrix.

**Conclusions:** Our recently presented biomarker-platform derived from a Pten knockout mouse model showed high feasibility for the identifica-
tion of predictive markers for therapy response to docetaxel chemotherapy in human patients with mCRPC. The analysis of the biomarker signature combi-
nation of these candidate biomarkers therefore warrants further investiga-
ion in a bigger collective of patients.

**Disclosure:** Martin Kälin: No conflict of interest disclosed.

Silke Gillessen: Honoraria: Patent application pending.

**P613**

**Mutations in the VHL tumor suppressor gene as prognostic factor for sporadic renal-cell carcinomas?**

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**Introduction:** Recently, drugs targeting the pVHL/HIF pathway have been shown to be effective for the treatment of sporadic clear-cell renal-cell carci-
nomas (cRCC). VHL inactivation has been found in more than 80% of ccRCC, provided the tumors have been studied comprehensively. Therefore, it is reasonable to argue that VHL alteration might be a prognostic or even pre-
dictive factor and could be applied as a biomarker in ccRCC. We previously described that VHL mutation or promoter hypermethylation are associated with advanced tumor stage.

**Methods:** We improved our detection rate of VHL alterations significantly by introducing new molecular genetic detection techniques, including whole gene sequencing. MLPA (Multiplex Ligation-dependent Probe Amplification), promoter methylation analysis and 3 p loss of heterozygosity studies. We extended out initial study also by the number of cases studied and the observa-
tion time.

**Results:** We included 129 cases of RCC being treated from 1992 – 1999 at the Department of Urology of the University with a follow-up time of 1 12 years. Genotype-phenotype association and survival curves demonstrate the prognostic importance of VHL inactivation mutation for ccRCC.

**Conclusions:** Since publication of our initial data, there have been at least 11 other studies on alteration status of VHL, some of which support the prognosis hypothesis, some do not. There are several reasons for this inconsistency, such as the numbers of cases studied, a limited observation time or the different detection methods applied. Here we present new data that extend our initial study by improving the methodology, i.e. applying the actually most compre-
hensive analysis techniques, also increasing the number of cases studied and the observation period. Our findings show the importance of VHL mutation status as a new molecular biomarker, as well as the limitations of this genetic parameter.

**Disclosure:** No conflict of interest disclosed.
Histology, metastases and palliative surgery in mRCC

**Introduction:** Renal cell carcinoma (RCC) is characterized by a variable clinical outcome within the same histological subtype, tumor stage or grading. Different parameters like TNM, performance status, nuclear grade or necrosis can help to predict the outcome in individual patients. We used array-CGH to identify regions of DNA copy number changes, which are associated with metastasis and clinical outcome in patients with the most frequent subtype of clear cell RCC (ccRCC).

**Methods:** 53 primary ccRCC including 31 metastasized and 22 non-metastasized tumors were analyzed by a custom made array-CGH DNA chip with a median resolution of 1:1.5 Mbp. For the validation of copy number aberrations (CNAs) with potential prognostic value, a fluorescence in situ hybridization (FISH) analysis was performed using commercially available fluorescent probes. Staging was carried out according to the TNM classification UICC2002.

**Results:** Overall, we detected 31 different recurrent CNAs which were present in >15% of all ccRCCs. The major differences in the groups were found on chromosome 7. The most frequent alteration in ccRCC detected by array-CGH was a deletion of the region 3p21.1-2p25.3. Additional deletions were localized on regions 8p, 1p4 and on chromosome 9. Five recurrent chromosomal aberrations were associated with metastasis: gains of 1q21.3, 1q13.12, 1q13.31q14.1 and 20q11.21q13.32 and loss of 9p21.3p24.1. Loss of 9p21.3p24.1 and gain of 1q21.3 and 20q11.2q13.32 were the strongest predictors for metastasis.

The strongest associations with shortened cancer-specific survival were observed for losses of 9p21.3p24.1 and 9q32q33.3 as well as gains of 7q36.3 and 20q11.2q13.32. Besides these alterations FISH analysis on the same cohort was performed for 4 selected regions such as 1q21.3, 7q36.3, 9p21.3p24.1 and 20q11.2q13.32 which clearly confirmed the array-CGH results.

**Conclusions:** Our data identified chromosomal alterations in ccRCC which might be used as predictors for the occurrence of metastasis and cancer-specific survival. For further scientific evaluation a combined FISH assay including these chromosomal bands should be verified within clinical trials.

**Disclosure:** No conflict of interest disclosed.

**Histology, metastases and palliative surgery in mRCC patients. Data from a clinical registry – RCC Registry**

**Introduction:** Current standard treatment of renal cell carcinoma includes nephrectomy for localized disease and metastasectomy for metastatic RCC. As most patients present with lymph node or distant metastases at the time of diagnosis, resection of isolated metastases reduces tumour burden and possibly prolongs survival. Furthermore, tumour histology has also been shown to affect survival.

**Methods:** The clinical registry on advanced or metastasized renal cell carcinoma (RCC Registry) conducted by the iOMEDICO AG in collaboration with the Arbeitskreis Klinische Studien (AKS) and Bund der Urologen (BuU e.G.) was established in December 2007. Among other information, the registry collects data on tumour histology, palliative surgery and metastases, including affected organ and time of detection. For patients with documented palliative surgery, additional information concerning the histology of the resected tissue was collected.

**Results:** From December 2007 to November 2010, 634 patients have been recruited. To date, for 554 metastasized RCC patients a total of 1392 cases of metastases have been documented with the most frequently affected organs being the lung (27%), bones (16%) and lymph nodes (15%). For 35% of the patients palliative surgery has been reported. So far, 29% of lung and bone metastases have been surgically resected. The majority of the metastasized RCC patients had a clear cell carcinoma (77%) as primary tumour. In 41% of the patients the histological profile of the resected tissue differed from the histology of the primary tumour.

**Conclusions:** Metastases from renal cell carcinoma are the most frequent localized in the lung and the bones. About 29% of these metastases were resected which might indicate potential benefit from a surgical intervention for almost one third of metastasized RCC patients. Furthermore this supports the role of metastatic resection in the era of targeted therapies. Further analyses will evaluate whether changes in the histological profile affect the choice of the antineoplastic therapy.

**Disclosure:** Lothar Müller: No conflict of interest disclosed.

Norbert Marschner: Advisory Role: ja – Medizinischer Berater der iOMEDICO AG

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Norbert Marschner: Advisory Role: ja – Medizinischer Berater der iOMEDICO AG
Dendritic cell-based multi-epitope immunotherapy in patients with castration resistant prostate cancer

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Background: The induction of specific immune responses towards tumor-associated antigens (TAA) provides a promising approach in cancer immunotherapy. Methods: Autologous monocyte-derived dendritic cells (DC), pulsed with peptides derived from multiple prostate antigens were used to vaccinate patients (pts) with castration-resistant prostate cancer (CRPC) bi-weekly for at least six times. A strict quality control concerning the expression of surface markers and the migratory capacity of the DC was performed before application.

Results: 21 pts were enrolled in the study. The results of six pts were published after a pilot-phase. Here we report on 15 pts, 12 of whom were evaluable for response as having received at least six vaccinations. Baseline characteristics are presented in table 1. Median number of vaccinations was 8 (range 1 – 23). The DC vaccine was well tolerated with low grade injection site reactions and itching being the most common side effects. Other adverse events included pain, abdominal symptoms, though most symptoms were not considered related to the study treatment or procedure. In almost all of the vaccinated patients remarkable strong immune responses to co-applicated recall antigens (influenza matrix protein, tetanus toxoid) were detectable. Although no immune responses against the prostate-specific antigens could be detected, 8 of 11 DC-treated patients did show a prolongation in the PSA-doubling time during vaccination compared to the doubling time before treatment. One patient experienced a PSA decline of >50% after 4 vaccinations and showed stable PSA levels for more than one year. Median overall survival was 26 months.

Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n = 15 (100 %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (range)</td>
<td>72 (58 – 86)</td>
</tr>
<tr>
<td>Performance Status 0, 1</td>
<td>13 (87%)</td>
</tr>
<tr>
<td>Serum PSA g/l (range)</td>
<td>75 (18 – 2'660)</td>
</tr>
<tr>
<td>Gleason score 8 – 10</td>
<td>7 (47%)</td>
</tr>
<tr>
<td>Prior chemotherapy</td>
<td>4 (27%)</td>
</tr>
<tr>
<td>Bone metastases</td>
<td>11 (73%)</td>
</tr>
<tr>
<td>Visceral metastases</td>
<td>2 (13%)</td>
</tr>
</tbody>
</table>

Conclusion: This study indicates that prolonged vaccination with an autologous DC vaccine is feasible and that this treatment leads to a clinical benefit. Equal contribution: First both authors.

Disclosure: No conflict of interest disclosed.

Studying the level of PSMA’s expression in prostate cancer

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Introduction: The PSMA (Prostate Specific Membrane Antigen) is a type 2 transmembrane glucoprotein expressed on the surface of prostate carcinomas as noncovalent homodimer. Although little is known about its molecular structure, it may be the base of a more effective marker in prognosis and diagnosis of prostate cancer. The present study attempted to prove the predictive value of PSMA as stemness marker as it is expressed either in human established prostate cancer cell lines (control cell lines) or in prostate cancer stem cells – like cell lines in higher levels.

Methods: Two methods were used in order to prove the above hypothesis. The first panel of the test included Reverse Transcription and Real – time PCR assays with PSMA- specific primers and in the second panel, a flow cytometric protocol was run using an anti-PSMA antibody conjugated with PE (NBL, K0142.5. mouse monoclonal). For this experiment, circulating tumor cells from prostate cancer patients (stage III and IV, Gleason rate 8) were isolated.

Results: From the experiment that has been performed, there was a statistic trend that stemness induces the expression level of PSMA in an analogue way.

Conclusions: Since PSMA is present to metastatic prostate carcinomas and the present study has pointed out that there is relevance between stemness phenotype development and expression rate of PSMA, the last antigen may have a significant prognostic value. Further studies need to be conducted in order to prove the above model.

Disclosure: No conflict of interest disclosed.

Sequential therapy with sorafenib and sunitinib in metastasized renal cell carcinoma (mRCC): a retrospective multi-center study and meta-analysis of available trials

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Background: Current treatment of mRCC offers a plentitude of choices for clinicians. Six novel agents have been approved in the last years. Since the advent of tyrosine kinase inhibitors (TKIs) for therapy of mRCC, sorafenib and sunitinib are among the best studied drugs in the clinic. Different centers have published their experience with sorafenib and sunitinib in form of mostly retrospective data dealing with the question of these TKI’s impact when used sequentially.

Methods: We performed a retrospective analysis of patients (n=28) in our and collaborating institutions that had received sequential therapy with these drugs. Our results were correlated with hitherto published data. We integrated all available data, total of 12 studies in a meta-analysis and examined differences in median progression-free survival (PFS) using weighted linear regression.

Results: As suggested by the majority of the published studies, a longer PFS for the sorafenib-sunitinib sequence compared to sunitinib-sorafenib was confirmed by the pooled analysis (median PFS on sunitinib-sorafenib was 12.0 months compared with 15.0 on sorafenib-sunitinib, 95% CI for difference 0.55 – 5.52, p = 0.02). No significant difference in the time to first progression (PFS1) was noted regardless which drug was initially used (median PFS1 was on average 0.2 months shorter on sorafenib-sunitinib, 95% CI for difference -2.4 – 2.0, p = 0.855). In second line, sunitinib showed a significant longer PFS than sorafenib (average increase of 3.1 months, CI 1.6 – 4.6, p = 0.0003), Impact on overall survival could not be assessed due to insufficient data reported by the studies.

Conclusions: This study confirms that both TKIs used sequentially result in a longer PFS compared to single TKI treatment. Sorafenib and sunitinib do not display relevant cross resistance. Sorafenib-sunitinib translates into a longer PFS im comparison to sunitinib-sorafenib. While this abstract was finalized another two studies – with significant number of study subjects – dealing with this topic were published. We have a confirmed collaboration with the respective study groups, thus this meta-analysis will be expanded and the updated data and the final analysis will be presented at the DGHO conference 2011.
Wissenschaftliches Symposium
Transplantation

V621
Controversy: Old sibling or young unrelated donor?

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Donor choice for patients scheduled for allogeneic stem cell transplantation (alloSCT) is determined by demographic, disease-specific and patient-specific factors. Due to the demographic development in Europe and in the US, the probability of finding a matched sibling donor will continuously decrease in the next decades. Recent data suggest, that outcome in patients with high-risk leukemia undergoing transplantation from matched unrelated donors (UD) are comparable to that of patients receiving grafts from matched sibling donors. This seems to be especially true after reduced-intensity conditioning which is mainly used in elderly patients. For certain diseases like indolent non-Hodgkin’s lymphoma and CLL, the incidence of relapse seems to be lower after transplantation from an UD, probably due to the more pronounced GvL effects. An advantage of younger donors might be the higher likelihood of mobilising more CD34+ cells. In the unrelated setting, donor age has been associated with outcome in retrospective studies. Additionally, higher donor age in the sibling setting is associated with a higher risk of being CMV seropositive. Finally, the availability of several UD donors may allow to select for non-HLA genes like NK cell receptors or pattern recognition receptors. After all, the risks associated with stem cell donation (cardiovascular/malignancies) are lower for younger donors.

Disclosure: Martin Bornhäuser: Advisory Role: Riemser; Financing of Scientific Research: Novartis, Celgene, Roche, Miltenyi; Expert Testimony: Roche, Novartis

V622
Allogeneic blood stem cell transplantation form alternative stem cell sources: Cord blood or haploidentical donor or adult unrelated mismatched donor

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As allogeneic stem cell transplantation represents the only curative treatment option for a variety of patients with lymphomas and leukemias the unavailability of a matched (related or unrelated) donor requires the use of alternative stem cell sources such as mismatched unrelated donors, haploidentical family donors or mismatched cord blood units. In the absence of a fully matched donor the use of single mismatched (related or unrelated) donors can be regarded as broadly accepted practice in high risk diseases. Optimized GvHD prophylaxis has helped to reduce graft-versus-host disease (GVHD) rates associated with this procedure. If such donors are not available the use of alternative donors should be considered in centers specialized for such transplant procedures. The introduction of double instead of single cord blood unit transplantation has helped to broaden the applicability of cord blood transplantation by improving engraftment and thereby reducing the rates of transplantation-related mortality (TRM). Nevertheless, the transplantation of a primarily naïve T lymphocyte repertoire with the cord blood is associated with increased rates of infectious complications and relapse and thus represents a drawback to its optimal use. The feasibility of haploidentical allogeneic stem cell transplantation largely depends on sufficient in vivo and ex vivo T cell depletion strategies to allow engraftment and to avoid severe GvHD. As a consequence TRM and relapse rates are increased, limiting the overall success of this approach, as well. Therefore novel strategies aim to improve post transplant immune reconstitution after haploidentical stem cell transplantation to reduce infectious complications and relapse of the underlying disease. In this context T cell depletion techniques of stem cell allografts targeting CD3 and CD19 to spare natural killer cells, introduction of suicide genes in donor-derived T cells allowing to stop GvHD in the case of need and ex vivo selective depletion of alloreactive T cells have become the focus of today’s translational transplant approaches.

Disclosure: Stephan Mielke: Expert Testimony: Scientific grant support by KIADIS Pharma, NL

V624
Prophylaxis and therapy of GVHD

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Graft-versus-host disease (GVHD) is a serious complication of allogeneic hematopoietic cell transplantation (HCT) and is associated with significant morbidity and mortality. With the introduction of new conditioning regimens and the use of peripheral blood stem cells instead of bone marrow the clinical presentation of GVHD has substantially changed. Acute GVHD occurs now more frequently as delayed acute GVHD or as an overlap syndrome with manifestations of both acute and chronic GVHD. The NIH consensus suggestions for staging and grading of chronic GVHD have considered these manifestations. The ability to prevent GVHD is crucial since treatment when prophylaxis fails remains suboptimal. However, the beneficial graft-versus-leukemia effects should be spared to allow efficient eradication of malignant disease by allogeneic HCT.

The most commonly used pharmacologic regimen for GVHD prophylaxis is the combination of a calcineurin inhibitor and methotrexate. Recently, mycophenolate mofetil (MMF) has been introduced for GVHD prevention as an alternative non-methotrexate (MTX) containing regimen. Currently, few data on the use of the mTOR-inhibitor sirolimus in combination with tacrolimus for GVHD prevention are available.

Despite major advances in the understanding of the pathophysiology of acute GVHD, corticosteroids remain the standard first-line therapy for patients who develop acute GVHD. Since only up to 40% of patients respond completely to first-line steroids, many still require additional salvage therapy for steroid-refractory acute GVHD and have a dismal prognosis. Antithymocyte globulin, monoclonal antibodies directed against cytokines such as tumor necrosis factor alpha or the interleukin-2 receptor or against CD3 have been frequently used in steroid-refractory acute GVHD achieving some responses but resulting in no improvement of survival due to increased infectious complications including Epstein-Barr virus lymphoproliferative disease. Using extracorporeal photopheresis (ECP) durable responses with favorable impact on survival and steroid-sparing were achieved in steroid-refractory acute GVHD patients. First-line therapy of chronic GVHD consists of steroids with or without calcineurin inhibitor. For salvage therapy of chronic GVHD ECP, mTOR inhibitors, MMF, MTX and rituximab have been frequently used. However, therapeutic responses are still unsatisfactory and more efficient treatment strategies are warranted to substantially improve patient outcome.