This phase III study assessed safety and efficacy of alemtuzumab consoli-
dation therapy (30mg i.v. TIW, 12 weeks) for patients with CLL in remis-
sion after initial treatment with fludarabine alone (F) or in combination with
cyclophosphamide (FC). Of 21 evaluable patients, 11 were random-
ized to alemtuzumab before the study was stopped due to CTC grade 3 or 4
infections in 7/11 patients who received alemtuzumab (1 grade IV pul-
monary aspergillosis; 4 grade III CMV reactivations; 1 grade III pul-
monary tuberculosis; 1 grade III herpes zoster). These infections were
successfully treated, and there was no significant association with dose or
duration of consolidation. At 6 months, in the alemtuzumab arm overall
response was higher (11/11 vs 7/10, P = .059). Furthermore, significantly
more patients converted to molecular remission in peripheral blood (alleg-
specific IgH RT-PCR, 5/6 vs 0/3, P = .048). At 15.5 months median follow-
up, there was a trend to longer progression-free survival (PFS) when pa-
tients received F/FC with alemtuzumab consolidation compared with F/FC alone (no progression vs mean 21.6 months, P = .069). At limited
median follow-up of 9.9 months, PFS is significantly improved when com-
paring consolidation to no further therapy (P = .0498). Based on the
promising results of this study, we are currently defining the optimal dose
for alemtuzumab consolidation of patients with CLL in remission after
fludarabine-based chemotherapy.

The prognosis of fludarabine-refractory CLL is poor with a median survival
time of 8 months despite various salvage regimens. Campath-1H (Mab-
Campath) was recently approved for fludarabine refractory CLL based on
a remission rate of 33%, a median survival time of 16 months in intent to
and, 32 months among responding patients (Keating et al., Blood 2002). Furthermore, CLL with 17p−/p53 mutation, predicting for chemore-
stance, may respond to Campath-1H (Stilgenbauer and Döhner, NEJM,
2002). While the standard route of Campath-1H administration is by 2 hour I.V. infusion three times weekly, the subcutaneous (S.C.) route was recently shown to be safe, efficacious, less time consuming and accompanied by less infusion/injection reactions in first line treatment of CLL (Lundin et al., Blood, 2002). Therefore, the CLL2H trial was initiated to evaluate the S.C.-
application of 3 x 30 mg Campath-1H weekly in fludarabine refractory CLL
after I.V. dose escalation to avoid large skin reaction after S.C. treatment
start. Currently 16 fludarabine-refractory CLL patients have been enrolled.
Treatment was not yet started in two patients due to active infection and in
one patient after withdrawal of consent. One patient refused further treat-
dment due to rigors and anxiety after the first I.V. dose of 3 mg while all of
the remaining 12 patients tolerated I.V. dose escalation accompanied by no
or mild (grade I-II) rigors and fever after premedication with paracetamol
and antihistamines. Continuation of treatment via the S.C. route was well
tolerated, with skin reactions, rigors, or fever (all grade I) in one patient
each and continued on an outpatient basis in all patients. Toxicity was most-
ly hematological with grade III/IV neutropenia (n = 6), grade I-II anaemia (n
= 4), and grade I IV thrombocytopenia (n = 3). Infections occurred in 3 pa-

tients with one case each of sepsis, pneumonia, and CMV reactivation re-
sponding to oral valganciclovir. There was so far one death (PD and sepsis)
on the trial. Response was evaluabel for 8 patients with PR (n = 3), SD (n
= 3) and PD (n = 2). In summary, Campath-1H given via the S.C. route after
on the trial. Response was evaluabel for 8 patients with PR (n = 3), SD (n
= 3) and PD (n = 2). In summary, Campath-1H given via the S.C. route after

Abstracts

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In summary, remissions can be attained with low cumulative doses of Campath-1H but are of limited duration. Therefore, we recommend flexible time intervals depending on the leukocyte counts, leading to prolonged treatment periods. However, whether a cumulative dosage according to 3 x 30 mg Campath-1H weekly for 12 weeks is needed remains to be clarified.

P869 Firstline treatment of advanced follicular lymphoma comparing fludarabine / Mitoxantrone and high-dose chemotherapy, each combined with CD20-antibody Rituximab


Gelsenkirchen, Düsseldorf, Ulm, Duisburg, Wuppertal, Remscheid, Hamburg, Neuss, Velbert, D

In this prospective randomized multicenter study, patients aged <60 years with stage III/IV follicular lymphoma were treated either with 6 courses of fludarabine (25 mg/m² d1–3) / mito-xantrone (10 mg/m² d1) or with 3 courses of conventional CHOP followed by two courses of HAM (AraC 2x2g/m² d1–2, mitoxantrone 10mg/m² d3) preceding high-dose therapy with BEAM (BCNU 300mg/m² d7, etoposide 300mg/m² d7 to d-4, AraC 400mg/m² d7 to d-4, melphalan 140mg/m² d-3) and autologous peripheral blood stem cell transplantation (ASCT). In both arms, CD20-antibody rituximab was added on day 0 except for the first course. For this interim analysis, 43 of 48 patients were evaluable. In the FM/R arm (n = 22), the rate of complete remission (CR) was 73% after 6 courses of chemo-immunotherapy. In the high-dose arm (n = 26), CR rate was 90% after final BEAM/AR chemotherapy. Municetopectomy was performed using t(14;18) nested PCR. In the FM/R arm, molecular remission (MR) of patients initially PCR positive in bone marrow and peripheral blood was observed in 57% after 6 courses. MR rate of PCR positive patients in the high-dose arm was 88%. At a median observation time of 21 months, event free and overall survival were not different comparing both arms. Peripheral stem cell mobilization was successful in 53% of FM/R treated patients as compared to 100% after CHOP/R and HAM/R treatment. Stem cell harvest was better in patients treated in the high-dose arm as compared to the FM/R arm (means: 13.1 vs 6.2×10⁶ CD34-positive cells/kg bw). Regarding toxicity, both treatment options were well tolerated, with 2% documented severe infections (CTC grade 3/4). As hematologic or biochemical (creatinine, AST, bilirubin) toxicity including grade 3/4 toxicity including grade 3 or 4 hematologic or biochemical toxicity according to the WHO classification, major bleedings, and infections. Secondary objectives were the rate of molecular complete remission and the overall rate of response (complete + partial remission). Results: Seven patients, 4 male and 3 female, aged mean 54.3 ± 8.8 years (range: 38 to 65 years) were considered eligible. Median time between diagnosis and start of treatment was 14.6 ± 9.3 months (range 3 to 30 months). There was a median of 5.3 ± 0.9 treatment cycles. R-FC was well tolerated with no episodes of Grade 3 or 4 hematologic or biochemical (creatine, AST, bilirubin) toxicity including severe infections, bleeds that required transfusion of packed red cells or platelets, there was no treatment with G-CSF. Six patients (86%) achieved an overall rate of response (4 patients (57%) achieved a molecular complete remission, 2 patients (29%) achieved a molecular partial remission), and in one patient minimal residual disease remained. The median duration of follow-up was 12.4 ± 8.0 months (range 4 to 24 months). Conclusions: Conventional chemotherapy of fludarabine monotherapy on five consecutive days every 4 weeks has been proposed the standard of first-line treatment in patients with B-CLL. This present trial underlines, that R-FC is well tolerated without significant Grade 3 or 4 toxicity. Because of the small number of subjects included in our study and the limited follow-up time, the promising results need to be confirmed in a larger controlled, prospective trial.

P871 Complete remission in a patient with autoimmune hemolytic anemia and chronic lymphocytic leukemia after treatment with Rituximab

M. Wiermann, M. Mohren, K. Jentsch-Ullrich, U. Dworschak, A. Franke

Magdeburg, D

Introduction: 10–15 percent of patients with chronic lymphocytic leukemia (CLL) develop autoimmune hemolytic anemia (AIHA) some time in the course of their disease. Until now therapy remains a challenge. We report on a CLL patient with AIHA, who achieved complete remission after four courses of CD-20-antibody (Rituximab). Patient: A 76-year-old female patient with a ten year history of CLL presented with severe AIHA of the warm antibody type (hemoglobin 4.0 g/dl). Past therapy of CLL included treatment with chlorambucil because of thrombocytopenia. AIHA therapy with prednisone and cyclophosphamide was as less effective as a bendamustine containing regimen. Due to ongoing transfusion dependance and leukocytopenia after receiving bendamustine we initiated therapy with four courses of weekly Rituximab. Within six days after the first course the hemoglobin increased and remained stable without any further blood transfusions. Normalization of the hemoglobin occurred after the forth cycle of Rituximab-therapy. AIHA remained in complete remission during a follow-up of four months. Discussion: AIHA in patients with CLL may show a course refractory to cytotoxic therapy. Additionally treatment-related leukocytopenia and thrombocytopenia can hamper therapeutic efforts. Rituximab is a CD20-antibody which has been increasingly employed in B-cell-lymphomas and immunocytopenias. There exist no reports on patients with B-CLL and AIHA of the warm antibody type treated with Rituximab single chemotherapy so far. The results in our patient show great promise and should be further investigated in prospective studies.

P872 Combined chemotherapy of Rituximab, fludarabine and cyclophosphamide is efficacious and safe in younger patients suffering from B-cell chronic lymphocytic leukemia

O. Ranze, M. Arland, H.-G. Hoeckfes

Fulda, D

Background: Fludarabine monotherapy is the standard first-line chemotherapy for patients with B-cell chronic lymphocytic leukemia (B-CLL). However, there is evidence that the combined chemotherapy of rituximab, fludarabine, and cyclophosphamide (R-FC) is superior in younger patients with B-CLL with respect to molecular remission rates. We present a case series treated with this regimen. Patients and Methods: Previously untreated and symptomatic patients received 4 to 6 cycles of combined chemotherapy of rituximab (375 mg/m² day (d) 1, fludarabine (25 mg/m²) and cyclophosphamide (250 mg/m²) from d 1 to d 4, IV every 4 weeks. Inclusion criteria were: diagnosis of B-CLL according to the Rai classification at stage I to IV, <70 years of age, ECOG status 0–1. Primary objectives were Grade 3 or 4 hematologic or biochemical toxicity according to the WHO classification, major bleedings, and infections. Secondary objectives were the rate of molecular complete remission and the overall rate of response (complete + partial remission). Results: Seven patients, 4 male and 3 female, aged mean aged 54.3 ± 8.8 years (range: 38 to 65 years) were considered eligible. Median time between diagnosis and start of treatment was 14.6 ± 9.3 months (range 3 to 30 months). There was a median of 5.3 ± 0.9 treatment cycles. R-FC was well tolerated with no episodes of Grade 3 or 4 hematologic or biochemical (creatine, AST, bilirubin) toxicity including severe infections, bleeds that required transfusion of packed red cells or platelets, there was no treatment with G-CSF. Six patients (86%) achieved an overall rate of response (4 patients (57%) achieved a molecular complete remission, 2 patients (29%) achieved a molecular partial remission), and in one patient minimal residual disease remained. The median duration of follow-up was 12.4 ± 8.0 months (range 4 to 24 months). Conclusions: Conventional chemotherapy of fludarabine monotherapy on five consecutive days every 4 weeks has been proposed the standard of first-line treatment in patients with B-CLL. This present trial underlines, that R-FC is well tolerated without significant Grade 3 or 4 toxicity. Because of the small number of subjects included in our study and the limited follow-up time, the promising results need to be confirmed in a larger controlled, prospective trial.
Background: Bendamustine and mitoxantrone have been shown to be potent cytotoxic drugs for the treatment of relapsed or refractory indolent lymphomas. The anti-CD20 monoclonal antibody rituximab has produced an overall response rate of 50% as a single agent in relapsed or refractory indolent lymphomas. We asked whether the combination of bendamustine, mitoxantrone and rituximab (BMR) could improve these results. **Objective**: Open label, single centre pilot study for patients (pts) with relapsed or refractory, CD20-positive indolent lymphoma or chronic lymphocytic leukemia. The therapy consisted of bendamustine (80 mg/m$^2$/d, day 1–3), mitoxantrone (10 mg/m$^2$/d, day 1), rituximab (375 mg/m$^2$, week 2, 3, 4, 5). Reduction of bendamustine dose according to pts’ clinical condition was permitted. BM was repeated on day 36 or when the hematological parameters had recovered. The maximum therapy consisted of 1 BMR-application, followed by 5 BM applications. Treatment was stopped when a partial response (PR) or a complete remission (CR) was achieved. **Results**: Between 3/99 and 04/03 54 pts received the BMR-regimen (6 secondary treatment for refractory or relapsed indolent lymphomas. Bendamustine/Mitoxantrone/Rituximab (BMR): A new effective phase-II study

H. Schulz, S.K. Klein, U. Rehwald, M. Reiser, S. Ibach, A. Engert on behalf of the German CLL Study Group (GCLLSG)

We present a follow up of a multicenter phase-II study combining rituximab (R) and fludarabine (F) in patients (pts) with fludarabine and antracycline-naive Chronic Lymphocytic Leukemia (CLL). 10 of 41 pts were treated in a pilot study and 31 in the main study of the German CLL study group. 24 were previously untreated and 17 relapsed. B-CLL pts were treated in a pilot study and 31 in the main study of the German CLL Study Group. 10 of 41 pts were Rai stage IV (Binet C) and 4 Rai stage II (Binet B). Overall response rate was 96% (52/54) with 22 patients achieving a CR (41%) and 30 pts achieving a PR (55%). Median time to progression is 26 months (0–48+) with a median observation time of 13 months (0–48+). Response is still durable in 31/54 pts (57%) (1+ to 48+ months after therapy). A symptomatic, reversible grade 3 or 4 hematotoxicity occurred in 10/54 pt’s (19%). A non-symptomatic grade 3 or 4 hematotoxicity was seen in 23/54 pts (43%). One hypersecretion reaction grade 3 was observed. **Conclusion**: BMR is a well tolerated, very effective outpatient treatment for relapsed and refractory indolent lymphoid malignancies. The suggested bendamustine dose for lymphoma pts is 90 mg/m$^2$, day 1 and 2, and for pts with B-CLL 80 mg/m$^2$, day 1 and 2, respectively.

P875

Rituximab in combination with fludarabine in patients with chronic lymphocytic leukaemia – update of a multicenter phase-II study

S. Irmer, M. Ritgen, S. Böttcher, M. Kneba

The simultaneous diagnosis of hairy cell leukaemia (HCL) and chronic lymphocytic leukaemia (CLL) is rare. We describe the case of a 52 year old male patient with massive splenomegaly and well characterized highly leukemic HCL in whom a subclinical monoclonal CLL population was detected at first diagnosis. Using flow cytometric analysis of CDS/CD20/CD23/CD103 and light chain kappa/lambda on CD19-gated B cells we identified a major HCL and minor CLL population. The immunophenotype of peripheral blood showed circa 42% of lymphocytes with HCL phenotype (CD19+/CD20+), whereas the CLL phenotype (CD19+/CD20−/CD103−/CD5−) was further characterized by CD23/CD103 and light chain kappa/lambda on CD19-gated B cells. Further investigation will be needed to uncover the cellular origin of both clones. The patient with severe anaemia, neutropenia and thrombocytopenia was treated with an escalated therapy of recombinant interferon-alpha, the monoclonal anti-CD20 antibody rituximab and the nucleosid analogon 2-chlorodeoxyadenosine (2-CDA). We compared the efficiency of three different potential substances in the treatment of HCL/CLL. Only after treatment with 2-CDA in the patient achieved a good response of the HCL with restoration of erythrocytosis, thrombocytosis and granulocytosis whereas the CLL population persisted.
P877 Anosmia, ageusia and autoimmune hypothyroidism in a patient treated with alpha interferon for secondary non-Hodgkin’s Lymphoma
B. Deschler, M. Reinicke, M. Engelhardt
Freiburg, D

We report on a 35-year-old patient (pt) who received alpha interferon (IFN) for maintenance therapy in Non-Hodgkin-Lymphoma (NHL). In 1981, he was diagnosed for stage IA Hodgkin’s Lymphoma (lymphocyte rich HD) which was treated by splenectomy, chemotherapy and radiation therapy. After 16 yrs of complete remission, he developed abdominal lymphomatosis, verified as secondary stage IIIB B-NHL (DLBCL). After standard-, he received high-dose-(HD)-chemotherapy (BEAM) with consecutive autologous stem cell transplantation (PBSCT). Already 1 year later, NHL disease relapsed. Following reinduction, a 2nd PBSCT was conducted with the same myeloablative regime and abdominal bulk radiation (38 Gy). For the remaining abdominal lymphomas, IFN was initiated (3Mio E/week) as maintenance therapy. After 3 yrs, severe hypothyroidism due to autoimmune thyroiditis became apparent. Thorough follow-up examinations further revealed a dry exanthema, bilateral hearing impairment and hypogonadotrophic azoospermia. When the pt (a professional taste tester for cereals) noticed a rapidly progressive anosmia and ageusia, IFN therapy was discontinued. One year later, hypothyroidism is well controlled, whereas hearing impairment, azoospermia, anosmia and ageusia persist. After intensive chemotherapy including 2 PBSCTs and consecutive IFN therapy, this pt shows typical as well as rarely observed treatment-induced side effects. Hypothyroidism is a well-known complication after PBSCT. Yet, due to the autoimmunome process detectable in our pt, we presume IFN to have triggered thyroiditis. In contrast, azoospermia as well as hearing impairment were most likely caused by intensive chemotherapy regimens. Pituitary dysfunction was ruled out. We presume that anosmia and ageusia were IFN-induced, since the onset occurred yrs after chemotherapy and persisted after treatment for hypothyroidism. To date, about a dozen pts have been reported with these IFN-induced complications. We conclude that reinduction HD-chemotherapy followed by maintenance IFN therapy is a potentially valid option for pts with recurrent NHL. 5 yrs after his second PBSCT (3.5 yrs under IFN therapy), our pt is in stable disease. This is of note, considering his unfavorable prognosis with secondary and recurrent NHL. Awareness of complex interactions of a variety of acute and long-term side effects after intensive therapy such as PBSCT and/or maintenance therapy must be practiced since differential diagnosis may be challenging.

P878 Composite lymphoma – B-CLL and Hodgkin’s disease: Case report and review of the literature

Introduction: Follicle episodes frequently occur in patients with chronic lymphocytic leukemia (CLL) and work up can be tedious, especially if fever takes a persistent course. Progressive disease, infection, autoimmunome phenomena as well as transformation into a secondary high grade lymphoma are the main underlying causes. We report on a CLL patient with persistent fever, who was found to have secondary Hodgkin’s disease (HD) on autopsy. Patient: A 59 year old patient was diagnosed with B-CLL stage Binet B (Lymphocytosis and splenomegaly) in an outside hospital. He was switched from oral chlorambucil to COP after 4 weeks due to progressive lymphocytosis (605 Gpt/l) and LAD. Therapy was stopped after 4 courses because of recurrent urinary tract infections with sepsis in neutropenia. At that time the CBC had normalized, bone marrow analysis showed only slight CLL infiltration and LAD and spleen size had decreased. 3 months later persistent fever with spikes of 39°C, night sweats and weight loss occurred. CT scans were consistent with constant LAD, a slight increase in spleen size, and failed to reveal an infectious focus. Repeat bone marrow specimen due to pancytopenia and were hypocellular Empiric antimicrobial therapy was initiated without amelioration of symptoms and the patient was transferred to our clinic. At that time the patient’s condition was severely compromised, he became comatous and died within very short time. Autopsy revealed Hodgkin’s disease (nodular sclerosis) with infiltration of bone marrow, spleen and lymph nodes. Discussion: Transformation into secondary high grade NHL is common in patients with CLL, but other malignancies, such as melanoma, lung, breast and colon cancer and HD may also occur. So far cytotoxic therapy has not been implicated in the pathogenesis of secondary malignancies. EBV infection as well as their origin in the follicular center of lymph nodes are thought to account for the concurrence of B-CLL and HD. Conclusions: Although rare, secondary malignancies such as described in our patient should be considered in CLL patients with persistent B-symptoms, in whom other causes such as progressive CLL or infection have been ruled out.

P879 Amyloid A due to non-Hodgkin’s lymphoma – a case report
Halle, D

Background: Amyloidosis is a term which encompasses a group of disorders with extracellular deposition of amyloid proteins due to different pathogenetic mechanisms. Amyloidosis may be a rare complication of Non-Hodgkin-s lymphoma (NHL). Mostly it appears as a systemic disease, sometimes with life-threatening complications. Rarely it occurs as localised amyloidosis with consecutive local dysfunction. Patient’s characteristics: We report a 64-year-old female patient with a history of long-standing, recurrent amyloidosis of the left lower leg. The first manifestation 15 years ago was localised in the soft tissue and peroneal muscle in association with a low grade lymphoma, which had been treated by subtotal resection followed by radiation therapy (30 Gy). The first relapse 11 years later had been treated again with subtotal resection. Histology presented a lymphoplasmacytoid immunocytoma with secretion of paraprotein A. The second relapse 3 years later has been treated with irradiation (40 Gy) and chemotherapy (6 courses of cyclophosphamide, vincristine, prednisolone combined with anti-CD20 monoclonal antibody (Rituximab)). The re-biopsy specimen revealed no evidence of lymphatic infiltration but a persistence of the amyloid process. At time of the first and second relapse there was neither evidence of systemic amyloidosis nor generalised NHL according to MRI, computed tomography, bone marrow puncture and rectal biopsy. The patient suffers from peroneal paresis due to the large tumour, growing slowly but infiltrative into the surrounding tissue. Conclusions: Localised low-grade NHL may be associated with localised amyloidosis secondary to local production and deposition of amyloid A (AA). The treatment of the NHL was effective against lymphoma but could not stop amyloid deposition. In this case progression of amyloidosis was the first indication of relapsed NHL.

P880 Repp86 expression in mantle cell lymphoma: A marker of cell proliferation and a prognostic factor for clinical outcome
Kiel, Essen, D

Purpose: Mantle cell lymphoma is a malignant lymphoma associated with a relatively aggressive clinical course and a median overall survival time of 3–4 years. Proliferation index is an important prognostic factor in the clinical outcome. The clinical relevance of the proliferation specific marker Repp86 (Ki-S2) could be demonstrated in other malignancies. Repp86 protein is expressed in cell cycle phases G2, S, M but not in G1, in contrast to Ki-67. We analysed the expression of Repp86 in relation to Ki-67 and the clinical course in mantle cell lymphoma. Materials and methods: Biopsy specimen from 94 untreated patients enrolled in two multicenter prospective trials were investigated immunohistochemically with monoclonal antibodies against CD20, CD5, CD3, CD23, cyclin D1, Repp86 (Ki-S2) and Ki-67 (Ki-S5). The positive cells were counted and compared with the overall survival data analysed according to Kaplan and Meier. Results: Patients with very low (0–1%) Repp86 expression (Ki-S2) had a median overall survival time of 71.0 months compared to 38.1 months for patients with more than 1 up to 5% positive cells. Patients with more than 5 up to 10% positive tumour cells had a median survival of 25.4 months. Very high expression (more than 10%) of Repp86 could be found in patients with a short survival (median: 15.0 months). The Ka-
plan-Meier analysis showed a significant difference (p<0.001) in the overall survival time between the patients with very high (>10%) and very low (0–1%) expression of Repp86 of the tumour cells. Conclusion: Based on these findings, the expression of Repp86 antigen in mantle cell lymphoma is an important prognostic factor.

**P881**

**Rapamycin induced cell cycle arrest in mantle cell lymphoma cells is accompanied by downregulation of cyclin D3 and cyclin A**

S. Hipp, F. Schneller, C. Peschel, T. Decker
Munich, D

**Objectives:** Mantle cell lymphoma (MCL) is characterized by overexpression of cyclin D1 as a consequence of the chromosomal translocation t(11;14)(q13;q32). MCL is still an incurable disease and combines unfavorable clinical features of aggressive and indolent lymphomas. Rapamycin has been reported to inhibit cell cycle in a broad range of human tumor cell lines. Therefore, we investigated the ability of Rapamycin to block cell cycle progression in MCL cells.

**Material and methods:** Two mantle cell lymphoma cell lines, Granta and NCEB, were analyzed in our study. Cell cycle analysis was done using Propidium Iodide (PI) staining, MTT assays and Thymidine incorporation. Apoptosis induction was analyzed using Annexin/PI and Tunel-Assays. Expression of cell cycle regulatory molecules was revealed in western blot experiments and activity of cyclin dependent kinases (cdk) was confirmed with in vitro-kinase assays.

**Results:** Rapamycin inhibited proliferation of MCL cell lines at doses which are readily achievable in vivo as confirmed by MTT assays and Thymidine incorporation. Cell cycle analyses revealed that cells were arrested in G1 phase of the cell cycle. NCEB cells were more sensitive to the anti-proliferative effect of Rapamycin than Granta cells. Cell cycle arrest was not accompanied by apoptosis induction in both cell lines. Western Blot experiments demonstrated that cyclin D1 expression was unchanged in Rapamycin treated MCL cells. However, cyclin D3 was downregulated in both cell lines, suggesting that cyclin D3 is the principal d-type cyclin in MCL cells. While cyclin A expression was strongly inhibited in NCEB cells, cyclin A was still detectable in Granta cells, corresponding to a larger fraction of cells in the S phase of the cell cycle in Rapamycin treated Granta cells. Our results were confirmed in purified MCL cells of one leukemic MCL patient. Rapamycin caused a G1 arrest in these cells, but cyclin D1 levels remained unchanged. In contrast, cyclin D3 expression was increased in activated MCL cells and was inhibited by Rapamycin.

**Conclusion:** Rapamycin is a drug that might be of interest in delaying disease progression in mantle cell lymphoma patients by causing cell cycle arrest. Given its favorable toxicity profile in transplant recipients, long term therapy might be an option in patients with this incurable disease.

**P882**

**Superior reduction of circulating lymphoma cells in mantle cell lymphoma patients treated with a combination of Rituximab and chemotherapy compared to chemotherapy alone**

C. Hirt, F. Schüler, C. Schwenke, M. Herold, G. Dölken für die Ostdeutsche Studiengruppe Hämatologie / Onkologie (OSHO)

In a randomized trial of the ‘Ostdeutsche Studiengruppe Hämatologie / Onkologie’ patients with stage III/IV follicular lymphoma (FL), lymphoplasmacytic lymphoma or mantle cell lymphoma (MCL) were randomized to receive either 8 cycles of chemotherapy alone (mitoxantrone, chlorambucil, prednisolone; MOP) or in combination with rituximab. We have tested 12 MCL patients for molecular relapse.

**Material and methods:** For the t(11;14) translocation and in 4 cases we were able to design a clone-specific primer for the CDR3 of the immunoglobulin heavy chain locus. In serial blood and bone marrow samples from 8 patients lymphoma cells were quantified by real-time quantitative PCR during therapy and follow-up. At diagnosis the median number of circulating lymphoma cells (CLC) were similar in both groups (95480/10^6 peripheral blood mononuclear cells (PBMCN) for patients treated with chemotherapy alone and 93848/10^6 PBMCN in the chemoinmunotherapy arm). After therapy median numbers dropped to 1469 in the chemotherapy alone arm compared to a median of 5.8 lymphoma cells per 10^6 PBMCN in the group that received additionally rituximab. Two patients in the rituximab arm achieved temporary complete molecular remissions lasting 17 and 73 weeks whereas none of the patients treated with chemotherapy alone showed a conversion to PCR negative results. Compared to FL patients with high initial numbers of CLC, the decrease in lymphoma cells was more slowly and less pronounced in MCL patients.

One patient who relapsed showed an increase of CLC from PCR negativity to 1313/10^6 lymphoma cells. Another patient who achieved a complete clinical response after 8 cycles MCP showed persisting CLC between 1469 and 10^5/10^6 PBMCN until clinical relapse 31 weeks later when no further increase in CLC could be observed. Two patients with slowly but steadily increasing numbers of CLC are still in clinical remission.

We conclude that our real-time quantitative PCR assay allows a rapid and exact molecular disease monitoring in MCL patients. By combining rituximab and chemotherapy a temporary clearance of CLC can be achieved in a proportion of MCL patients. The clinical significance of molecular remissions in MCL patients has to be further evaluated and might lead to treatment strategies based on the results of molecular disease monitoring like in childhood ALL and CML after allogenic stem cell transplantation.

**P883**

**Therapy of molecular relapses in patients with malignant non-Hodgkin’s lymphoma after allogeneic blood stem cell transplantation**

F. Schüler, C. Hirt, T. Kieler, G. Döhlen
Greifswald, D

A molecular relapse was detected by quantitative clone-specific PCR in two patients with malignant Non-Hodgkin lymphoma after allogeneic blood stem cell transplantation.

The first patient (38 yrs.) had a mantle cell lymphoma stage IVB with bone marrow infiltration and leukemic generalization. After 2x CHOP and 5x DHAP with rituximab the patient achieved a clinical, but not a molecular remission (20 lymphoma cells/100,000 peripheral blood mononuclear cells, PBMCN) and was treated with allogenic BSCT. The second patient (60 yrs.) presented with variant hairy cell leukemia (>100,000 circulating hairy cells/µl blood). He was resistant to 2-CdA, alpha-interferon and various chemotherapy regimens, achieved a clinical remission lasting 12 months after therapy with rituximab, but finally had progressive disease.

The criterion for molecular relapse was an increase of about three orders of magnitude of circulating lymphoma cells with no symptoms or signs of a clinical relapse.

In both patients a molecular relapse could be detected within 20 days and 3 months after transplant based on the detection of circulating lymphoma cells by real-time quantitative PCR using the lymphoma clone specific IgH CDR3 (VDJ) sequence as the target.

The rapid reduction of immunosuppressive therapy in combination with rituximab has led to the disappearance of circulating lymphoma cells in both patients within 30 – 50 days. Acute GvHD only observed in the first patient was successfully treated with CSA and steroid at a lower dose. No donor lymphocyte infusions were given. Now, 12 and 17 months after molecular relapse, both patients are in complete clinical and molecular remission.

It is important to note that both patients were treated with rituximab before allogeneic BSCT, but in spite of clinical efficacy it did not result in a molecular remission before BSCT. In both patients a graft-versus-lymphoma effect leading to a molecular remission has been demonstrated by real-time quantitative PCR after reduction of immunosuppressive therapy for molecular relapse.
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Background: MALT lymphoma is a relatively common type of lymphoma arising in various tissues throughout the human body. Currently, there is no standard chemotherapy for advanced stage MALT lymphoma. This has prompted us to retrospectively analyse our experience with the MCP-regimen (mitoxantrone, chlorambucil and prednisone) in patients with MALT lymphoma. Patients and methods: Patients with histologically verified MALT lymphoma undergoing chemotherapy with MCP were retrospectively evaluated. The MCP regimen consists of mitoxantrone 8 mg/m² i.v. day 1 + 2, chlorambucil 3 x 3 mg/m² p.o. day 1 – 5 and prednisone 25 mg/m² p.o. day 1–5. Information analysed included localization of the lymphoma, clinical stage, pretreatment, number of chemotherapy cycles administered, toxicity, response to treatment, follow-up time, relapse and survival. Results: A total of 15 patients (6 female/9 male aged between 34 – 88 years) with histologically ascertained MALT lymphoma undergoing treatment with the MCP regimen were identified from our records. Ten patients had extragastric lymphoma, while 5 patients suffered from gastric MALT lymphoma. All patients were chemotherapy-naive, while two had been locally irradiated before application of MCP for recurrent disease. A total of 74 cycles was administered to our patients, with a median number of 5 cycles per patient. Eight (53%) patients achieved complete remission after a median follow-up time of 16 months (range: 12 – 29). Conclusions: Our data suggest that MCP is an effective and well tolerated regimen for treatment of patients with MALT lymphoma irrespective of localization. Judging from our data, MCP also appears to be a feasible regimen in elderly patients.

Remission of lymphoma of large granulated lymphocytes after treatment with fludarabine, cyclophosphamide and mitoxantrone: A case report

C. Waterhouse, C. Nerl
Munich, D

In a 44 year old patient who presented with anaemia, granulocytopenia and mild splenomegaly lymphoma of large granular lymphocytes was diagnosed. 44% of blood lymphocytes showed an immunophenotype of T cells positive for CD2, CD3, CD5, CD7, CD8 and CD57. Bone marrow and spleen were involved. Evidence of monoclonality of the Tcell receptor g-chain was only found in the aspirate of the spleen. Clinically the patient became dependent on transfusions. Between Dec. 1999 and March 2001 65 units of packed erythrocytes were transfused. Ferritin levels reached 4430ng/ml. Several sequentially used immunosuppressive and cytoreductive treatment regimens failed to show any change in transfusion frequency; prednisolone for three months, started with 100 mg daily, cyclosporine A 400 mg daily for three months, methotrexate 20mg weekly for three months. It was then decided to treat the patient with CHOP(cyclophosphamide, doxorubicin, vincristine and prednisolone). The anaemia improved and after 2 of 6 cycles of CHOP the patient needed no more transfusions. Because of remaining granulocytopenia and persistence of a small fraction of LGL-cells in the blood and bone marrow, interferon maintenance therapy was started with 3Mio IU a-interferon (IFN) three times weekly. This treatment was not well tolerated and did not lead to any further improvement. Granulocytopenia became worse (neutrophils 200/ml) and the haemoglobin level started to fall (from 15 g/dl to 12 g/dl). After 3 months IFN was therefore discontinued and polychemotherapy with fludarabine, cyclophosphamide and mitoxantrone (FCM) was started. Four cycles of this regimen were given. As a result all blood values normalized and the spleen size decreased. A small population of the pathological LGL clone could only be detected by PCR in the bone marrow which was histologically normal. After reaching this stable partial remission, regular erythropoiesis has been performed since August 2002 in order to reduce the massive transfusion induced iron overload. Upl till now 8 times 500ml erythrocyte concentrate were removed. The ferritin level fell from 4430 ng/ml to 1200 ng/ml. Clinically the patient is doing well and is able to lead a normal life.

Splenomegaly and pancytopenia in NK-type LGL leukemia with an unusual phenotype – a case report

H. Thomssen, R. Nanan, A. Marx
Bremen, Würzburg, D

Natural killer (NK) cell neoplasms are a rare group of Non-Hodgkin lymphoma. One of these is NK-type LGL leukemia which is characterized by increased numbers of LGL associated with tissue invasion of marrow, spleen and liver. The clinical symptoms probably arise from autoimmunity. We report on a 64-year-old man who was admitted with pancytopenia, hepatomegaly and a progressive splenomegaly. In the peripheral blood there were agranulocytosis, lymphocytosis, thrombocytopenia, anaemia and hypogammaglobulinaemia. The bone marrow (smear and trephine biopsy) showed hyperplasia of the granulopoiesis with a maturation stop at the stage of promyelocytes, an increase in erythropoiesis, and hyperplasia of the megakaryopoiesis. There were signs of haemophagocytosis. Infiltrating lymphoid cells were mainly part of a CD3 positive T cell population. There was no clonality for immunoglobulin heavy chain or for the T-cell receptor (TCR). Since the cause of the pancytopenia and hepatosplenomegaly remained unclear, splenectomy (weight 2800g) and a liver biopsy were performed. Since lymphoid cells were seen throughout the spleen and in the sinuses of the liver. By immunohistochemistry (IHC) and flow cytometry, the cells were positive for CD2, CD7, CD16, TIA-1, granzyme B, CD45 and CD94, while other killer-inhibitory and killer activating receptors were negative. The cells were negative for CD1a, sCD3, CD4, CD5, CD8, CD34, CD56, CD57, TdT and Epstein Barr Virus. Analyses of TCR rearrangements showed polyclonal bands. Strong lytic activity against K562 cells was demonstrated. The cells were positive for CD2, CD3, CD5, CD7, CD8, CD34, CD56, CD57, TdT and Epstein Barr Virus. Analyses of TCR rearrangements showed polyclonal bands. Strong lytic activity against K562 cells was demonstrated. The cells were negative for CD1a, sCD3, CD4, CD5, CD7, CD8 and CD56. The T-cell receptor (TCR) was expressed on the surface of the leukocytes, suggesting that the cell population was of lymphoid origin.

Successful treatment of lymphoid granulomatosis with anti-CD20 monoclonal antibody – rationale and a case report

W. Grothe, K. Jordan, T. Kegel, H.-J. Schmoll
Halle, D

Rationale: Lymphoid granulomatosis originally described by Liebow 1972 is a rare angiocentric and angiodestructive Epstein-Barr positive B-cell lymphoproliferative disorder. Treatment options include corticosteroids, antiviral therapy with interferon alpha and ganciclovir as well as combination chemotherapy however long term prognosis is very poor. Current available data suggest a beneficial role of Rituximab.

Case report: A 21-year-old white woman was admitted to the University of Halle for evaluation of a mediastinal bulk (50 x 60 mm) diagnosed in May 2002. Except chronic cough her health status was not impaired. Thoracotomy and biopsy specimen was revealed after uncertainty of the diagnosis: CD20+–lymphomatoid granulomatosis with clear EBV-association. Bronchoscopy was done and cytologic examination showed no malignant cells. Bone marrow aspirate showed a hypercellular and plasmacytoid bone marrow with normocellular bone marrow. The plasma cells were negative for CD20.

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marrow biopsy specimen were normal. Computed tomographic scans showed no hilar and abdominal lymphadenopathy. In regard of the EBV association and lack of standard therapy options, we initiated an antiviral therapy with Valganciclovir. After 2 months of continuing treatment and no change of the mediastinal bulk, treatment was completed with interferon-alpha. After responding to that combination therapy shortly for 1 month with a moderate decrease of the tumour mass, MR-imaging of the thorax in October 2002 showed progression of the mediastinal bulk. On the basis of positive expression of CD 20 on the lymphoid cells we started treatment with Rituximab with four weekly doses of 375 mg/m² followed by a monthly dose schedule. After 3 months of treatment MR-imaging of the thorax showed an impressive remission of the bulk from 80 × 60 mm to a residual tumor mass with a size of 10 × 15 mm. During the whole treatment period the patient did not experience any therapy-associated side effects and was able to work in a full time job. For consolidation radiotherapy of the initial bulk was performed. Conclusion: Rituximab is a well tolerated treatment option with a favourable toxicity profile even in rare aggressive CD20+/CD10+/bcl2-, CD10-/bcl2+/bcl6- variants: germinal center B-cell-like (GC), activated B-cell-like (ABC) and a ‘third’ type. The present study was designed to analyze the relationship between immunophenotype, clinical features and outcome in DLBCL with respect to its gene expression profile. A TMA comprising 113 different cases of DLBCL with complete clinical follow-up (mean observation period 40 months) was constructed. Expression of CD10, CD20, bcl-2, bcl-6 and Ki-67 was assessed by immunohistochemistry. Disease specific survival (DSS) was analyzed with the Kaplan-Meier method. Nonparametric tests were applied to demonstrate correlations between immunoprofiles and stage, IPI, LDH, Beta2-microglobulin, age, sex and primary site of involvement. Multivariate analysis for the effect of each expressed factor as well as for clinical parameters was performed using a Cox stepwise regression analysis. Samples were segregated into GC-(bcl-6+/CD10+/bcl2-, n = 46 41%), ABC-(bcl-6-/CD10-/bcl2+, n = 24, 21%) and unclassifiable DLBCL (all other expression profiles, n = 43, 38%). Median DSS of patients with GC-DLBCL was 109 months compared to 69 months in unclassified cases and 51 months in the ABC-type. Clinical parameters, except for involvement of more than one extranodal site (GC=ABC p = 0.003), were similarly distributed in the different DLBCL-groups. Multivariate analysis showed IPI (p = 0.027), age (p = 0.013) and and lymphoma subtype (p = 0.024, Figure 1) to be of independent prognostic significance for DSS in DLBCL. Younger patients and patients with low IPI (0-1) as well as with GC-DLBCL show a better DSS. This might be important in stratifying patients with DLBCL in regard to risk-adjusted treatment.

P888
Prognostic impact of clinical and immunohistochemical parameters in 113 cases of diffuse large B-cell lymphoma – a tissue microarray based study
F. Augustin, A. Pehrs, A. Zimpfer, P. Went, M. Fiegl, R. Greil, S. Dinrhofer, A. Tzankov
Innsbruck, A; Basel, CH

Gene expression profiling of DLBCL revealed three distinct phenotypic variants: germinal center B-cell-like (GC), activated B-cell-like (ABC) and a ‘third’ type. The present study was designed to analyze the relationship between immunophenotype, clinical features and outcome in DLBCL with respect to its gene expression profile. A TMA comprising 113 different cases of DLBCL with complete clinical follow-up (mean observation period 40 months) was constructed. Expression of CD10, CD20, bcl-2, bcl-6 and Ki-67 was assessed by immunohistochemistry. Disease specific survival (DSS) was analyzed with the Kaplan-Meier method. Nonparametric tests were applied to demonstrate correlations between immunoprofiles and stage, IPI, LDH, Beta2-microglobulin, age, sex and primary site of involvement. Multivariate analysis for the effect of each expressed factor as well as for clinical parameters was performed using a Cox stepwise regression analysis. Samples were segregated into GC-(bcl-6+/CD10+/bcl2-, n = 46 41%), ABC-(bcl-6-/CD10-/bcl2+, n = 24, 21%) and unclassifiable DLBCL (all other expression profiles, n = 43, 38%). Median DSS of patients with GC-DLBCL was 109 months compared to 69 months in unclassified cases and 51 months in the ABC-type. Clinical parameters, except for involvement of more than one extranodal site (GC=ABC p = 0.003), were similarly distributed in the different DLBCL-groups. Multivariate analysis showed IPI (p = 0.027), age (p = 0.013) and and lymphoma subtype (p = 0.024, Figure 1) to be of independent prognostic significance for DSS in DLBCL. Younger patients and patients with low IPI (0-1) as well as with GC-DLBCL show a better DSS. This might be important in stratifying patients with DLBCL in regard to risk-adjusted treatment.

P889
Claoip (Liposomal Doxorubicin Modified Chop) in patients with high grade NHL: A phase I study with 14 day intervals
U. Keilholz, G. Hütter, J. Siehl, A. Schmittel, E. Thiel
Berlin, D

CHOP is the most efficient and least toxic treatment for the first line treatment of high-grade Non-Hodgkin's lymphoma (NHL). However, because of the cardiotoxicity of doxorubicin the use of CHOP is restricted in patients being at risk for cardiotoxicity (e.g. age, pre-existing cardiac dysfunction). Previously, we have shown that a 21-day CHOP regimen with 20 mg/m² of Caelyx can safely be administered to patients with high-grade NHL with cardiac impairment. We now performed a phase I study in patients with high grade NHL and risk factors for cardiac toxicity with the CLAOIP14 regimen given every 2 weeks (Cyclophosphamide 750 mg/m² day 1, Vincristine 1 mg i.v. day 1, Prednim 100 mg p.o. day 1-5) with G-CSF support and escalating doses of Caelyx, beginning with 20 mg/m² day 1. Seventeen patients with a median age of 78 years (range 49-92) have entered the study. 78 cycles (median 5, range 2 to 6) have been given. Main side effects were haematologic: leucopenia grade III/IV in 2 patients, thrombopenia grade III/IV in 3 patients. However, at the dose level of 25 mg/m², frequent infectious episodes, and palmar-plantar erythema (PPE) were observed. No other organ toxicity greater grade II and especially no cardiac cardiotoxicity was noticed. Currently a third cohort of patients is accrued with 2 mg/m² of Caelyx and a single injection of Peg-G-CSF (Neulasta) on day 5, and in the initial 3 patients, neutropenia could be as effectively prevented with Peg-G-CSF as in the previous cohort by conventional G-CSF. All patients are evaluable for efficacy. Remission rate is NOSR (53%), 4 PR (23%), and 9 CR (24%). CLAOIP14 is safe and effective in patients with high grade NHL and cardiac risk. The recommended dose for randomized trials is 20 mg/m². The substitution of conventional G-CSF by Peg-G-CSF appears to be feasible, and offers easier management and compliance, especially for elderly patients.

P890
Application of risk factors for prognosis in patients with human-immunodeficiency-virus-related non-Hodgkin's lymphoma
M. Ruhnke, C. Lüke, J. Unseld, D. Huhn, K. Possinger
Berlin, D

Background: The association between Human Immunodeficiency-Virus (HIV) infection and the development of lymphoma has been known since the early days of AIDS. While the introduction of highly active antiretroviral therapy (HAART) was able to dramatically reduce the incidence of several HIV-related diseases like opportunistic infections and some malignancies (e.g. Kaposi-Sarkoma), the incidence of AIDS-related Non Hodgkin lymphomas (AIDS-NHL) is steadily increasing. For HIV-related NHL, the treatment strategies are still controversial and therefore prognostic indicators are particularly important. The international prognostic index (IPI) was created in the 1998ies as an instrument to estimate the individual prognosis of patients with aggressive NHL to adjust therapy regimens. As the relevance of prognostic indices in HIV patients is not clear, we compared the practicability of three scores used in these patients: 1. IPI, 2. the adapted age-adjusted-IPI, 3. High-versus standard-risk score (German HIV-lymphoma study group). Methods: Seventy-four patients with HIV-related systemic NHL, attended at a single institute over a 12 year period, were analysed retrospectively. Univariate and multivariate methods were used for statistical analysis. Results: According to IPI (age-adjusted IPI) 42% (20%) of the evaluated patients were assigned to the low risk group, 20% (26%) to the low intermediate group, 32% (43%) to the high-intermediate group and 6% (11%) to the high risk group in the whole study population. The correlation of the initial estimation according to the IPI with the course of the disease was generally poor. Especially in the group of patients with short survival (<12 month) the risk was underestimated: merely 12% of patients were initially classified for high risk group, but 25% for low risk group. Nearly the same results were obtained for the age-adjusted IPI (Low: 3%, low intermediate: 25%, high intermediate: 50% high:22%) as well as for the German-HIV-lymphoma-group-score (standard risk = no risk factor 47% or one risk factor 29%; high risk (= two or three risk factors) 24%) respectively. Conclusion: The present-
ed data indicate, that the International Prognostic Index for aggressive NHL as well as the age-adjusted IPTI and the German lymphoma group score tend to underestimate risk in HIV-positive individuals. A modification for this group of patients might be reasonable.

### P891

**Efficacy of CHOP regimen in Post-transplant lymphoproliferative disorders (PTLD). A retrospective study on 25 cases**

S. Oertel, M. Papp-Vary, S. Choquet, V. LeBlond, B. Dörken, H. Riess

**Berlin, D; Paris, F**

**Background:** PTLD represent a rare complication of organ transplantations, ranging from 1% to more than 15%, depending on the immunosuppression and on the type of transplant. Until now, there is no consensus on treatment modalities and the mortality remains very high. **Aims:** To proof the efficacy of the lymphoma gold standard chemotherapy, the CHOP regimen, in PTLD, as a first line. **Methods:** A retrospective analysis of 25 PTLD, treated in two centers, Pitie Salpetriere, Paris, France, and Charite Campus Virchow, Berlin, Germany, has been made. **Results:** On the 25 patients, 8 were females and 17 males, with a mean age of 45 [22–68]. The transplanted organ was a heart in 9 cases, a kidney in 8, liver in 4 and lungs in 3. The PTLD was monomorphic in 21 cases and polymorphic in 4. The B phenotype was predominant (22/25) and the tumor was EBV positive in 10 cases, negative in 14, not done in one case. At presentation, the mean number of localizations was 3 [1–7], and PTLD were mainly stage IV (15 cases) from the Ann Arbor classification (4 stages III, 5 stages II, 1 stage I). CHOP regimen has been used in standard dosages, for one to six cycles, depending on the clinical response. The total response rate was 96% (12 complete remissions, 4 partial remissions), with a median overall survival of 329 days [13–3294]. Nine patients are still alive; six (24%) died from disease progression, eight (32%) from infection, one from major hepatic toxicity, and one from graft rejection. Between the responders, six relapsed, with a mean time to progression of 332 days [6–2124]. **Conclusion:** CHOP regimen, despite a rather good response rate, remains a toxic first line therapy in PTLD. It is associated with a therapy-related-mortality of 1/3, mainly infections which makes the use of growth factors like GCSF necessary. In these particularly fragile patients, alternative treatments need to be developed, as monoclonal antibodies alone or before CHOP.

### P892

**Treatment of patients with post-transplant lymphoproliferative disorder with a sequential treatment consisting of anti CD20 antibody Rituximab and CHOP + G-CSF chemotherapy**


**Berlin, D; Vienna, A; Paris, F**

**Background:** Immunosuppressive therapy in organ transplant recipients relates to an increased risk of developing malignancies, e.g. with an incidence of 1 – 10% for B-cell PTLD. **Aims:** Showing a low toxicity profile, treatment with the anti-CD20 monoclonal antibody rituximab is a promising alternative. **Methods:** We conducted a multicentre phase II trial investigating rituximab as monotherapy in 25 pts. with PTLD. The treatment consisted of four infusions rituximab 375 mg/m² on days 1, 8, 15 and 22. **Results:** (23.0–73.1) with a mean follow up time of 15.6 months. (0.6–53.6). Histology comprised 18 diffuse large B-cell-, 2 marginal zone-, 1 Burkitt lymphoma and 4 polymorphic lymphoproliferations. The mean overall survival is 42.2 months with 20 pts. still alive. One pt. died from chronic rejection and one due to progressive PTLD. In total 13 pts. (52%) achieved a complete remission (CR) with a mean duration of 25.1 months. Partial remission was observed in 1 pt., minor remission in 2 pts, no change in 8 pts. and 1 pt. experienced progressive disease. Overall response rate is 64%. Early relapse (3, 6 and 12 months) occurred in 3 pts. after CR (23%). Switch to chemotherapy resulted in CR in all 3 pts. (49+, 11+, 7+). **Summary/conclusions:** Monotherapy with rituximab proved to be well tolerated and effective in the treatment of PTLD. Adverse events were moderate and infrequent (12 complete remissions, 4 partial remissions). **Discussion and study proposal:** Due to the rate of early relapse (23%) an international multicentre phase II trial (100 pts expected) was initiated in 2003 and is open to participation investigating the efficacy, safety and the tolerability of a sequential therapy consisting of rituximab 375 mg/m² on days 1,8,15 and 22 followed after a 4 weeks interval by CHOP chemotherapy + GCSF every 3 weeks at days 50, 72, 94 and 116. In case of disease progression during rituximab phase the patients directly enter CHOP chemotherapy. We speculate, that after the pre-phase treatment with rituximab, CHOP chemotherapy will reduce the rate of relapses and will be less toxic due to the lower tumor burden and improved performance status of the pts. Furthermore the total number of cytoxic cycles of CHOP-therapy is reduced from 6 or 8 to 4 cycles and hopefully treatment related severe or lethal toxicities, frequently reported in patients with PTLD undergoing cytoxic chemotherapy, may be prevented.

### P893

**Dexamethasone, high-dose cytobarine, and cisplatin (DHAP) in combination with Rituximab as salvage treatment for patients with refractory or relapsed aggressive non-Hodgkin’s lymphoma**


**Bonn, Aschaffenburg, Marburg, Nuremberg, Heidelberg, Hildesheim, Dessau, D**

**Background:** With commonly used salvage chemotherapy regimens like DHAP, response and overall survival rates are unsatisfactory. Therefore, we designed a multicenter phase II trial to evaluate the safety and efficacy of the combination of the immunotherapeutic agent rituximab with the DHAP regimen in patients who relapsed after or were resistant to a CHOP-like regimen. **Methods:** 43 patients with relapsed or resistant aggressive B-cell NHL were included so far. Median age was 64 years. Of evaluable patients, 26 were in first relapse; 21 had recurrent disease within the first 12 months post treatment, 8 patients had second or subsequent relapse, 9 had primary refractory disease. Rituximab infusions (375 mg/m² per dose) on day 1 were followed by dexamethasone 40 mg d3–6 (d3–5 in first cycle), cytobarine 2 X 2000 mg/m² d 4 (2 X 1000 mg/m² in first cycle), and cisplatin 25 mg/m² d3–6 (d3–5 in first cycle) for a maximum of 4 cycles. When hematological toxicity did not allow continuation of chemotherapy on day 22, rituximab was given as single agent on day 22 and the next cycle was postponed until day 29. **Results:** The overall response rate in evaluable patients was 43.0%. 26.0% of patients experienced a complete response, 17.0% of patients had a partial response, 17.0% of patients had stable disease. 39.0% of patients showed progressive disease. Grade 3/4 nausea and vomiting were the only severe toxicities attributed to rituximab in one patient. Main toxicities were neutropenia and thrombocytopenia leading to dose reductions in 17 of 43 patients, primarily after the first cycle. Grade 3/4 neutropoetoxy occurred in 2 patients. **Conclusion:** Analysis of the data suggests that the combination of rituximab with the DHAP regimen in the treatment of relapsed or refractory aggressive lymphoma is feasible and effective. However, due to the compromised bone marrow reserve in this intensively pretreated patient population, dose reductions were mandatory during the first cycle in order to avoid severe hematological toxicities. In view of the very unfavorable prognostic factors in our patient population, results with rituximab in combination with the DHAP-regimen are encouraging.

### P894

**Salvage chemotherapy with dexamethasone, cytobarine and oxaliplatin (DHAOx) in recurrent non-Hodgkin’s lymphoma**

K. Friedrichsen, S. Nesper, M. Teich, A. Hänel, R. Herbst, M. Hänel

**Chemnitz, D**

**Introduction:** Based on the DHAP protocol the DHAOx regimen was developed by the substitution of cisplatin with oxaliplatin. Encouraging results with DHAOx in 24 patients (pts) with relapsed or refractory non-Hodgkin’s lymphoma (NHL) were reported by Chau et al. (Br J Haema- tol 2001, 115: 736–792). Furthermore we report our single center experiences with DHAOx in recurrent NHL. **Patients and methods:** Between 09/2002 and 03/2003 a total of 12 pts (8 male, 4 female) with chronic lymphoctic leukemia (n = 4), mantle cell lymphoma (n = 2) and diffuse large B cell lymphoma (n = 6). were treated. Median age of the pts was 60 years.
Abstracts

P896

Germicidamine, vinorelbine and prednisone for refractory or relapsed aggressive lymphoma, results of a phase II single center study

H. Müller-Beißenhirtz, C. Kasper, H. Rückel, U. Dührsen
Essen, D

Introduction: The optimum salvage therapy for patients with relapsed or refractory aggressive non-Hodgkin's lymphomas (NHL) not qualifying for platinum-based and/or high-dose chemotherapy is not known. Both gemcitabine and vinorelbine have reported single agent activity in patients with advanced aggressive NHL. We conducted a prospective phase II study evaluating a novel regimen consisting of gemcitabine 1000 mg/m² and vinorelbine 30 mg/m² i.v. on days 1 and 8, and prednisone 100 mg/d p.o. on days 1–8 (GVP). Dose reduction was applied according to haematological or other toxicities. A maximum of 6 cycles at 21 day intervals was planned with response evaluation after every second cycle.

Patients: Between November 1999 and July 2002 15 patients with relapsed (n = 7) or refractory (n = 8) aggressive NHL (4 transformed) were enrolled. The median age was 68 years (25–75). Diagnoses included: B lymphoblastic (n = 1), diffuse large B cell (n = 10), large anaplastic T cell (n = 2) and peripheral T cell NHL (n = 2). LDH levels were elevated in 11 patients (73%). The median international prognostic index (IPI) score was 3 (6 patients with an IPI score of 4 or 5). Patients had a median of 3 previous therapies (range: 2 – 4).

Results:

A total of 49 cycles were administered, with 7 patients receiving more than 2 cycles. Five patients (33%) achieved a complete remission (CR) and 3 patients (20%) a partial remission for an overall remission rate of 53%. The median overall survival and the median time to next treatment were 8.9 (range: 1 – 32) and 4.4 months (range: 1 – 17), respectively. The principal toxicity was myelo-suppression leading to dose reduction in 21 cycles (43%). Haematological toxicities of World Health Organisation grades III/IV were: leukopenia in 57%, thrombocytopenia in 33% and anaemia in 16% of all courses. The most common non-haematological toxicity was phlebitis, occurring in 5 patients (33%). Three patients had grade 3 infections. There was no treatment-related mortality. Conclusion: GVP shows substantial activity in heavily pretreated patients with prognostically unfavourable relapsed or refractory aggressive lymphomas. The regimen is generally well tolerated and can be given in an outpatient setting, but haematological toxicity is common and dose-limiting.

P897

Sequential high-dose chemotherapy with autologous stem cell support in combination with the anti-CD20 antibody Rituximab is feasible and effective in relapsed aggressive non-Hodgkin's lymphoma

J.O. Staak, J.-P. Glossmann, V. Diehl, A. Engert, A. Josting
Cologne, D

Introduction: Patients (pts) with aggressive Non-Hodgkin's lymphoma (NHL) can be cured by combination chemotherapy, but those with relapse still have a poor prognosis. High-dose chemotherapy (HDCT) with autologous stem cell support (ASCT) improves outcome of these pts as shown in the precursor study with response rates of 48% (32% CR, 16% PR) and FTT/OS of 42%/54% at the final evaluation. Chemosensitivity and achievement of minimal disease status prior to HDCT are important prognostic factors in NHL pts treated with HDCT. Rituximab demonstrated encouraging activity in aggressive NHL and showed low toxicity in the setting of combined immunochemotherapy. Patients and methods: The intensified salvage program was modified by addition of rituximab to the chemotherapy cycles followed by a final myeloablative course with stem cell reinfusion. Eligibility criteria include pts with age 18–65 years and eligible for HDCT with histologically proven CD20+ relapsed NHL. Treatment program consists of 2 cycles DHAP (dexamethasone, cytarabine, cisplatin) plus rituximab (375mg/m²); pts with PR or CR receive cyclophosphamide (4g/m²) plus rituximab followed by PBSC harvest; methylxate (8g/m²), vincristine (1.4mg/m²) plus rituximab; and etoposide 2g/m² plus rituximab. The final myeloablative course BEAM plus rituximab followed by ASCT. Results: 16 pts (median age 49 years, range 22–64) with relapsed aggressive NHL have been enrolled (stage I/II: 6, stage III/IV: 10) so far. All pts had CHOP or CHOP-like regimens as first-line therapy, 5 had prior radiation. Median time to progression was 12 months. This chemoimmunotherapy combination regi-
men was well tolerated in all pts without side effects exceeding the toxicity expected from chemotherapy alone. Treatment was discontinued in one pt due to severe heart failure. 13 pts were available for restaging after 2 cycles DHAP with 3 CR, 9 PR, 1 PD. Stem cell harvest was successful in 11/11 patients. 9 pts were available for final restaging evaluation with 6 CR, 3 PR. Conclusion: Preliminary results suggest feasibility and efficacy of rapid sequential administration of DHAP and high doses of cyclophosphamide, mitotrexate and etoposide, each in combination with rituximab without affecting the tolerability of the final myeloablative BEAM. The combination regimen allows adequate mobilization of stem cells and is effective in pts with relapsed aggressive NHL.

P998
Allogeneic and autologous stem cell transplantation for patients with relapsed or primary refractory lymphoma: A single center experience
A.N. Hünerleitköglu, G. Kobbe, R. Fenk, R. Kronenwett, R. Haas
Düsseldorf, D
We report on a group of 82 pts with malignant lymphoma who received high-dose therapy followed by autologous PBSC or allografting from an HLA matched sibling or unrelated donor. Common denominator for all patients is the failure to first line therapy, either they did not respond or relapsed. According to the WHO classification 23 patients had follicular NHL, 5 patients mantle zone lymphoma, 4 patients intermediate NHL, one patient had B-CLL, one marginal zone NHL (low grade NHL N = 34), 30 patients had a diffuse large B-cell NHL, 5 patients T precursor lymphoblastic NHL, 4 patients a Burkitt NHL, 8 patients precursor lymphoblastic and one patient anaplastic large cell lymphoma (high grade lymphoma n = 48), respectively. High dose therapy consisted of BCNU, etoposide, cytosine-arabinoside, melphalan (BEAM), or a combination of cytoxan, etoposide, BCNU and idarubicine (I-CVB) or total body irradiation and cytoxan. At the time of this writing (median follow up time = 52 months), 24 of 34 patients with low grade NHL are in remission (21 CR/3PR), whereas 9 patients died of progressive disease. There is one patient alive with progressive disease (overall survival 70%). In 8 patients the 14:18 translocation was examined. In 4 patients the translocation was detected at the time of high-dose therapy. 7 patients had a clearing of t (14:18) after the high dose therapy (median follow up time = 10.5 months). All these patients were treated with interferon for maintenance therapy.

P999
Autologous tandem transplantation with high-dose etoposide mobilized peripheral blood stem cells in patients with primary progressive Hodgkin’s and aggressive non-Hodgkin’s lymphoma
Cologne, Lübeck, Cottbus, D
Background: Patients with primary progressive Hodgkin’s (HD) or aggressive non-Hodgkin’s lymphoma (NHL) have a particularly poor prognosis. Here we report the results of a phase II study using autologous tandem transplantation. Patients and methods: We investigated the effectiveness of tandem high-dose chemotherapy (HDCT) followed by autologous stem cell transplantation (ASCT) in patients aged 18–50 years with primary progressive HD and aggressive NHL. Progressive disease was defined as progression during induction treatment or within 90 days after the end of treatment. Patients received high-dose etoposide (2000 mg/m²) followed by peripheral blood stem cell harvest (PBSC). The first HDCT (TM3) consisted of thiopeta (75mg/m²), mitoxantrone (40mg/m²) and carboplatin (900mg/m²). Patients with no change (NC), partial remission (PR) and complete remission (CR) after the first HDCT then received BEAM with Carmustin (300mg/m²), etoposide (1200mg/m²), cytarabine (1600mg/m²) and melphalan (140mg/m²). Patients with bulky disease (> 5 cm) or residual lymphoma 30 days after the second HDCT therapy additionally received involved field radiotherapy.

Results: Twenty-eight patients were included. PBSC harvest with more than 4 x 10⁶ CD34+cells/kg was successful in all patients. At the time of this interim analysis 10 patients with HD and 15 patients with aggressive NHL were evaluable for treatment outcome. The median age was 35 years (range 22–50 years). Six patients (24%) had stage II disease, 4 patients (16%) stage III and 15 patients (60%) stage IV disease. Two patients with HD achieved a CR and 5 patients a PR, resulting in an overall response rate (RR) of 70% for HD patients. Three patients (30%) had a treatment failure including one death due to septicemia during treatment. Six patients with aggressive NHL were in CR, two patients in PR (RR 53%). From the 7 patients (47%) with treatment failure, one died due to infection during treatment. Freedom from treatment failure (FFTF) and overall survival (OS) for all patients after 12 months was 28% and 40%, respectively. There was no outcome difference patients with HD and patients with NHL. Conclusion: Tandem HDCT followed by ASCT is tolerable and effective in this poor prognostic patients.

P900
Extensive cutaneous CD30-positive high grade T cell lymphoma in a patient with hyper-IgE-syndrome
K. Namberger, A. Lux, R. Hein, M. Kremer, F. Fend, C. von Schilling, D. Juyster, C. Peschel
Munich, D
We report an unusual case of a CD30-positive high grade T-cell-lymphoma with extensive skin manifestation and no visceral involvement refractory to chemotherapy and immunotherapy in a patient with a long lasting Hyper-IgE-Syndrome. The hyper-IgE-syndrome is a rare, complex immunoregulatory disorder characterized by hyperimmunoglobulinemia E, recurrent bacterial infections and chronic eczematoid dermatitis usually manifesting in early life. There is no proven evidence of increased risk for lymphomas in hyper-IgE syndrome.

A 38-year old female patient with hyper-IgE-syndrome for more than 20 years developed a CD30-positive cutaneous T-cell-lymphoma involving arms, legs, body, head and neck. Visceral involvement could be excluded. Histology showed evidence of secondary transformed mycosis fungoides as an aggressive lymphoma with regression of CD 30. However cells did not look big enough to diagnose large cell anaplastic lymphoma. We also found a malignant T-cell clone in skin biopsies at early stage of the disease.

There was neither response to variant chemotherapies such as CHOP, IEV, FCM, nor to immunotherapy with the monoclonal CD52-antibody Campath. Finally the patient died of progressive lymphoma and sepsis (multiresistant pseudomonas aeruginosa) from ulcerative skin infections. In conclusion high grade cutaneous T-cell lymphoma could be a rare but crucial manifestation in hyper-IgE syndrome showing no response to chemotherapy or immunotherapy.
of chemotherapy while still being in complete remission (CR). **Patient:** A 67 year old male patient with stage IVB DLCL (generalized lymphadenopathy with infiltration of the left kidney and the skin) received 6 cycles of CHOP21 and has remained in CR since. However, 2 years after completion of chemotherapy he complained about increasing pain in his wrists, elbows, shoulders, knees, MCP, PIP and MTP joints bilaterally and morning stiffness of approximately 1 h duration. On physical exam there was swelling of all tender joints and a palpable effusion of the right knee.

Radiographic studies showed extensive destructive lesions of both hands and feet. Rheumatoid factor and antineuclear antibodies were present at high titers. Therapy with methotrexate and corticosteroids was initiated with quick alleviation of symptoms. **Discussion:** Chronic B cell stimulation due to persistent inflammation on the joint level and further progression of monoclonal B cell proliferation, the latter possibly augmented by immunosuppressive therapy, is being considered as the major etiologic factor in the development of RA in patients with AD. Since infection with EBV has been implicated in the pathogenesis of RA as well as NHL, viral agents may be of additional significance. In contrast to this our patient developed symptomatic RA 2 years after the diagnosis of lymphoma, although considering the severity of the radiologic findings arthritic inflammatory changes were only incidentally observed. In our patient, diagnosis of lymphoma prior to symptomatic rheumatoid arthritis suggests that there may be a so far unrecognized factor predisposing to both RA and NHL.

**Conclusions:**

Diagnosis of lymphoma in the absence of NHL in patients with AD. Since infection with EBV has been implicated in the pathogenesis of RA as well as NHL, viral agents may be of additional significance. In contrast to this our patient developed symptomatic RA 2 years after the diagnosis of lymphoma, although considering the severity of the radiologic findings arthritic inflammation must have been preexistent some time before articular swelling and pain started. **Conclusions:** Diagnosis of lymphoma prior to symptomatic rheumatoid arthritis in our patient suggests that there may be a so far unrecognized factor predisposing to both RA and NHL.

**P904**

Impact of age at diagnosis in childhood and adolescent non-Hodgkin’s lymphoma: Treatment results in the pediatric multicenter studies NHL-BFM


We analysed whether in the co-operative NHL-BFM trials on Non-Hodgkin-Lymphoma (NHL) the probability of event-free survival (pEFS) differs according to patient’s age at diagnosis. From 10/1986 to 03/2001, a total of 1,904 patients (pts) up to 18 y of age suffering from all entities of NHL were registered in the multicentre trials NHL-BFM 86, 90, 95, 339 pts (18%) were 0–4 y old, 728 pts (38%) were 5–9 y old, 631 pts (33%) were 10–14 y old and 206 pts (11%) were >15 y of age. Treatment was stratified according to lymphoma entity. Pts with lymphoblastic lymphomas (LBL) received an ALL-type strategy consisting of an 8-drug induction, consolidation, re-induction, maintenance and cranial radiotherapy (advanced stages only). All other pts received 2 to 6 five-day therapy courses based upon dexamethasone, cyclophosphamide, ifosfamide, MTX, cytarabine, doxorubicin, etoposide and i.t. therapy. With a median follow-up of 5.3 y (0.4–15.9 y), pEFS at 5 y for the whole group of 1904 pts was 84.1%.

Distribution of histological subtypes and pEFS according to age group was as follows:

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Pts (%)</th>
<th>pEFS (5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–4 y</td>
<td>381</td>
<td>96.1 (%)</td>
</tr>
<tr>
<td>5–9 y</td>
<td>728</td>
<td>93.5 (%)</td>
</tr>
<tr>
<td>10–14 y</td>
<td>631</td>
<td>92.2 (%)</td>
</tr>
<tr>
<td>&gt;15 y</td>
<td>206</td>
<td>84.0 (%)</td>
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</tbody>
</table>

**P902**

Unusual manifestation of Burkitt-like lymphoma in a young female patient

K. Horvát-Karajz, L. Flóró, G. Múzes, L. Sréter

Budapest, H

A 21-year-old woman met her dentist with toothache and swelling of the gingiva and extraction of the aching tooth was performed. Because of parodontitis antibiotic therapy was started but neither local nor systemic treatment was effective. Later on, fever, weight loss, a painful, large mass in the abdomen and swelling of breasts were present. Ultrasonography indicated a 160×120×105 mm large, hypervascularised ovarian tumor with peritoneal metastases so hysterectomy and adnexectomy was performed. Histological analysis revealed Burkitt-like lymphoma. Central nervous system manifestation was also present on liquor sample cytology. The HIV test was negative. Systemic and intrathecal chemotherapy concerning to B-ALL was administered. After six cycles of drug administration the patient was free of symptoms. She has got no HL-identic donor.

**Conclusions:**

Careful histological examination even of dental samples are important. In our case, hystrectomy could have been prevented if the correct diagnosis had been drawn earlier.

**P903**

Pathologic splenic rupture in aggressive non-Hodgkin’s lymphoma

L. Petersen, A.A.N. Giagounidis, M. Heinisch, R. Kasperk, C. Aul

Duisburg, D

Spleenic ruptures in hematological malignancies are rare events. When occurring without abdominal trauma in a spleen affected by neoplastic disease they are referred to as pathologic splenic ruptures. In a review of the medical literature since 1861 we have been able to identify 19 pathologic splenic ruptures in acute lymphatic leukemia (ALL) and 35 in Non-Hodgkin’s Lymphoma (NHL) excluding chronic lymphocytic and hairy cell leukemia. We present two cases of pathologic splenic rupture occurring in men, one with Burkitt-like leukemia and one with diffuse large cell NHL. Both patients had highly proliferative disease with rapidly rising lactate dehydrogenase (LDH) levels of 1700 U/l and 9800 U/l, respectively. Spleenic size was 20×13×5 cm and 21×16×10 cm, respectively. Spleenic rupture occurred before commencement of systemic chemotherapy. After splenectomy, both patients received cytotoxic therapy and went into ongoing complete remission. Three pathogenetic factors have been described for splenic rupture: Coagulation disorders leading to intravascular accumulation and splenic infarcts leading to loci of reduced resistance. Chemotherapy might additionally lead to release of proteolytic enzymes that affect splenic tissue structure. However, this mechanism is excluded in our cases, as cytotoxic treatment was begun later. Most pathologic ruptures of the spleen reported in the medical literature have occurred in enlarged spleens of 750 to 1500 g. Only two spleens weighed less than 200 g. Both our patients had spleen size and weight within this range. It seems that rapid growth of the spleens contributed to tissue damage and ultimately led to rupture. Diagnosis is usually based on abdominal ultrasound, CT-scan and aspiration of abdominal fluid. It is interesting to note that in both our cases, abdominal ultrasound did not reveal splenic disintegration. Diagnosis was based on aspiration of abdominal fluid. At surgery, one case showed a fissure that was located at the subhepatic fraction of the spleen and was therefore not visible to ultrasound; the other case had multiple small lesions of a maximum of 1.5 cm as well as two small fissures. Therapy for splenic rupture is splenectomy. 40 of 43 patients not undergoing splenectomy in the medical literature have died.
The MLT/MALT1 gene was recently discovered due to its involvement in the translocation (t(11;18)(q21;q21) associated with extranodal marginal zone B-cell lymphoma (MZBCL) of the MALT-type and characterizes about one third of the cases. The t(11;18) leads to a fusion of the apoptosis inhibitor gene API2 on chromosome 11 and the novel MLT/MALT1 gene, a human paracaspase, on chromosome 18, resulting in activation of the NF-kappaB signaling pathway. In order to screen for variant translocations and amplifications of MLT/MALT1, we have developed a novel, undirected two-color interphase FISH assay with two PAC clones flanking the MLT/MALT1 gene (PACs 117B5 and 59N7). A separation of the hybridization signals of the two PAC probes indicates a disruption of the MLT/MALT1 gene, for example by a translocation, whereas a colocalization or fusion of the hybridization signals is found in cases with an undisrupted MLT/MALT1 gene. This assay was applied to 108 MZBCL comprising 72 extranodal MALT lymphomas from various locations, 19 splenic, and 17 nodal MZBCL. In 19 MALT lymphomas (26% of MALT lymphomas), but in none of the splenic or nodal MZBCL, separated hybridization signals of the MLT/MALT1 flanking probes were found. Further FISH analyses with API2, IGH, and MLT/MALT1 specific probes showed that 12 of these 19 cases displayed the classical t(11;18) and the remaining 7 cases revealed the novel t(14;18)(q21;q21) involving the MLT/MALT1 and IGH genes. The frequency at which these translocations occurred varied significantly with the primary location of disease. The t(11;18) was mainly detected in gastrointestinal MALT lymphomas (28% of the cases), whereas the t(14;18) occurred in MALT lymphomas of the parotid gland and the conjunctiva (23% of the cases). Amplification of MLT/MALT1 was not observed in any of the lymphomas analyzed. Trisomy or polysomy of chromosome 18 evidenced by the presence of three to five MLT/MALT1-, BCL2-, and centromere 18-specific hybridization signals, was found in 13 MZBCLs. We conclude, that the translocations t(11;18)(q21;q21) and t(14;18)(q21;q21) represent the most structural aberrations involving MLT/MALT1 in MALT lymphomas, whereas true amplifications of MLT/MALT1 occur rarely in MZBCL.

The BCL10 and the MALT1 genes are involved in independent chromosomal translocations in MALT lymphoma. BCL10 is typically translocated to the Ig heavy chain locus and MALT1 fuses to the API2 gene. Physiologically BCL10 and MALT1 proteins are able to form a subcellular complex and the two molecules can cooperate to signal activation of NF-kappaB transcription factors. However, the precise molecular function and the hierarchy of BCL10 and MALT1 in signal transduction are not well understood. To identify and characterize the cellular pathways that are controlled by the BCL10/MALT1 complex, we generated Bcl10 deficient mutant mice by gene targeting. We found Bcl10 to be essential for the induction of adaptive immune responses in vivo. In lymphocytes Bcl10 acts as a central regulator of antigen induced cell proliferation. After antigen receptor ligation on B or T cells Bcl10 transmits cell-activating signals through a novel and specific pathway to NF-kappaB activation. We found biochemically that Bcl10 induces NF-kappaB through the activation of the I-kappaB kinase complex to mediate I-kappaB degradation. To investigate whether BCL10 operates up- or downstream of Bcl10, we overexpressed patient derived IAP2-MALT1 fusion proteins in cells from Bcl10 deficient and wild type control mice. Here we saw NF-kappaB activation in cells of both genotypes to the same extend. This demonstrates that IAP2-MALT1 can activate NF-kappaB in a Bcl10 independent fashion and suggests that MALT1 might be a downstream effector of Bcl10. These results might contribute to a better understanding of the molecular basis of MALT lymphoma.

Recently, the t(14;18)(q21;q21) involving the MLT/MALT1 gene and the immunoglobulin heavy chain (IGH) locus has been identified as a new recurrent chromosomal translocation in extranodal marginal zone B-cell lymphoma of the MALT-type. The t(14;18) characterizes MALT lymphomas in locations, such as liver, skin, parotid gland, and ocular adnexa which rarely harbor the t(11;18)(q21;q21). Other recurrent chromosomal aberrations in MALT lymphomas include trisomies of chromosomes 3, 12, and 18. Trisomy of chromosome 3, either complete or partial, represents the most frequent numerical chromosomal abnormality in MALT lymphomas and has been detected in up to 85% of cases using interphase fluorescence in situ hybridization (FISH). Despite of the high frequency of trisomy 3 in MALT lymphomas, this aberration is not specific for this lymphoma subtype and represents other numerical abnormalities not seldomly a clonal evolution event. In contrast to the t(11;18)(q21;q21), which almost always occurs as the sole chromosomal abnormality, the t(14;18) has been described in two cases as part of a complex karyotype and was associated with trisomy 3 and/or 18 in 4 of 12 cases analyzed (Steube et al., 2003). In the present study, 27 cases of MALT lymphomas of the parotid gland were analyzed by interphase FISH using differentially labeled probes hybridizing immediately downstream of the MLT/MALT1 gene (PAC 59N7) and within the alpha constant region of the IGH locus (cosmid C alpha1). Using this assay, the t(14;18) was identified in 5 of the 27 cases (19%). These 5 cases were further analyzed with alpha-satellite probes hybridizing to the centromeric regions of chromosomes 3, 12, and 18. In none of the cases an aneuploidy or trisomy of the analyzed chromosomes could be detected. According to our results, secondary numerical abnormalities of chromosomes 3, 12, and 18 are not frequent in t(14;18)-positive MALT lymphomas of the parotid gland, however, more of these rare cases need to be investigated to draw final conclusions.

Common variable immunodeficiency (CVID) is a primary immunodeficiency disease characterized by hypogammaglobulinemia, recurrent infections and heterogenous features including development of malignancies, especially non-Hodgkin’s lymphoma (NHL). The phenotypic defect is a failure in B-cell differentiation with impaired secretion of immunoglobulins, but various T cell abnormalities are also present. The primary genetic defect is unknown, the impaired interplay between T and B cells could be of considerable importance. We report on a 35-year-old female patient suffering from CVID since she was 19 years old. Despite treatment to intravenous immunoglobulines (IVIG) she had a history of recurrent sinopulmonary and gastrointestinal infections. Six years after the diagnosis of CVID she developed progressive lung infiltrates involving the alveolar interstitium. Open lung biopsy showed a lymphoid interstitial pneumonitis (LIP). This rare entity is known to be associated with various immunodeficiency states including CVID. It has a variable clinical course and progressive respiratory failure may occur due to pulmonary fibrosis. For many years the patient responded to corticosteroid therapy. Nine years later the patient developed severe progression of lung disease with dyspnoea, cough and fatigue in the absence of new bacterial, fungal or viral infection. High resolution thoracic CT scans showed progressive irregular peribronchovascular nodules, areas of bronchiectasis, hilar and mediastinal lymphadenopathies. Video-assisted thoracoscopic lung biopsy revealed extranodal marginal zone B-cell lymphoma of mucosa-associat- ed lymphoid tissue (MALT). Abdominal CT scans and ultrasound examination showed a splenomegaly, but there was no evidence of further lympho...
phadenopathies. Bone marrow aspiration including immunophenotyping revealed normal hematopoiesis without invasive lymphoproliferative disease. An optimal treatment program in patients with this rarely described low grade B-cell MALT lymphoma associated with CVID has not been defined. As chemotherapy adds to the immunosuppression intrinsic to CVID we chose Rituximab, a monoclonal antibody directed against the pan B cell antigen CD 20, for treatment. The patient continued to receive IVIG replacement. Four cycles of Rituximab led to continuous remission with resolution of lung nodules, lymphadenopathies and splenomegaly for more than one year without any serious side effects or deterioration of the CVID-related symptoms.

Pression was prolonged for the following weeks. Endoscopic ultrasound (EUS) showed thickening and infiltration of all layers of mucosal wall without lymph node involvement. On repeated controls no malignant tissue was found, yet healing was prolonged. Not before 7 weeks after initial presentation biopsies taken during gastroscopy revealed primary lymphoma of the stomach (stage E12 according to EUS). The tissue was reviewed by an experienced pathologist (HS) and diagnosis of an extranodal marginal zone B-cell lymphoma was made. There was LGGL with transition to HGGL. There was plasma-cell-differentiation and monotypical light-chain-lambda immunoglobulin expression. Cells were CD 20+, revealed aberrant expression of CD 43 and of BCL-2-oncoprotein. Growth fraction was estimated 20–50%. After three months gastroscopy revealed complete remission (multiple biopsies – gastric mapping). During all the time the patient remained on the transplantation list and NTX took place 5 months after first presentation. Now 10 months later the patient is free of tumour and clinically doing well. Conclusion: This case supports that H.pylori eradication may induce complete remission in patients with partial HGGL. The case presented here shows prolonged remission even after immunosuppression following NTX.

**P909**

**Treatment of primary cardiac B-cell lymphoma with systemic chemotherapy, immunotherapy and radiotherapy – a case report**


Halle, D

Primary cardiac lymphoma (PCL) is an extranodal Non-Hodgkin’s lymphoma exclusively located in the heart or pericardium. It is extremely rare in immunocompetent patients, but more common in immunosuppressed patients. Here we discuss the case of a 65-year-old immunocompetent woman who was admitted with dyspnea and superior vena cava syndrome. Transesophageal echocardiography (TEE) demonstrated two large tumors in the right atrium invading the superior vena cava. This was confirmed by computed tomodigraphy and magnetic resonance imaging. Triage to biopsy of the intracardiac tumor was unsuccessful. Therefore, histological diagnosis was obtained by thoracotomy and revealed CD20 positive diffuse large B-cell lymphoma. The patient subsequently underwent 6 cycles of chemotherapy with CHOP (Cyclophosphamid 750 mg/m² d1, Doxorubicin 40mg/m² cycle 1), Caelyx (Doxorubicin liposomal) 20 mg/m² d1 (cycle 2–6), Vincristine 1,4 mg/m² d1, Prednisonol 100 mg/m² p.o. d 1–5). In addition, she received CD20 positive monoclonal antibody Rituximab (375mg/m²). Ventricular arrhythmia developed the day after completion of the first course of chemotherapy. This was thought to be due to involvement of the cardiac conduction system by the tumor. Therefore we administered the CHOP protocol with liposomal anthracyclin in course 2–6. After 6 cycles of chemotherapy, local radiation therapy was administered. The patient has been in complete remission for 9 months after her initial diagnosis without recurrence of the tumor on transthoracic echocardiography. No further cardiac arrhythmias have been detected during the follow-up period.

In conclusion, diagnosis of primary cardiac lymphoma is difficult due to its rarity and its non-specific clinical manifestations and should be considered in the differential of any patient with a cardiac mass. The authors believe that PCL should be treated in similar fashion to aggressive lymphomas arising at other anatomic sites.

**P910**

**Regression of stage I high grade primary gastrointestinal lymphoma after eradication of H – pylori and renal transplantation**

S. Sahm, B. Krumme, H. Stein

Wiesbaden, Berlin, D

**Introduction:** Eradication of H.pylori has been established as therapy for stage I low grade gastrointestinal lymphoma (LGGL) with response rates between 70–80% and long term remission of lymphoma up to 60%. There are rare occasional reports about the effect of H.pylori eradication in patients with high grade gastrointestinal lymphoma (HGGL), i.e. aggressive lymphomas. We report a patient with mixed LGGL – HGGL and complete remission after H.pylori eradication. The case is of even more interest because the patient received renal allograft and immunosuppressive treatment shortly after obtaining clinical remission. Clinical course: A 62 y old male patient with terminal renal insufficiency and chronic hemodialysis was admitted to the gastrointestinal/oncology unit for chronic abdominal pain. On gastroscopy a flat large ulcer was found. The lesion was suspicious of neoplastic tissue. Despite multiple biopsies no neoplastic tissue was found. H.pylori was found histologically and eradication therapy (metronidazol, clarithromycine, pantoprazol) was initiated and acid-suppression was prolonged for the following weeks. Endoscopic ultrasound (EUS) showed thickening and infiltration of all layers of mucosal wall without lymph node involvement. On repeated controls no malignant tissue was found, yet healing was prolonged. Not before 7 weeks after initial presentation biopsies taken during gastroscopy revealed primary lymphoma of the stomach (stage E12 according to EUS). The tissue was reviewed by an experienced pathologist (HS) and diagnosis of an extranodal marginal zone B-cell lymphoma was made. There was LGGL with transition to HGGL. There was plasma-cell-differentiation and monotypical light-chain-lambda immunoglobulin expression. Cells were CD 20+, revealed aberrant expression of CD 43 and of BCL-2-oncoprotein. Growth fraction was estimated 20–50%. After three months gastroscopy revealed complete remission (multiple biopsies – gastric mapping). During all the time the patient remained on the transplantation list and NTX took place 5 months after first presentation. Now 10 months later the patient is free of tumour and clinically doing well. Conclusion: This case supports that H.pylori eradication may induce complete remission in patients with partial HGGL. The case presented here shows prolonged remission even after immunosuppression following NTX.

**P911**

**Ki-1+/ALK-anaplastic T cell lymphoma in a Crohn’s patient after Infliximab treatment**

C. Bucher, L. Degen, P. Went, S. Dirnhofer, M. Pless, C. Rochlitz, R. Herrmann

Basel, CH

A 61-year-old patient with a 30-year history of intensely treated Crohn’s disease presented with rapidly increasing stool frequency, urgency, diarrhea, rectal bleeding ulcers and recto-rectal fistulas. Biopsies of the ulcers showed an anaplastic Ki-1+/ALK-negative-T-cell lymphoma. Staging was IAE as evidenced by CF- and PET scans. Treatment was with 5 cycles of CHOP-Chemotherapy. Restaging with a PET-scan and biopsies 3 months after the last CHOP-treatment showed complete remission.

To our knowledge this is the first case of an anaplastic Ki-1+/ALK-T-cell lymphoma in a Crohns patient.

We summarize the literature on the frequency of lymphoma in crohns patients and briefly describe the published cases of lymphoma after infliximab treatment.

**Poster session: Hodgkin’s lymphoma**

**P912**

**Inhibition of cell proliferation by tyrophostins – Mode of action in Hodgkin’s lymphoma cells**

N. Kussebi, B. Stürzenhofecker, E.-M. Choi, M. Vockeroth, L. Trümper, D. Kube

Göttingen, D

Tyrophostins, specific protein tyrosine kinase inhibitors, are effective inhibitors of proliferation for a growing number of human tumors. Recently, it was shown that treatment of diverse Hodgkin lymphoma (HL) cell lines with tyrophostin AG490 went along with decreasing levels of constitutive STAT3 phosphorylation as well as reduced DNA binding. In our report we analysed the inhibitory effect of additional tyrophostins on HL cell proliferation, survival and STAT activation.

In contrast to other tyrosine kinase inhibitors treatment with a subset of tyrophostins led to a dramatically reduced phosphorylation of STAT3 and STAT6. Inhibition of STAT phosphorylation went along with a cell cycle arrest in HL cell line L428. This event was not accompanied by caspase associated apoptosis within 72h. However, tyrophostin treatment sensitized L428 cells to a certain extent for undergoing programmed cell death mediated by CD95 activation. Furthermore, the expression of several pro- and antiapoptotic factors was affected.

For a comprehensive description of the tyrophostin action in HL a gene expression profile analysis using cDNA arrays containing 36.000 target genes is in progress. This work underlines the role of constitutively activated STAT molecules in the pathophysiology of HL cell lines and may provide new therapeutic perspectives.
Abstracts

P913
Growth inhibition of Hodgkin’s lymphoma cells by the natural prostaglandin derivative 15dPGJ2 is independent of PPARgamma and IkappaB-mediated inhibition of NKappaB

R.K. Thomas, O. Mani, T. Zander, V. Diehl, J. Wolf
Cologne, D

Constitutive activity of the transcription factor NFkappaB is a common feature of many B cell lymphomas, including Hodgkin’s lymphoma (HL). Inhibition of NKappaB in such tumors leads to induction of apoptosis. Thus, therapeutic strategies targeting the NKappaB pathway are promising.

Thiazolidinediones are anti-diabetic drugs known to induce apoptosis in a variety of human cancers by inhibition of NKappaB after ligation of PPARgamma, a nuclear hormone receptor.

Here, we used HL derived cell lines with constitutive activity of NKappaB as a model for investigating the potential role of PPARgamma agonists as a therapeutic strategy in malignant lymphoma. These cell lines either harbor mutated or wildtype IkappaB-alpha and -epsilon genes that are important in negative regulation of NKappaB and are therefore ideal in vitro models for studying NKappaB targeting drugs.

Incubation of HL cell lines with varying concentrations of the physiological PPARgamma ligand 15dPGJ2 lead to massive inhibition of proliferation. Surprisingly, this effect was also seen in a cell line with mutated IkappaB-genes, suggesting that inhibition was independent of IkappaB-mediated inhibition of NKappaB. To analyze if this inhibition was mediated by ligation of PPARgamma, we determined expression of this gene in a panel of 11 B lymphoma cell lines (incl. 4 HL lines) by RT-PCR and western blot. Since different anti-PPARgamma antibodies yielded inconsistent results, we overexpressed PPARgamma in Hela cells and selected an antibody specifically recognizing the overexpressed gene in lysates from these transgenes. In contrast to several other cancer entities, PPARgamma was only expressed in 3/11 B lymphoma cell lines tested.

Therefore, growth inhibition of HL cells by 15dPGJ2 was not mediated by ligation of PPARgamma. Consequently, the synthetic PPARgamma agonist Cigitazine (Cig) did not mimic the effects of 15dPGJ2 in HL cells.

Growth inhibition occurred only weakly at very high doses of Cig and the PPARalpha agonist (VH and VL domain of the Ki-4 monoclonal antibody (mab) by a flexible peptide linker (G4S)3 (~30kD). Furthermore, we developed a scFv from the murine anti-CD30 antibody Ki-4, fusing VH

We conclude that PPARgamma is downregulated in B lymphomas and that synthetic PPARgamma ligands might therefore not represent a potential treatment option for these neoplasms. In contrast, 15dPGJ2 is a potent growth inhibitor in HL cells with constitutive activity of NFkB and this effect appears not to be mediated by ligation of PPARgamma and phosphorylation of IkappaB proteins.

P915
Lymphocyte-predominant Hodgkin’s disease in clinical stage IA: Review of treatment options in three study generations of the German Hodgkin Study Group

Cologne, D

Introduction: The pathology and clinical presentation of Lymphocyte-predominant Hodgkin’s disease (LPHD) differs from other forms of Hodgkin’s disease (HD); the classical type of HD included. LPHD is a rare entity accounting for 3 to 8% of all HD cases in western countries. A recent report from the task force on HD has suggested that patients (pts) in early stage LPHD should be treated with a reduced treatment intensity. However, relevant clinical studies are still lacking. The German Hodgkin Study Group (GHSG) has thus reviewed all LPHD-cases registered in the last studies (HD4, HD7 and LPHD observation study) for early stages (CS IA) and compared the results of different treatment approaches.

Patients and methods: 165 pts with LPHD treated within the GHSG trials of HD4 (n = 23), HD7 (n = 37) and the LPHD observation study (n = 105) in clinical stage IA without risk factors such as large mediastinal mass, extranodal involvement, ESR > 50 and without massive spleen involvement (additional for HD4 and HD7) were analysed retrospectively. Pts in HD4 were randomised between both the 40 Gy extended field (EF) and the 30 Gy EF plus 10 Gy involved field (IF). Pts in HD7 received either 30 Gy EF + 10 Gy IF or 2x ABVD + 30 Gy EF + 10 Gy IF. In the LPHD observation study, a more flexible strategy was chosen, including IF-radiother-apy, watch and wait or combined modality treatment (randomised between 2x ABVD + IF and 4x ABVD + IF). Results: 22/23 patients from HD4 were evaluable, of which 11 received IF radiotherapy and the other 11 received 30 Gy EF + 10 Gy IF radiotherapy. 37/37 patients from HD7 were evaluable, of which 22 received EF radiotherapy and 15 patients were treated with 2x ABVD + EF. In the LPHD observation study, 70/89 pts qualified, of which 68 received IF radiotherapy and 4 combined modality treatment. After first line therapy 86% pts achieved CR/CURs in HD4, 95% in HD7 and 97% in the LPHD observation study group. The full analysis of these data will be presented.

Conclusion: The early stages of LPHD may be treated effectively with reduced dose intensity of therapy modalities as presented above.

P916
Development of new recombinant radio-immunoconjugates for the treatment of Hodgkin’s lymphoma

Cologne, D

Rationale: CD30 has been shown to be an excellent and promising target for antibody-based immunotherapy in Hodgkin’s lymphoma (HL) due to its overexpression on the malignant Hodgkin-Sternberg-Reed cells (HS-RR). Major limitations of monoclonal antibody (mab) conjugates as therapeutic agents are inadequate tumor targeting, poor tumor penetration and immunogenicity. The smaller size of single-chain antibodies (scFv) and the absence of Fc segments may contribute to lower immunogenicity and better tumor penetration.

Materials and methods: We genetically constructed a scFv from the murine anti-CD30 antibody Ki-4, fusing VH (heavy chain variable) and VL (light chain variable) domain of the Ki-4 mab by a flexible peptide linker (G4S)3 (~30kD). Furthermore, we developed a dimeric scFv (~60kD) consisting of two scFvs linked by a (G4S)2

Abstracts

P914
Salvage treatment for relapse after radiation therapy of early stage Hodgkin’s disease – results of the German Hodgkin’s Lymphoma Study Group (GHSG)

K. Wingbermühle, J. Glossmann, J. Franklin, L. Nogova, K. Behringer, A. Engert, V. Diehl, A. Josting
Cologne, D

Introduction: Patients with early stage favorable Hodgkin’s disease (HD) who relapse after extended field (EF) radiotherapy (RT) have relative satisfactory results when treated with conventional chemotherapy at relapse. The present analysis retrospectively analysed patients with relapsed HD after initial radiation therapy to determine treatment outcome and prognostic factors.

Methods: In the HD4 and 7 trial of the German Hodgkin Study Group 945 patients in localized stages without risk factors received either 40 Gy extended field (EF) RT or 30 Gy EF RT followed by an additional 10 Gy to involved lymph node regions. 107 patients relapsed and received salvage therapy after first-line RT. Characteristics of these 107 patients at relapse were as follows: median age 34 years (range 18–75), 31% were female, 69% were male. Histologic types at first diagnosis were NS in 44%, MC in 41%, LP in 13%, LD in 1 case, unknown in 1 case. The majority of patients were treated with conventional chemotherapy (93%), 60% were treated with COPP/ABVD, 21% with BEACOPP, 4% with COPP/ABV/IMEP, 3% with ABVD, 2% with COPP and 3% received various other regimens. 7% were treated with RT alone.

Results: The median follow-up after relapse was 45 months. FF2F and overall OS were: 81% and 89% for patients relapsing after RT, respectively. Patients relapsed after a median of 19 months (range 4–98 months) after first diagnosis, 27% had early, 73% had late relapse with 11% of all patients relapsing after 5 years. Stage at relapse was: stage I in 39%, II in 23%, III in 10% and IV in 28%. 20% of the patients had B-symptoms at the time of relapse, 23% presented with infeld relapse, 47% with outfeld relapse and 30% with both, in- and outfeld relapse. In multivariate analysis age, B symptoms and extranodal involvement were significant in terms of OS. Age, B-symptoms and salvage chemotherapy were significant factors for FF2F. Conclusion: The long-term outcome of patients relapsing after EF RT is excellent. Age, B symptoms, extranodal involvement and salvage chemotherapy were identified as prognostic factors.
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sequence, and a trimer (~90kD). The fusion proteins were inserted into an
expression vector and transformed into the eukaryotic cell line L293T. Supernatant of producing cells was collected and purified by com-
binations of Ni-NTA affinity, anion exchange and molecular size-chro-
matography, supported by compatible solutes. Recombinant proteins
were analysed by Coomassie and Western blotting. Binding properties on the
HL derived cell line L540 were assessed by FACS analysis, BIACore and
immunofluorescence staining. To further determine the C30 cluster
to which the recombinant scFvs bind, competition ELISA was performed,
using either Ki-4 mab (cluster A) or Ki-3 mab (cluster C) as a reference.

Results and conclusion: Expression and purification of the mono- and bi-
valent scFvs has been demonstrated. Purification of the trimer resulted in
contamination with mono- and bivalent fragments, probably due to enzy-
matic cleavage. All recombinant scFvs showed specific binding to CD30
cluster A as determined by competition ELISA. Data on conjugation of
scFvs to iodine-131 and other isotopes (Indium-111, Yttrium-86), in vivo
biodistribution and activity will be determined in solid / disseminated
xenografted models of HL in SCID mice. The results will be presented.

P917
A complete remission for two years of relapsed mixed
cellularity Hodgkin's disease after treatment with Rituximab
Leipzig, D

Introduction: Cure rates of Hodgkin's disease (HD) with chemotherapy
and/or radiotherapy are high. However, a few patients are refractory to

treatment or relapse. It is suggested that the lymphocyte predominant
(LP) type is composed of clonal B-lymphocytes derived from germinal
center B-cells. In contrast, the lineage and clonality of HRS cells in classic
HD are still controversial. Somatic mutations within rearranged Ig genes
indicate that HRS cells were derived from germinal center B-cells. B-cell
markers are reported in 80% of classic HD. CD20 expression in HRS cells
in classic HD varied from 21%-80%. Only a very few patients with classic
HD treated with Rituximab have been described. We report a patient
with mixed cellularity (MC) type HD with frequent relapses. As all
Hodgkin’s or Reed-Sternberg (HRS) cells expressed CD20, treatment
with mixed cellularity (MC) type HD with frequent relapses. As all
Hodgkin’s or Reed-Sternberg (HRS) cells expressed CD20, treatment
with Rituximab was given. Patient and methods: In 1992, a 55 years old
male presented with lymphadenopathy. Biopsy revealed HD of MC type.
According to the Ann Arbor classification, Stage IVA was present (cervi-
cal and retroperitoneal lymphadenopathy, splenomegaly, bone marrow
and liver involvement). CR was achieved after 6 cycles of ABVD alter-
nating with the COPP regimen. The first relapse occurred 12 months later
and was treated with DEXA-BEAM followed by an autologous peripheral
blood stem cell transplantation. A second CR was obtained. In 2000, a
second histologically proven relapse was diagnosed. Stage IVA was again
present (supraclavicular, mediastinal and abdominal lymphadenopathy
in addition to liver involvement). A partial remission was induced with 2
DEXA-BEAM cycles. 4 months later, the disease progressed. Despite
treatment with Gemicetabine for 5 months no response was observed. As
all HRS cells and CD20 positive, Rituximab at a dose of 375mg/m² was
given once a week for 4 weeks in an outpatient setting. Treatment was
well tolerated. Results: CR documented 3 months after Rituximab by
computer tomography was achieved. 24 months later, the now 66 years
old patient is still in CR with a Karnofsky Index of 100%. No infectious
episodes occurred. Bone marrow B-cells analyzed by FACS normalized
after 18 months. Reduced IgG and IgM levels are still present. Conclu-
sion: An indication for the treatment with Rituximab in refractory or re-
lapsing patients with classic HD expressing CD20 is rational. Durable re-
sponses as occurred in our patient may be achieved.

P918
Pericardial mesothelioma after first-line therapy of Hodgkin's disease
P. Krenn, E.K. Koller, A.C. Chott, R.G. Grill, H. Hanak, M. Gessner,
E. Pittermann, M. Pleifstöcker
Vienna, A

Hodgkin’s disease has a high potential for cure, therefore avoidance of
and surveillance for secondary malignancies is of high importance. In this
report we present a case of unusual manifestation of a malignancy early
after first line therapy. A 31-year old woman presented with indolent cer-
vical lymph nodes, a lymphnode biopsy revealed Hodgkin’s disease of
nodular sclerosis subtype. Staging showed collar lymphnodes and lymph-
nodes in the upper mediastinum, no B-symptoms could be explored. Be-
cause of Ann Arbor stage Stage I/2A we administered 4 courses ABVD
and involved field radiotherapy (30Gy) inducing complete remission.
19 months later the patient was admitted with retrosternal pain and mild
dyspnea. X-ray showed pericardial effusion, cardiac echo confirmed the diagnosis of
pericardial effusion, the CT-scan of thorax and abdomen showed no other abnormalities especially no lymphnode enlargement. A pericardial
punctio was performed, testing for viral or bacterial infection and cytology
was negative. The symptoms grew worse with atrial fibrillation, cardiac
decompenation and small infiltrates of the lung. Because of bad clinical
condition and the presumption of relapse of Hodgkin’s disease we started
with one cycle BEACOPP. The patient’s condition improved, but there
was no response in the cardiac echo. Therefore we took a pericardial
biopsy. The histopathological diagnosis was pericardial mesothelioma,
positive for cam5.2, vimentin and calretinin in immunohistochemistry.
We started chemotherapy with platin and gemcitabine. After a short period of
stabilization there was massive progression and one cycle pemetrexed was
given. The patient died shortly afterwards, the autopsy showed a huge car-
diac tumor and infiltration of the lung and bone marrow by the mesothe-
loma, no manifestations of Hodgkin’s disease were detectable. As there
was no risk factor for mesothelioma except radiation therapy of the medi-
astinum we have to suspect this complication as early secondary malign-
ancy after therapy for Hodgkin’s disease.

P919
Thyroid diseases after therapy for Hodgkin's lymphoma
H. Mocikova, D. Fettl, T. Kozak, J. Markova
Prag, CZ

We retrospectively reviewed thyroid function in 165 patients (pts) with
primary Hodgkin’s lymphoma (HL) who have been treated with 3rd and
4th generation trials according to German Hodgkin Lymphoma Study
Group for early, intermediate and advanced stages of Hodgkin’s lymph-
oma (HD7 – HD12) between 1995 and 2003. Patients and methods: Median age at the time of treatment was 51 years (range 18 – 66), 3 pts
(1.8%) were treated with radiation alone, 135 pts (82%) chemotherapy
and radiation, 27 pts (16.2%) chemotherapy alone. 7 pts (4%) died: 5 of
progressive HL (3%) and 2 of causes other than HL (1.2%). Serum free
thyroxine (FT4) and thyrotropin (TSH) were measured at least once a
year. In case of thyroid dysfunction ultrasound examination and fine nee-
dle aspiration cytology was performed and thyroid autoantibodies were
measured. Results: during the follow – up 33 cases of hypothyroidism
(20%) were confirmed: 14 cases of manifest clinical hypothyroidism
(8%) and 19 cases of subclinical hypothyroidism (12%). Graves’ hyper-
thyroidism developed in 2 pts (1.2%). 3 pts underwent thyroidectomy
(1.8%) including one patient with benign adenoma. No thyroid cancer
was found in our group of pts. Only one patient with thyroid dysfunction
was treated with chemotherapy alone, others were treated with radiation
 +/- chemotherapy. Conclusion: Due to frequent thyroid diseases after
therapy for Hodgkin’s lymphoma it is recommended to perform clinical
and biochemical examination of thyroid gland regularly at least once a
year.

Poster session: Breast cancer

P920
Hepatic intra-arterial chemotherapy with Gemcitabine:
An ongoing phase II study in patients with liver metastases of breast cancer
K. Eichler, S. Zangos, T. Lehnert, C. Herzog, M. Mack, J. Balzer,
T. Vogl
Frankfurt, D

Purpose: To evaluate the efficacy and tolerability of hepatic intra-arterial
chemotherapy with gemcitabine as cytostatic agent in patients with
inoperable liver metastases of breast cancer. Material and methods: This
is an ongoing, open-label, single center study. Patients are required to
have histologically confirmed breast cancer with inoperable liver meta-
stases. The results will be presented.
tases, an adequate bone marrow reserve, sufficient liver and renal function, no active CNS metastasis, a Karnofsky performance status >70%, and a life expectancy of >12 weeks. The cytostatic suspension, consisting of 1200 mg/m² gemcitabine, 10 ml/m² of iodised oil (lipiodol) and 5 ml of a starch microsphere (Spherex) suspension, is administered intra-arterially up to 3 times in 4-week intervals. Dose-limited toxicity (DLT) is defined as grade 4 thrombocytopenia, neutropenia, or any nonhematologic toxicity higher than grade 3. Tumor response is evaluated by magnetic resonance tomography (MRT) and computed tomography (CT) imaging. Results: So far, 10 patients have been enrolled (median age 58 years, range 48–65 years). All patients tolerated the treatment well, no dose limiting toxicity was observed. Four patients (40%) achieved a partial response, five had stable disease, and one patient had progressive disease. Conclusion: First results of this ongoing study indicate that hepatic intra-arterial chemotherapy with gemcitabine is well tolerated and can achieve encouraging response rates in patients with liver metastases of breast cancer. This study is supported in part by Eli Lilly Oncology.

P921
Dose escalation and pharmacokinetics of weekly administered epirubicin and paclitaxel in patients with advanced breast cancer
R.M. Mader, B. Rizovski, C. Wenzel, R. Bartsch, C.C. Zielinski, G.G. Steger
Vienna, A

Background: As alternative to the therapy with anthracyclines and taxanes every three weeks, we administered epirubicin and paclitaxel on a weekly schedule and compared the pharmacokinetics at the beginning and at the end of the first therapeutic cycle. Material and methods: In a dose escalation study, epirubicin was administered as i.v. infusion over 30 minutes starting at 20 mg/m² followed by paclitaxel given as i.v. infusion over 3 hours starting at 70 mg/m² with standard premedication. This combination was administered weekly for 6 weeks followed by one week of rest (=1 cycle) with tumour reassessment after 2 cycles of therapy. Dose escalation in steps of 5 mg epirubicin/m² and 5 mg paclitaxel/m² was done, if toxic side effects were not higher than grade 3 according to WHO criteria in 2 of 3 patients per dose level. To evaluate pharmacokinetics, both compounds were monitored at week 1 and 6 in each patient using an on-line HPLC method. Results: The pharmacokinetics of epirubicin and paclitaxel were similar in week 1 (anthracycline and taxane naive patients) and week 6 when comparing the following parameters: area under the concentration-time curve (AUC), maximum plasma concentration (Cmax), and total Clearance (Cl tot). Cmax of epirubicin and paclitaxel were closely correlated (r² = 0.69). Considering dose escalation, there was a statistically significant reduction in the total clearance of both agents indicating non-linear pharmacokinetics at the administered dosage. For instance, Cl tot of epirubicin decreased from 162 to 57.4 L/h and that of paclitaxel from 61.4 to 18.9 L/h when escalating the dose. Noteworthy, the metabolism of epirubicin was not dose dependent in the range from 20–30 mg/m². Conclusions: Reduced clearance observed after administration of 30 mg epirubicin/m² and 80 mg paclitaxel/m² may contribute to the dose limiting leukopenia (WHO grade 4 in 2 of 3 patients). Since the dose of 25 mg epirubicin/m² and 75 mg paclitaxel/m² was well tolerated under a weekly schedule, we recommend this dose as a starting point for future clinical trials. Once established, there is no need for dose adjustment caused by a shift of pharmacokinetic parameters, which were stable over at least six weeks. This study was supported by EBEWE Pharma, Austria.

P922
Liver metastases in conjunction with breast cancer: Local ablation of hepatic tumours with RITA allows the consolidation of tumour control
P. Abitabile, U. Hartl, J. Lange
Liestal, St. Gallen, CH

Introduction: The surgical resection of hepatic metastatic breast cancer is in most cases not indicated. This as a result of the assumption that it is a question of a systemic dissemination. Only in cases which exhibit a stable condition and limited hepatic disease is metastatic surgery indicated. Radiofrequency ablation presents an alternative to classical surgery. It makes possible a selective, efficient and minimally-invasive destruction of tumours by means of a percutaneous or laparoscopic approach. Methodology: Under sonographic control, a monopolar electrode is inserted into the liver metastasis. The application of radiofrequencies causes a local increase in the temperature and results in necrosis of the targeted tumours. The success of the ablation can be sonographically monitored by means of the steam given off. All the patients were checked within 1 week of ablation with a 3-phase CT. Further checks are carried out 3.6,12.18 und 24 months after the ablation. In the absence of tumours, an annual check is sufficient, otherwise according to the recommendation of the oncologist in attendance and the state of the tumour. Results: Since April 1998, we have treated 8 patients, suffering from local hepatic metastatic breast cancer and exhibiting stable conditions over several months of systemic therapy, with RITA. A total of 16 liver tumours were locally removed. The average maximum tumour size was 3.74 cm. In all but one case, the post operative CT-Control showed a complete necrosis. Apart from a temporary increase in transaminase and occasional fever, the patients exhibited no complications. The average follow up at present is 21.9 months (range 1–48). 5 patients have remained free of tumours during the observation period, a further 2 show stable disease over 24 and 32 months. One patient, with initial not completely coagulated 9cm metastases, showed tumor progress after 2 months and died 14 months after RITA. Conclusions: When used by an experienced surgeon, RITA combines the efficiency of radical surgery with the modest burden of a minimally invasive operation. In combination with systemic therapy, local radiofrequency ablation of liver metastases in conjunction with breast cancer consolidates a stable disease condition.

P923
Genetic abnormalities in micrometastatic tumor cells of patients with breast and prostate cancer
Hamburg, D

Metastasis is responsible for most deaths from cancer. In patients with early stage cancer, metastatic relapse is caused by the occult dissemination of tumor cells, either systemically, regionally or both. The bone marrow is a relevant site of micrometastatic disease in patients with breast and prostate cancer. Using monoclonal antibodies to epithelial cytokeratins, individual carcinoma cells can be detected on cytologic bone marrow preparations at frequencies of 0.00001 to 0.000001. Since little is known about genetic abnormalities in these micrometastatic cells, we have developed a strategy for in-depth characterization of genomic changes of these cells: unique tumor cell lines were established from bone marrow of 3 patients with breast cancer and two patients with prostate cancer. Remarkably, all of these patients were free of histopathological lymph node metastasis (pN0) as well as clinical signs of overt systemic metastases in distant organs (Mo) at the time of bone marrow analysis. The cell lines were analyzed by multiplex fluorescence in situ hybridization (M-FISH) and comparative genomic hybridization (CGH). M-FISH revealed that the cells were characterized by complex numerical and structural chromosomal abnormalities. Some of these aberrations resulted in chromosomal gains and losses. Material of chromosomes 8q, 11q, and X (2 cases each) was most frequently gained, whereas losses mainly involved chromosomes 8p and 18q (2 cases each). Interestingly, high-level amplifications were detected in two cell lines and involved the regions 8q23–24. Interphase FISH was performed in one cell line with an amplification of 8q using a probe specific for the CMYC gene located on 8q24. More than 60% of the analyzed interphase cells showed 4 to 7 hybridization signals of the CMYC specific probe. These results suggest a pathogenetic relevance of the 8q23–24 amplon including the CMYC gene in the cell lines analyzed. The analysis of micrometastatic cells opens a new way to assess the molecular determinants of both early tumor cell dissemination and subsequent outgrowth into overt metastases.
**P924**

**Response to thirdline and following cytostatic therapies in patients with metastatic breast cancer**


Berlin, Ulm, D

**Background:** Patients with progression of metastatic breast cancer after second line therapy and good performance status often wish further therapy. As there are numerous cytostatic substances with proven effect in metastatic breast cancer, cytostatic therapy in third or following line is occasionally applied. However, little is known about the benefit such therapies in this situation, as most regimens have been evaluated as first- or second-line, and in some cases as third-line therapy. **Patients and methods:** In a retrospective two-center study, we evaluated 86 patients (pts.) with breast cancer, receiving at least three cytostatic therapies. Main endpoints were response, TTP, toxicity, and time to progression (TTP). Hormonal therapy was not taken into account in this analysis. **Results:** We found objective response up to the 5th line therapy (3 PR), and stable disease up to the 10th line therapy. High grade toxicity was occasionally found, however in higher lines this was rare. Median survival was 42.56 months from first diagnosis of metastases. Summary is given in table 1. TTP given as median values. **Conclusions:** Objective response to cytostatic therapy in metastatic breast cancer can be achieved up to the 5th line. Stabilization of the disease is possible even in heavily pretreated patients up to 10th line therapy. High grade toxicity in this situation is rare. These data suggest, that cytostatic therapy beyond the third line might be a reasonable option for selected patients with metastatic breast cancer.

**P925**

**Clinicopathological correlations of genomic aberrations in breast cancer: A comparative genomic hybridization (CGH) analysis of 237 patients**


Ulm, Heidelberg, Tübingen, Munich, D

Little is known about correlations of clinical and pathological characteristics with genomic aberrations in patients with breast cancer. Therefore we analyzed 237 patients with breast cancer using Comparative Genomic Hybridization (CGH). The median age was 41 (range, 21 to 76 years). Tumor Size was T1 in 79 cases, T2 in 123 cases, T3 and T4 in 35 cases. Lymph node metastases were present in 105 out of 237 patients. Grade 1 and 2 was present in 135 patients, 102 patients had grade 3 tumors. Hormone receptor-positive, and 64% were progesterone receptor-positive. Ge- nomec aberrations were detected in 207 out of 231 patients (89%). The most frequent gains (more than 10% of cases) affected chromosome arms 1q (109 cases), 8q (94 cases), 11q (27 cases), 16p (34 cases), 17q (77 cases) and 20q (46 cases). The most frequent losses mapped to 6q (27 cases), 8q (30 cases), 11q (42 cases), 13q (50 cases) and 16q (31 cases). For clinicopathological correlations, only data with a p-value < 0.01 are presented. Gains on 8q, 17q and 20q were associated with very young age at diagnosis (below 35 years; p=0.001 for all aberrations; Fisher’s exact test). Lower histopathological grade (Grade 1 and 2) were associated with gains on 16p (p = 0.0015). More aggressive histopathological patterns (grade 3) were associated with gains on chromosome arms 8q, 10p and 17q (p<0.0001; p = 0.0091; p = 0.0001, respectively). Negativity of both estrogen and progesterone receptor status was found more frequently in cases with gains on 10p (p<0.0001). Positive nodal status was associated with gains on 8q and 17q as well as with losses on 8p, 9p, 11q and 14q (p=0.001 in all cases). These data indicate that genomic aberrations are strongly associated with clinical and pathological characteristics.

**P926**

**An unusual presentation of a common disease**


Bonn, D

A variety of rheumatic syndromes have been recognized as paraneoplastic conditions. We report a case of occult metastatic breast cancer which presented with symptoms and signs of adult onset Still’s disease. **Case report:** A 52-year-old Caucasian woman presented with a four week history of high, spiking fevers that were accompanied by a transient macular rash, myalgia and arthralgia involving most joints. Initially, there had been a sore throat and painful lymphadenopathy in the left supraclavicular fossa. A lymph node was removed for histopathological examination. The physical and gynecological examinations were normal. There was no peripher- al lymphadenopathy. Blood tests showed a Hb of 10 g/dl, a leukocytosis of 12.3 G/l with 85% neutrophils, raised C-reactive protein (91 mg/l) and ESR (94/120) as well as an exceptionally high serum ferritin level (21762 ng/ml). Testing for bacterial, viral or fungal infection and autoantibody screens were consistently negative. Additional work-up (ultrasound, CT scan, bone marrow aspirate) revealed only splenomegaly and increased numbers of normal-sized lymph nodes in the left axilla. These findings justify the clinical diagnosis of adult onset Still’s disease (ASD) based on the presence of all the diagnostic criteria proposed by Yamaguchi et al. with aspinir% (3 g/day) accordingly resulted in a gradual resolution of fever. However, the histopathological examination of the lymph node removed initially revealed a small breast cancer metastasis. Further inves- tigation including mammography, ultrasound and MRI of both breasts revealed a 15 mm suspicious mass in the left breast. A lumpectomy and lymph node dissection was carried out. The lesion was diagnosed as a ductal adenocarcinoma. After tumor resection, fever and all other symptoms of ASD, including serological markers of inflammation such as ESR, CRP and ferritin, returned to normal levels without further treatment with aspinir% The patient is currently receiving adjuvant chemotherapy. **Discussion:** Symptoms of ASD have very rarely been described as the first symptoms in patients with breast cancer. Since the symptoms of ASD re- solved entirely after start of treatment of breast cancer, they may reflect an unusually strong anti-tumor immune response. In practical terms this unusual presentation (ASD) of an all-too-common disease (metastatic breast cancer) should again remind us that rheumatic diseases can sometimes be clues to occult malignancies.

**P927**

**Preoperative secondline chemotherapy induces objective responses in primary breast cancer**


Vienna, A

**Purpose:** Preoperative chemotherapy in patients with primary breast cancer results in high response rates, allowing breast conserving surgery in patients primarily not suitable for this procedure. Patients failing to re- spond to preoperative chemotherapy are thought to be chemotherapy-re- sistant. We questioned this hypothesis and treated 13 patients, who were resistant to preoperative anthracycline-containing first-line treatment. Patients failing to re- spond to preoperative chemotherapy are thought to be chemotherapy-re- sistant. We questioned this hypothesis and treated 13 patients, who were resistant to preoperative anthracycline-containing first-line treatment. Patients failing to respond to preoperative chemotherapy are thought to be chemotherapy-resistant. We questioned this hypothesis and treated 13 patients, who were resistant to preoperative anthracycline-containing first-line treatment. Patients failing to respond to preoperative chemotherapy are thought to be chemotherapy-resistant. We questioned this hypothesis and treated 13 patients, who were resistant to preoperative anthracycline-containing first-line treatment. Patients failing to respond to preoperative chemotherapy are thought to be chemotherapy-resistant. We questioned this hypothesis and treated 13 patients, who were resistant to preoperative anthracycline-containing first-line treatment. Patients failing to respond to preoperative chemotherapy are thought to be chemotherapy-resistant. We questioned this hypothesis and treated 13 patients, who were resistant to preoperative anthracycline-containing first-line treatment. Patients failing to respond to preoperative chemotherapy are thought to be chemotherapy-resistant. We questioned this hypothesis and treated 13 patients, who were resistant to preoperative anthracycline-containing first-line treatment. Patients failing to respond to preoperative chemotherapy are thought to be chemotherapy-resistant. We questioned this hypothesis and treated 13 patients, who were resistant to preoperative anthracycline-containing first-line treatment. Patients failing to respond to preoperative chemotherapy are thought to be chemotherapy-resistant. We questioned this hypothesis and treated 13 patients, who were resistant to preoperative anthracycline-containing first-line treatment. Patients failing to respond to preoperative chemotherapy are thought to be chemotherapy-resistant. We questioned this hypothesis and treated 13 patients, who were resistant to preoperative anthracycline-containing first-line treatment. Patients failing to respond to preoperative chemotherapy are thought to be chemotherapy-resistant. We questioned this hypothesis and treated 13 patients, who were resistant to preoperative anthracycline-containing first-line treatment. Patients failing to respond to preoperative chemotherapy are thought to be chemotherapy-resistant. We questioned this hypothesis and treated 13 patients, who were resistant to preoperative anthracycline-containing first-line treatment. Patients failing to respond to preoperative chemotherapy are thought to be chemotherapy-resistant. We questioned this hypothesis and treated 13 patients, who were resistant to preoperative anthracycline-containing first-line treatment. Patients failing to respond to preoperative chemotherapy are thought to be chemotherapy-resistant. **Pa-
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gram. Interestingly, this was not associated with G2/M progression and forced conditional expression of a constitutively active p34cdc2 kinase lying basis for resistance to irradiation-induced cell death in MCF-7 cells. Then fails to induce apoptotic cell death. In contrast, anticancer drugs target functionally closely linked phenomena. Ionising irradiation induces senescence. Here, we report that senescence and apoptosis are not distinct death or activation of cell cycle arrest programs including premature senescence. Thus, such cells appear to be pre-terminal but fail to undergo apoptosis unless damage-induced cell cycle arrest programs are abrogated.

P930

Phase II-data of chemoendocrine therapy with exemestane and trofosfamid (ACIX I) in metastatic breast cancer

H.-J. Illiger
Oldenburg, D

Anticancer chemotherapy is thought to be effective by means of direct cytotoxicity against tumor cells. But we must kill to cure? Cancer is a process characterized by regulatory imbalance rather than autonomy. Imbalance is potentially reversible. Killing strategies may be counterproductive because they impair host response.

A new paradigm for dosing of chemotherapy is based on targeting the vascular system of the tumor as well as the induction of the apoptosis than the tumor itself by using low dose continuous chemotherapy and antiangiogenic drugs. Preclinical studies indicate that Exemestane (EXE) inhibit angiogenesis and potentiates the antitumor effect of subtoxic doses of various antineoplastic agents. Low dose alkylating and other cytotoxic agents also have antiangiogenic activity and can induce apoptosis.

Based on these data we initiate a phase I/II study to test a target orientated metronomic two drug regime of the alkylating agent Trofosfamid (TRO) combined with Exemestane (EXE) in postmenopausal patients with metastatic breast cancer. Our primary goal was to determine the DLT of TRO in combination with EXE. Starting dose of TRO was 100 mg and was increased to 150 mg daily dose. During the 2002-DGHO-Meeting we presented phase I data of this study and early clinical response data in 9 of 11 patients. This time we will present phase II data of 20 patients.

Results (May 03): 4 CR (6–12 +months), 9 PR (6–14 +months), 3 NC (at least 6 months), 4 PD (with in 3 months), Clinical benefit: 16/20 patients Quality of life is not influenced: toxicity is very few, CTC grade I and II was seen in few patients (myelosuppression). Conclusion: The combination of EXE and TRO is a highly effective therapy in particular for low risk metastatic breast cancer with low toxicity.

P929

Selective effects of the two phytoestrogens genistein and kaempferol in human breast cancer cells and osteoblasts

H. Tullberg-Reinert, B. Asemota, R. Bruggisser, B. Dusseiller, W. Schaffner, G. Jundt
Basel, CH

The use of phytoestrogens as an alternative to estrogen for the treatment of postmenopausal women has attracted increasing interest since a recent longterm study (Womens Heath Initiative Trial USA, 2002) indicated that hormone replacement with estrogen and progesterin displayed higher risk for breast cancer in longterm users (JAMA, 2002), whereas beneficial effects were found for bone (lower fracture risk).

In the present study we selected genistein, the major phytoestrogen in soy and kaempferol, a phytoestrogen found in many fruits, vegetables and medical plants, as representatives for this class of compounds. Both agents were tested for their interference with growth and viability of two human breast cancer cells with different estrogen receptor (ER) profiles: MCF-7 (ER alpha-positive) and HS 578 (ER alpha-negative). In addition, a human periosteal cell line with predominant ER beta was chosen to test the effects of the two agents on collagen synthesis.

Cell growth was quantified by crystal violet, viability was estimated by MTT tetrazolium reduction, and collagen synthesis was determined by a Sirius Red-based microassay. As expected, at lower concentrations genistein (0.3–10 microM) and kaempferol (1.2–38 microM) displayed partial estrogen-like growth-stimulatory activity in the ER alpha–positive MCF-7 cells whereas interestingly both agents were clearly growth inhibitory with the ER alpha-negative HS 578 cells at the same concentrations. In addition, kaempferol at 5 microM stimulated collagen synthesis in the periostatal cells, a marker for increased bone formation. Thus, kaempferol displays characteristics of a ‘Phyto-SERM’ with negative effects against breast cancer cells but positive effects towards osteoblastic cells.

The presented model will help to screen for new plant-derived agents with the wanted tissue specific activity profile.

P931

Oral cyclophosphamide and methotrexate +/- prednisone (CMP) is effective, very well tolerated, and cost efficient in the treatment of metastatic breast cancer

C. Walter, A. Schneider, J. Hense, S. Seeber
Essen, D

Because intravenous chemotherapy for women with metastatic breast cancer is often inconvenient, several oral chemotherapy regimens have been developed. Some of them are comparably new and therefore include more expensive agents. With the objective of increasing tolerability, convenience, quality of life and cost efficacy, we would like to report our experience with the combination chemotherapy of two well known oral available agents with proven antitumor activity in patients with metastatic breast cancer (BC). Patients and methods: We retrospectively reviewed the charts of patients currently seen in our outpatient clinic. We identified 40 patients with metastatic BC who received CMP between 1998 and 2003. Median age was 58.5 years (42–76 years), most patients (78%) were hormone receptor positive and had failed prior tamoxifen and/or aromatase inhibitors, 16 patients had premenopausal BC at diagnosis. Nineteen patients had received adjuvant chemotherapy (10 CMF, 8 anthracycline-based, 1 high dose regimen). Disease free interval from diagnosis of BC to occurrence of first metastasis was on average 7.3 years (range 1–24) and 8 pts. presented with synchronous metastases. The regimen consisted of cyclophosphamide (C) 200 mg fixed dose p.o. day 1–4, methotrexate (M) 10mg fixed dose p.o. d1+2 and d8+9, and leucovorin 15mg p.o. d3+10 every four weeks. All received prednisone (P) and if necessary alizaprid was added as an antiemetic. Of the 40 patients 22 (22/40 or 55%) had failed prior chemotherapy and 10 pts. presented with synchronous metastases. Disease response rate was 52%, which was comparable with published results. Cost effectiveness analysis per month of treatment showed a mean cost reduction of about 25% compared to standard intravenous chemotherapy. Conclusion: Oral cyclophosphamide and methotrexate +/- prednisone is effective, very well tolerated, and cost efficient in the treatment of metastatic breast cancer.
average of 4.6 (range 1–16) cycles of CMP; there were 1 complete remission (CR) and 7 partial remissions (PR) for an overall response rate of 21% and an overall clinical benefit (CR+PR+stable disease > 8 weeks) of 68%. There were no major toxicities except 2 grade 3 leukopenias and 1 grade 3 fatigue. Significant hair loss occurred only in 7%. Time to progression was evaluable in 32 patients and averaged 4.8 (range 1–16) months. Time to progression was not evaluable in 8 patients: 2 were taken off therapy early due to toxicity (hair loss, fatigue), 2 received currently CMP (one PR, one stable disease), 4 patients (1 CR, 3 PR) remain progression free on aromatase inhibitors (3) or MPA (1) for 11, 22, 30 and 43 months. The median survival time from the onset of metastatic disease is 4.1 (range 1.2–10) years. Conclusion: Oral CMP is an effective, very well tolerated and cost efficient regimen (approx. 50–75 U/cycle) for the treatment of patients with metastatic breast cancer.

P932
An approach to standardize the detection circulating tumor cells comparing different methods for analysis in peripheral blood and bone marrow

K. Pachmann, J. Clement, K. Höffken, U. Pachmann
Jena, Bayreuth, D

We have compared different methods for analysis of circulating tumor cells in blood and bone marrow in order to get closer to a standardized procedure to timely monitor the numbers of circulating tumour cells and their response to therapeutic regimen. There are already vast differences in the preanalytic treatment of the samples which may lead to differing results. Red blood cell lysis was compared to separation over density gradients, revealing that, unlike breast cancer cell line cells, most circulating epithelial cells from breast cancer patients sediment with the granulocytes and the red blood cells in ficoll gradients and are lost from the mononuclear fraction. Different magnetic bead enrichment procedures were also compared and the yield in epithelial-antigen positive cells compared per volume of the initial sample. Whereas Miltenyi beads yielded highly pure populations of epithelial-antigen positive cells retained in the columns, however with a simultaneous high loss of positive cells into the washing buffer, the purification was low with Labsoft beads but with a much higher yield. Thus depending on the problem to be solved beads with higher potential in purification or in yield should be used. Analysis can be performed either by immunocytochemistry or by immunofluorescence. Intra-cellular antigens, such as cytokeratin stained by indirect methods will result in much more intense staining than analysis of surface antigens using direct staining. There is, however still no good correlation between the results from different investigators even in exchange analyses and it is difficult to discriminate between unspecific and specific staining. In addition, in fixed cells it is not possible to distinguish between dead and live cells. Immunofluorimetry of surface antigen staining, in contrast has the advantage of unequivocally recognizing live and using Laser Scanning Cytometry allows for quantification of epithelial-antigen positive cells per volume and per white blood cells unenriched and enriched smiples. Using the method breast cancer patients are now routinely evaluated for their response to adjuvant chemotherapy. Thus this method allows to determine therapy response of tumour cells in vivo and hopefully will enable individual tailoring of chemotherapy in cancer patients before development of metastases.

P934
The new somatostatin analogue SOM230: A potent inhibitor of the GH/IGF-1 axis

Basel, CH

Somatostatin is expressed in many tissues throughout the body including the CNS, the gastrointestinal (GI) tract and the pancreas. Somatostatin exerts effects on target cells via activation of 5 SRIF receptors (sst1 to sst5) and inhibits effectively growth hormone and insulin-like growth factor-I (IGF-1) release. Natural somatostatins (SRIF 14 or 28) bind with high affinity to all sst receptors, however their use is limited by the rapid proteolytic degradation in plasma (t1/2 3min). The octapeptide Sandostatin (SMS 201–995) is successfully used in 2/3 of acromegaly patients and patients with gastroenteropancreatic (GEP) tumors, although desensitization of the inhibitory response occurs in GEP tumor patients after prolonged treatment. SOM230 is a new SRIF analog which binds with nanomolar affinity to sst1–3 and sst5.

In short term (1 h) rat experiments SOM230 and SMS 201–995 inhibit GH release with similar potency; however, the inhibitory effect of SOM230 on GH release was 4-fold more potent at 6 h post injection than SMS 201–995, indicating its increased metabolic stability. In fact, PK studies in rats demonstrated a plasma half-life of SOM230 of 23 h, as compared to 2 h for SMS 201–995. The improved metabolic stability of SOM230 was confirmed in monkeys and humans. Continuous treatment of rats with SOM230 at 10 μg/kgcdot h, decreased IGF-1 plasma levels on day 2 by 90% while under SMS 201–995 treatment plasma IGF-1 levels decreased only by 49%. After a 2-week infusion of somatostatin analogue in rats the suppression of GH and IGF-1 levels by SOM230 was still pronounced, while the response to SMS 201–995 was largely lost. This enhanced effect of SOM230 on IGF-1 plasma levels was confirmed in an 8-week study where both analogues were infused at the high dose of 50 μg/kg/h in rats. The marked suppression of plasma IGF-1 levels corresponded with potent inhibition of body weight gain of rats. In acute studies in Rhesus monkey, SOM230 and SMS 201–995 treatment resulted in GH inhibition in 1h with ID50 values of 0.5 and 0.4 μg/kg respectively, but plasma IGF-1 levels were only lowered by SOM230 at this early time point (53% at 24h post injection). In Cynomolgus monkeys a 2-week infusion of SOM230, but to a much lesser extent SMS 201–995, lowered plasma GH levels significantly (from 16.3 to 1.8 ng/ml).

In conclusion, SOM230 has a unique structure, binds almost universally to plasma GH levels significantly (from 16.3 to 1.8 ng/ml).

Poster session: New drugs

P933
Phase I study with the dolastatin-10-analogue TZT-1027 in patients with solid tumors

P. Schoffski, B. Thate, G. Beutel, O. Boltz, M. Hofmann, A. Gaenser, A. Jenner, P. Cheverton, M. Satomi
Hannover, D; London, UK; Tokio, J

TZT-1027 is a semisynthetic dolastatin-10 analogue derived from Dolabella auricularia with antineoplastic properties in various preclinical models. The present study evaluated the safety, toxicity, activity and pharmacokinetics of TZT-1027 given as a 30mg/m2 q3w in pts with solid tumors. Pts had histologically verified measurable disease, at least 18 years old, had an ECOG PS <2 and adequate bone marrow, liver, and renal function. Dose limiting toxicity (DLT) was defined for haematological and non-haematological toxicities. The MTD was defined as ≥2 pts experiencing DLT Over 12 months, 21 fully eligible and evaluable pts (19 male, 2 female) were enrolled in this single institution trial. The median age was 56 yrs (range 39–68) and the median no. of previous regimens 4 (range 1–12). Colorectal (11), renal ca. (3) and soft tissue sarcoma (3) tumors were the most common tumor types. All patients had previously received chemotherapy. Dose levels of TZT-1027 ranged from 1.35–3.0 mg/m2. The median no. of cycles was 2 (range 1–4). DLTs were observed in 4 pts at the 3.0 mg/m2 dose level, including neutropenia, fatigue, and a short-lasting, reversible neurotoxic syndrome of mandibular cramps, pain of arms/legs, paresthesias, insomnia and agitation. The most common non-haematological toxicities in cycle 1 were alopecia (8 pts), constipation (5), appetite loss (4), fatigue (4), nausea (4), anorexia (3), abdominal pain (3), pain of neck and mandible (3), and taste irritation (3). Hypernatremia (6), GPT increase (4), hypochloremia (4), creatinine increase (3), GOT elevation (2), and hyperbilirubinemia (2) were seen. Hematologic events were mainly neutropenia (10) and leukopenia (7). The best response (RECIST criteria) was stable disease in 2 renal cell ca. pts, lasting for 2 and 4 cycles. There was no evidence of early metabolic response on serial 18FDG-PET scans according to EORTC criteria. PK evaluation revealed a t1/2 of approximately 7 hrs and linear kinetics. The recommended dose for further clinical trials with TZT-1027 is 2.7mg/m2 q3w.
P935 New thermosensitive liposomes – in vitro and in vivo results

Munich, Göttingen, D

Introduction: Hyperthermia has been used to improve the effectiveness of chemotherapy generally by increasing blood flow, membrane permeability and drug uptake. Thermosensitive liposomes which exclusively release their entrapped drugs in heated tissue thereby reduce drug distribution in drugs to healthy organs. In order to revolutionize the treatment of tumors, the influence of a new synthetic 1,2-dipalmitoylglycero-3-phospho(1,2-diacylglycerol) (DPPGOG) on classic thermosensitive liposomes consisting of DPPC and DSPC was studied. Methods: Thermosensitive liposomes containing carboxyfluorescin (CF) at quenching concentrations were prepared using the lipid film hydration method. In vitro-assays, CF-release of liposomes during incubation in fetal calf serum at different temperatures and for different time periods was measured using a spectrofluorometer. For in-vitro-testing, CF-release of liposome in heated tumor-tissue was measured using a fluorescence camera in a hamster skin flap window chamber. Serum pharmacokinetics was determined by fluorometric analysis of subsequent blood samples. Results: DPPGOG facilitates drug release in a dose dependent manner. DSPC content is responsible for serum stability leading to drug release temperatures between 40 and 43°C. In vivo-experiments of DPPGOG/DSPC/ DPPC-liposomes (3:2:5) showed no drug release at body temperature. Circulation half-life was about 10 h. Applying heat (42°C) at a distinct region of the hamster lead to continuous accumulation of drug in the warmed tissue. Interestingly, drug concentration was not only higher than the peak concentration of freely administered drug but also persisted over several hours in the tissue whereas freely administered drug disappeared completely within 1 h. Conclusion: DPPGOG demonstrates favorable properties in classic thermosensitive liposomes and is the smallest molecule shown to prolong circulation half-life of liposomes. Additionally, DPPGOG facilitates drug release of DSPC/DPPC-liposomes in a dose dependent manner. DSPC in these liposomes could be used to titrate the temperature for drug release between 40 and 43°C. In vivo-experiments demonstrate selective drug delivery into heated tumor-tissue combined with prolonged tumor-half-life of drugs. These liposomes may be candidates for anticancer therapy as well as for non-invasive thermometry.

P936 The cdk inhibitor Roscovitine induces apoptosis in chronic lymphocytic leukemia cells via activation of caspase 3 and modulation of bcl-2 family proteins

I.N. Hahtrow, F. Schneller, M. Oelsner, C. Peschel, T. Decker
Munich, D

Objectives: A new class of cell cycle inhibitors is currently entering clinical trials. These drugs exert their activity by inhibition of cyclin dependent kinases (cdk) and induce cell cycle arrest and apoptosis in cycling cancer cells. Although B-CLL cells are resistant to G0 phase of the cell cycle, B-CLL cells have been described to be susceptible to apoptosis induction by the cdk-inhibitor Flavopiridol. However, Flavopiridol was equally cytotoxic towards normal mononuclear cells. Therefore, we analyzed the effect of Roscovitine, a cdk inhibitor which is currently in preclinical evaluation, on B-CLL cells as well as normal lymphocytes. Material and methods: B cells were isolated from the peripheral blood of patients with clinically and immunologically defined diagnosis of B-CLL. Different assays like Annexin-V/Propidiumiodid (PI), DIOC/PI and TUNEL assays were used for quantitation of apoptotic cells, changes in the mitochondrial membrane potential and DNA strand breaks. Caspase 3 involvement was demonstrated by intracytoplasmatic staining with antibodies specific for active Caspase 3. Expression of zap70, p53 and Bak was increased and Bax cleavage was observed in Roscovitine treated cells. Expression of the proapoptotic protein Bak was increased and Bax cleavage was observed in Roscovitine treated B-CLL cells while antiapoptotic proteins McI-1 and Xiap were downregulated. In contrast, the expression of BCL-2 remained unchanged. Conclusion: In contrast to previous reports in cancer cell lines, Roscovitine treatment was not accompanied by nuclear accumulation of p53.

P937 Successful treatment of refractory metastatic ovarian cancer with the EGFR-tyrosine kinase inhibitor Iressa

Dresden, Halle, Wuppertal, D

Iressa (ZD 1839) a selective inhibitor of the epidermal growth factor receptor (EGFR)-tyrosine kinase seems to be a promising new agent which allows growth inhibition in a variety of solid tumors including lung, prostate, breast, colon and ovarian cancer. A 33-year-old woman with a epithelial ovarian cancer, first diagnosed in 1991, relapsed 4 times after several cytotoxic therapies including platinum, taxol, paclitaxel, topotecan and high-dose chemotherapy with autografting (three courses). In order to induce a graft-versus tumor effect an allogeneic hematopoietic stem cell transplantation (HSCT) from her HLA-identical brother was performed after dose reduced conditioning. She developed acute graft-versus host disease grade II (GVHD) and six months after HSCT all tumor manifestations had disappeared. 14 month after HSCT a intraabdominal and iliacal relapse was diagnosed. Immunohistological staining of an iliacal metastatic lymph node showed an 10–20% expression of EGF-R (HER-2/Neu negative). Iressa was started with 250mg p.o. daily.

After 4 weeks of continuous therapy two out of three tumor manifestations regressed as confirmed clinically and by computed tomography. In addition, CA125 decreased. Therapy was well tolerated with a mild acniform rash and diarrhoea.

This case supports pre-clinical data which show antiproliferative effects of this EGFR-tyrosine kinase inhibitor against ovarian cancer in experimental tumor models. Future trials evaluating the efficacy of Iressa as mono- or combination therapy together with cytostatic or immunotherapeutic treatment modalities are warranted.

P938 Final report of the phase I clinical program of the novel Raf kinase inhibitor BAY 43–9006 in patients with refractory solid tumors

Essen, Wuppertal, D

Raf-1 is a protein kinase that acts as a downstream effector of the Ras signal transduction pathway. BAY 43–9006 (BAY) is an orally active small molecule inhibitor of Raf-1. Here we present a complete report of the 4 single agent phase I clinical trials of BAY in patients (pts) with advanced refractory solid tumors. Four phase I studies were initiated to determine the MTD, DLT, pharmacokinetics (PK), pharmacodynamics and recommended phase II dose of BAY given orally, twice daily, according to 4 dosing schedules: continuous dosing, 28 days on/7 days off, 21 days on/7 days off, 7 days on/7 days off. A total of 172 pts were entered across the 4 studies (median age 55; M/F: 96:76; colon 74, breast 12, HCC 12, ovary 11, GIother 10, renal 9, pancreas 6, melanoma 5, H&N 2; PS 0:1:2 69:82:19). 80% of the pts had stable disease for at least 12 weeks. Median time on drug was 9.6 weeks; 28 pts remained on study for > 6 months and 9 pts remained on study for > 1 year; 10 pts are still ongoing. Furthermore, 2 confirmed partial responses were observed at DL 400 mg bid containing tumor (HCC) and 600 mg bid 21 days on 7 days off (RCC). Additional 4 pts (1 colorectal, 2 ovarian, 1 renal) achieved tumor shrinkage >=20%. Toxicities have been mild-moderate and included reversible skin rash, hand-foot syndrome, diarrhea and fatigue. DLT was diarrhea (DL 400 mg bid, skin
toxicity (DL 800 mg bid 7 days on/7 days off; DL 600 mg bid in all other), nausea, and hypertension (DL 800 mg bid 7 days on/7 days off). With respect to PK, Cmax and AUC increased with increasing doses; interpatient variability was high. Steady state plasma levels of BAY were reached after day 7. There was evidence of inhibition of the Raf kinase pathway in pts treated at higher doses. In summary, the single agent phase I program for BAY is complete. Preliminary analysis of the data show evidence of antitumor activity and a favorable safety profile. These phase I data suggest that BAY is a promising antimetastatic agent, and phase II clinical trials are ongoing at 400 mg bid continuous dosing.

P939
A novel mechanism for potentiation of drug-induced apoptosis: The chemopreventive agent resveratrol sensitizes tumor cells for anticancer drugs through cell cycle arrest and survivin depletion
S. Fulda, K.M. Debatin
Ulm, D
Since resistance of tumors to current treatments protocols remains a major concern in oncology, novel strategies are necessary to target resistance. Naturally occurring dietary compounds such as resveratrol, a polyphenolic phytoalexin found in wine, have gained considerable attention as cancer chemopreventive agents. Here, we identified a novel function of the chemopreventive compound resveratrol: resveratrol acts as a potent sensitizer for anticancer drug-induced apoptosis by inducing cell cycle arrest, which in turn resulted in survivin depletion. Concomitant analysis of cell cycle and apoptosis revealed that pretreatment with resveratrol resulted in cell cycle arrest in S phase and apoptosis induction preferentially out of S phase upon subsequent drug treatment. Likewise, cell cycle arrest in S phase by cell cycle inhibitors enhanced drug-induced apoptosis. Resveratrol-mediated cell cycle arrest sensitized for apoptosis by downregulating survivin expression through transcriptional and posttranscriptional mechanisms, e.g. enhanced proteasomal degradation. Similarly, downregulation of survivin expression using survivin antisense oligonucleotides sensitized for drug-induced apoptosis. Importantly, downregulation of survivin and enhanced drug-induced apoptosis by resveratrol occurred in various human tumor cell lines derived from leukemia, neuroblastoma, malignant brain tumors, melanoma, colon or breast carcinoma independently of p53 status. Also, synergy between resveratrol and cytotoxic drugs was found in patients’ derived primary tumor cells. By demonstrating that drug-induced apoptosis is strongly enhanced by resveratrol through cell cycle arrest and survivin depletion, our findings may have important clinical implication. Thus, this combined sensitizer (resveratrol)/inducer (cytotoxic drugs) concept may be a novel strategy to enhance the efficacy of anticancer therapy and to overcome resistance in a variety of human cancers.

P940
Induction of second complete remission with arsenic trioxide in recurrent acute promyelocytic leukemia
M. Kondakci, R. Fenk, L. Henze, T. Gräf, R. Haas, N. Gattermann
Düsseldorf, D
Background: Arsenic trioxide is capable of inducing second complete remission in the treatment of relapsed patients with acute promyelocytic leukaemia, and only mild adverse effects are reported. The exact role of ATO in the treatment of relapsed APL is currently being evaluated. We studied the therapeutic and adverse effects of ATO in two patients of relapsed APL. Patients and methods: In two patients (41-year-old male, 42-year-old female) with relapsed t(15;17)-positive APL, induction therapy was started with ATO plus all-trans retinoic acid. As first-line therapy both had received several cytotoxic regimens combined with ATRA, and both patients had reached cytological and molecular CR. No maintenance therapy was given. Relapse occurred after 13.5 months. ATO was administered to both patients at a dosage of 0.15 mg/kg over 30 days. The concomitant ATRA treatment had to be discontinued in patient 1 after one week because he developed a pulmonary capillary leak syndrome. He had had similar problems with ATRA during his first-line induction therapy. Results: Patient 1 achieved cytogenetic and molecular CR after induction with ATO. Patient 2 showed a cytological remission and a cytogenetic remission by conventional cytogenetics, but there were 2% residual abnormal cells on FISH analysis. No significant adverse effects of ATO occurred in patient 1. The pulmonary problems must have been related to the administration of ATRA since they resolved after ATRA was stopped, but the patient went through a cytopenic phase (WBC < 1000/µl for two weeks, only one platelet transfusion required). Patient 2 had no severe side effects from ATO during induction, but discontinued ATO during a second (consolidation) course, because of worsening of a pre-existing polynephropathy. Conclusion: Treatment for relapsed APL is not yet standardized but should include ATO which was successful in our two patients. Regarding adverse effects, we saw a worsening of symptoms in a patient with preexisting polynephropathy. ATO-induced leukocytosis in the other patient was effectively treated with low-dose chemotherapy. It remains to be seen which type of consolidation therapy should follow successful ATO treatment of relapsed APL.

P941
P-glycoprotein mediated drug efflux is a resistance mechanism of chronic myelogenous leukemia cells to treatment with STI571
Dresden, D
STI571 (Gleevec) is an intracellular acting drug that shows high activity against chronic myelogenous leukemia cells in vitro and in vivo (CML) or acute lymphoblastic leukemia (ALL). However, many of the patients especially with advanced disease develop drug resistance. Here we show by a novel high performance liquid chromatography (HPLC) based method that intracellular levels of STI571 decrease in P-glycoprotein (Pgp) positive chronic myelogenous leukemia. Importantly, downregulation of survivin and enhanced drug-induced apoptosis by resveratrol occurred in various human tumor cell lines derived from leukemia, neuroblastoma, malignant brain tumors, melanoma, colon or breast carcinoma independently of p53 status. Also, synergy between resveratrol and cytotoxic drugs was found in patients’ derived primary tumor cells. By demonstrating that drug-induced apoptosis is strongly enhanced by resveratrol through cell cycle arrest and survivin depletion, our findings may have important clinical implication. Thus, combined sensitizer (resveratrol)/inducer (cytotoxic drugs) concept may be a novel strategy to enhance the efficacy of anticancer therapy and to overcome resistance in a variety of human cancers.

P942
Oral administration of Gefitinib (‘Iressa’, zD1839) is active against brain metastases in patients with non-small-cell lung cancer
E. Roggero, G. Busi, A. Palumbo, A. Pedrazzini
Locarno, CH
The epidermal growth factor receptor (EGFR) is expressed in a wide range of tumor cells, and expression correlates with disease progression, resistance to chemotherapy, and tumor invasion. The orally active EGFR tyrosine kinase inhibitor (EGFR-TKI), gefitinib (‘Iressa’, zD1839) blocks signal transduction pathways implicated in the proliferation and survival of cancer cells. We report the case of a 77-year-old non-smoking woman with advanced NSCLC in whom we observed regression of brain metastasis after oral administration of gefitinib (‘Iressa’, zD1839). This patient was first diagnosed in January 2001 with a NSCLC with metastases in the liver and contralateral lung. She was highly symptomatic (never ending cough, asthma and dyspnoea at rest) and received two different types of polychemotherapies for one month each (vinorelbine/cyclophosphamid as first line and irinotecan/gemcitabine, as second line). Her general condition deteriorated. Leukocytosis was continued. Leukocytosis and the patient complained about headaches. Tumour progression was confirmed in brain (diffuse and cerebellar lesions) lung and liver. The patient was included into the Iressa Expanded Access Program and started therapy in April 2002 (once daily doses of 250mg). Her performance status was WHO Grade 2. After 2 months of Iressa therapy, a significant radiological reduction in primary tumour, brain and liver metastases was documented on chest X-ray and CT-scan. Her symptoms of fatigue, cough and pain had improved and the patient had a good performance status for...
approx. 7 weeks. During Iressa therapy she presented with a herpes zoster who rapidly regressed after 10 days of aciclovir administration. Iressa intake was interrupted for 6 days. The patient died in September 2002 due to rapid progression of liver metastases. The levels of expression of EGFR were determined: 100% of tumor cells displaying membrane staining with EGFR. 70% of tumor cells displaying the highest intensity of membrane staining and 90% of tumor cells displaying cytoplasmic EGFR staining.

This observation suggests that gefitinib can benefit patients with NSCLC that overexpresses EGFR and provide evidence that gefiTinib can penetrate blood-brain barrier and can be active in patients with central nervous system metastases.

**Abstracts**

**P943**

**Synergistic activity of imatinib and the heat-shock-protein-90 inhibitor 17-AAG in Imatinib-resistant leukemia cells overexpressing Bcr-Abl**

J. Topaly, M. Schad, S. Laufs, B. Schultheis, J. Melo, W. J. Zeller, S. Fruehauf

Heidelberg, D; London, UK

Overexpression of the BCR-ABL protein is one of the mechanisms of imatinib resistance which accounts for 10–15% of resistant cases. As combination therapy may allow to overcome drug resistance, we were interested in the effect of combination treatment with imatinib and 17-allylamino-17-demethoxygeldanamycin (17-AAG), an inhibitor of the heat shock protein 90 (Hsp90) chaperone complex. In imatinib-sensitive CML cell lines, combination index values (CI) obtained using the method of Chou and Talalay indicated additive (CI = 1) or slightly antagonistic (CI > 1) effects following simultaneous treatment with imatinib and 17-AAG. Additivity was also seen in primary chronic-phase CML cells using a colony forming assay. In contrast, the agents acted synergistically in imatinib-resistant cells (CI = 0.6, 75% growth inhibition level). Annexin V / propidium iodide staining showed that the activity of imatinib and 17-AAG is mediated by apoptosis after 24 and 48 hours of incubation. In imatinib-resistant cells BCR-ABL mRNA levels and BCR-ABL protein expression were increased compared to imatinib sensitive parental cells. 17-AAG alone lowered BCR-ABL protein levels in both cell lines as expected. Combination treatment was even more effective in this respect. Interestingly, on CRKL and phosphotyrosine immunoblots of imatinib-resistant cells a significant decrease of CRKL phosphorylation was only observed in the high-dose combination therapy group (imatinib 1 µM, 17-AAG 2 µM) whereas in all other monotherapy and low-dose combination therapy groups no such effect was observed. In the imatinib-sensitive parental cells the combination also was not more effective than imatinib alone. These new findings suggest that combination of imatinib and 17-AAG may be useful to overcome imatinib resistance caused by BCR-ABL overexpression.

**P944**

**Circadian variability of MAP kinase activation affects pharmacodynamic monitoring of signal transduction inhibitors**


Essen, D

The Ras – Raf pathway is involved in abnormally elevated signalling of many common solid tumours. The downstream extracellular signal-regulated kinase (ERK) serves a shuttle protein into the nucleus and thus initiates the cell-proliferation. BAY 43–9006 is a novel potent and orally active inhibitor of Raf kinase and thus directed toward a specific molecular target misregulated in many tumours. It was the purpose of this study to develop a method for the quantification of the inhibitory potency of this new compound, measuring the phosphorylated (activated) ERK as a biomarker.

Evidence of Raf kinase inhibition was measured by suppression of PMA-stimulated ERK phosphorylation. Specifically, about 300 blood samples from 30 volunteers were analyzed in the absence and presence of PMA for the development and validation of the biomarker assay. We detected a circadian rhythm in the extend of inducible phosphorylated ERK1/2 proteins. Analyzing the measured values, we were able to describe a sinusoidal distribution of the maximum inducible ERK1/2 phosphorylation at 12 o’clock (midday).

Following our observation, the biomarker studies within a phase I study were conducted using peripheral blood lymphocytes (PBLs) collected from 66 patients with advanced cancers treated with BAY 43–9006 at various dose levels. PBLs, taken in a time window within two hours around midday, were monitored for BAY 43–9006-dependent inhibition of ERK phosphorylation using our flow cytometry technique. Blood samples were collected for analysis before treatment with BAY 43–9006 and on days 1, 2 and 10–21 post treatment to allow comparisons among patients at different dose levels.

The data presented demonstrate inhibition of ERK phosphorylation in patients treated with BAY 43–9006. Substantial inhibition of PMA stimulated ERK phosphorylation was obtained in CD-7 positive T-cells in 4 out of 6 patients following continuous treatment with 400 mg bid, in 6 out of 14 patients following continuous treatment with 600 mg bid, and in 6 out of 6 patients following continuous treatment with 800 mg bid.

Considering the observed circadian rhythm, phosphorylated ERK1/2 may serve as a biomarker for drugs targeting the MAP-kinase cascade. Our results demonstrate that BAY 43–9006 administered at dose levels > 200 mg bid inhibits PMA-stimulated ERK phosphorylation in treated patients and indicate that PBLs are suitable surrogate tissues for biomarker studies in future trials.

**P945**

**In vitro efficacy of combined treatment depends on the underlying mechanism of resistance in imatinib-resistant Bcr-Abl positive cell lines**

P La Rosée, K. Johnson, A.S. Corbin, E.P. Stoffregen, E.M. Moseson, M.W. Deininger, B.J. Drucker

Portland, USA

Imatinib mesylate (Glivec (R), formerly STI571) is an effective therapy for all stages of chronic myelogenous leukemia (CML). While responses in chronic phase CML are generally durable, resistance develops in many patients with advanced disease. Major mechanisms of resistance are Bcr-Abl amplification of Bcr-Abl and mutations within the Ab1 kinase domain that interfere with drug binding. We evaluated arsenic trioxide and decitabine for their potential to overcome resistance in cell lines with imatinib induced Bcr-Abl amplification (AR230-r1, Ba/Fc/BLR-r1; Mahon et al., 2000) or in cell lines expressing mutant Bcr-Abl (Ba/F3p210 Y253F, M351T, or T315I; La Rosée et al., 2002), representing various levels of residual sensitivity to imatinib. Using cell proliferation assays, we investigated whether different mechanisms of resistance to imatinib would alter the efficacy of arsenic trioxide (As2O3; Trisenox (TM)) or 5-Aza-2-deoxycytidine (decitabine) alone and in combination with imatinib.

Our results indicate that resistance to imatinib induced by Bcr-Abl overexpression or by engineered expression of clinically relevant Bcr-Abl mutants does not induce cross-resistance to As2O3 or decitabine. Combined treatment with these agents and imatinib is additive or synergistic in cell lines that have residual sensitivity to imatinib monotherapy (AR230-r1, Ba/Fc/BLR-r1, Y253F- and M351T- expressing Ba/F3p210 cells), at doses of imatinib that overcome resistance. Cells expressing T315I, which is completely insensitive to imatinib, showed no benefit from the addition. However, when imatinib was used at low dosages, combination treatment with imatinib leads to antagonism. Apoptosis studies using flow cytometric analysis of activated caspase-3 suggest that this can be explained in part by the reduced pro-apoptotic activity of imatinib in resistant cell lines. Quantitative analysis of kinase activity using anti-phosphotyrosine immunoblots revealed that apoptosis is induced if kinase activity is reduced to < 50% of baseline levels. This data underlines the importance of resistance-testing and quantitative assays of kinase inhibition to optimize treatment results.
P946

Leukemic stem cells from patients with acute myeloid leukemia with and without activating FLT3 mutations are highly sensitive to the protein tyrosine kinase inhibitor SU6614

N. Arseni, K. Speikermann, C. Buske, S. Schnittger, W. Hiddemann, M. Feuring-Buske

Munich, D

Constitutively activating length mutations (FLT3-LM+) and point mutations of the receptor tyrosine kinase FLT3 can be detected in up to 40% of patients with acute myeloid leukemia (AML) and represent the most frequent single genetic abnormality in patients with this disease. It has been shown that transformation of hematopoietic cell lines by FLT3 mutants is depending on its kinase activity. Thus targeting of the FLT3 activated kinase by FLT3 protein tyrosine kinase inhibitors (PTK) is a promising treatment approach. Aim of our study was to test whether the PTK inhibitor SU6614 not only eliminates leukemic blasts but more importantly leukemic stem cells while sparing their normal counterparts. AML blasts from patients at diagnosis with FLT3-LM+, D835+ and non-mutated FLT3 as well as CD34+ enriched (>98%) normal BM cells were incubated for 24 hrs with 0–10μmol SU6614. After pre-incubation cells were washed and the mean% kill of AML colony-forming cells (CFC), long term culture-initiating cells ( LTC-IC), and suspension culture-IC (SC-IC) was evaluated. In the control arm colony growth could be obtained in 3/3 D835+, 3/3 FLT3-LM+ and 2/2 normal AML samples. SU6614 inhibited the growth of AML CFC in 2/3 D835+ and 3/3 FLT3-LM+ AML samples (77–100%). PCR analysis from single residual CFC from 1 patient with FLT3-LM+ showed the absence of the FLT3-LM. In contrast CFC growth was largely unaffected in 2 samples with non-mutated FLT3 indicating that SU6614 is able to eliminate leukemic blasts in the majority of patients with constitutively activated FLT3. On the level of leukemic stem cells SU6614 induced a total eradication of AML SC-IC and LTC-IC in 2/3 D835+ and 3/3 FLT3-LM+ samples. Intriguingly, SU6614 also eradicated leukemic stem cells from AML patients with non-mutated FLT3 (89–100%). However, SU6614 showed a high activity on stem cell candidates from healthy donors with an eradication of all BM SC-IC (n = 3). Our data indicate that SU6614 is a potent drug for the elimination of leukemic stem cell candidates from patients with FLT3-LM+, D835+ AML as well as those with non-mutated FLT3. However, SU6614, which is known to target different kinases such as FLT3, VEGFR-2 and Kit, is not sparing normal stem cells. These data underline the necessity to test the impact of kinase inhibitors on normal and leukemic stem cells in appropriate assays and point to a potentially considerable stem cell toxicity of agents inhibiting multiple stem cell relevant kinases.

Poster session: Melanoma / sarcoma

P947

Prognostic factors for development of metastases in uveal melanoma: > 5 years follow-up of 271 patients


Berlin, D

The frequency of metastases after therapy of uveal melanoma is 15–40%, which is associated with a very poor prognosis. Since modern vaccination strategies are promising in high-risk cutaneous melanoma patients, the determination of prognostic factors in ocular melanoma is urgently required to be able to perform adjuvant vaccination trials with innovative regimens. We analysed the course of patients with uveal melanoma, who were first diagnosed between 1994 and 1995 in our hospital. Data on the primary tumor, primary therapy and follow-up were collected. 271 patients with a mean age of 57 (8 – 86) years were treated. In 59% of patients the tumor was located anterior of the equator, of which 41% had involvement of the ciliary body. 10 out 271 patients had an extracocular tumor growth (EOG). The mean of the largest tumor diameter (LTD) was 12.7 mm (standard deviation 3.5 mm) and the mean thickness was 6.1 mm (standard deviation 3.0 mm). 85% and 71% of patients were available for a potential follow up of >4 and >5 years. A total of 50 (18.5%) developed metastases during the follow-up period. 35.4% of patients with ciliary body involvement, 70% with EOG and 32.4% with an LTD >15 mm developed metastases. These 3 factors were associated with a decreased time to progression (log-rank test for both factors p<0.01). This study defines prognostic factors as a basis for adjuvant therapy protocols.

P948

Peptide vaccination can induce high-frequency T cell responses and long-term freedom from relapsing skin metastases


Berlin, D

Peptide vaccination can induce regression of melanoma which was mostly reported in patients with skin metastases. We have vaccinated 8 melanoma patients with multiple cutaneous relapses with tyrosinase peptides alone or in combination with MAGE-3 peptides at our institution. Peptides were applied intradermally and subcutaneously in combination with the adjuvants OM-CSF alone or in combination with KLH. While patients had 3 or more cutaneous, subcutaneous or mucosal relapses (median 6, range 3 – 10 relapses) during the year before vaccination, 4 of the 8 patients have experienced long-term freedom from recurrence of cutaneous metastases after vaccination was initiated for currently 12+, 33, 60+, and 35+ months. Two of these 4 patients had developed postvaccination however, a single site lymph node or small bowel metastasis, respectively, which could be completely resected. In another patient with > 10 mucosal relapses vaccination was effective to control mucosal relapses while multiple lymph node and bone metastases occurred. Using intracellular cytokine staining and tetramer staining induction of up to 2.0% tyrosinase peptide-specific T cells could be demonstrated in 5 of 6 patients receiving more than 6 vaccinations. Further phenotypic analysis revealed that tyrosinase-specific T cells belong to both effector T cell (CD45RA+CCR7-) as well as memory T cell subsets. Tyrosinase-specific memory T cells were enriched in the bone marrow in 2 of 2 patients analyzed. Our results show that peptide vaccination can be effective in preventing cutaneous metastases and induces sustained effector and memory T cell responses.

P949

Vaccination with a mimotope of the high molecular weight melanoma-associated antigen induces antibodies inhibiting melanoma tumor cell growth


Vienna, A; Buffalo, USA

Tumor-associated antigens (TAA) are potential targets for the development of immunotherapeutic approaches. For melanoma the human high molecular weight-melanoma-associated antigen (HMW-MAA) has been described as such a TAA. Like most of the TAAs the HMW-MAA also represents a self-antigen. One approach to apply specific immunotherapy for malignant melanoma is to break the tolerance to this self-antigen. We used the anti-HMW-MAA monoclonal antibody 225.28S for screening of a linear pVIII-9aa and a circular pIII-10aa phage peptide library to develop peptide mimics of the HMW-MAA. After three rounds of panning specific phages were enriched and the DNA coding for the displayed peptides was sequenced. The deduced amino acid sequences of the peptides showed no homology to the amino acid sequence of the HMW-MAA. In ELISA experiments 16 phages inhibited the binding of the 225.28S antibody to the HMW-MAA. These 3 factors were associated with a decreased time to progression (log-rank test for both factors p<0.01). This study describes a potential follow up of >4 and >5 years. A total of 50 (18.5%) developed metastases during the follow-up period. 35.4% of patients with ciliary body involvement, 70% with EOG and 32.4% with an LTD >15 mm developed metastases. These 3 factors were associated with a decreased time to progression (log-rank test for both factors p<0.01). This study defines prognostic factors as a basis for adjuvant therapy protocols.
High dose chemotherapy with autologous peripheral blood stem cell transplantation for bone and soft tissue sarcomas

B. Kasper, C. Harter, A.D. Ho, G. Egerer
Heidelberg, D.

The role of high-dose chemotherapy (HDCT) with autologous peripheral blood stem cell transplantation (PBSCT) in the treatment of bone and soft tissue sarcomas is not defined. 

From August 1998 to March 2003 21 patients (pts.) with bone and soft tissue sarcomas received HDCT and PBSCT (osteosarcoma n = 6, ewing sarcoma family n = 7, MPNST n = 3, liposarcoma n = 2, rhabdomyosarcoma n = 1, meningo sarcoma n = 1 and synovial sarcoma n = 1). 12 pts. were female, 9 pts. were male. Median age at date of transplantation was 26 years [range: 13 – 44].

HDCT was performed in 6 pts. as 1st line therapy. Following conventional chemotherapy and surgery CR (n = 9), PR (n = 6), SD (n = 2) and PD (n = 4) were reached prior to PBSCT. For HDCT different conditioning regimens were used: ICE/PEI (Ifosfamid 2000 mg/m²/d 1–6; Carboplatin 200 mg/m²/d 1–6; Etoposid 200 mg/m²/d 1–6) (n = 9), Melphalan 60 mg/m² 1–3 + Etoposid 1000 mg/m² dia 1–3 (n = 3), Melphalan 140 mg/m² + Busulfan 600 mg/m² (n = 6), or Melphalan 200 mg/m² alone (n = 1). Samarium 153 + Carboplatin/Etoposid (Carboplatin 150 mg/m² dia 1–4; Etoposid 150 mg/m²/d 1–4) (n = 2). Two pts. were treated with tandem-PB SCT. One pt. died due to complications related to HDCT were observed. Three pts. died within 6 months after PBSCT due to PD, in another 5 pts. disease re-occurred after PBSCT and led to death, 12 pts. are still alive.

Despite a short follow-up time, median progression free survival (PFS) in all pts. is 11.6 months [range: 0 – 59]. Nine pts. in CR before PBSCT showed no evidence of disease (NED) after PBSCT and demonstrated a PFS of up to 50 months [range: 3 – 50]. Pts. in PD before PBSCT could not benefit from the HDCT.

In summary, although the role of HDCT with PBSCT in the treatment of sarcomas is not defined, a subgroup of pts. who achieved CR before HDCT could benefit from this strategy.

Treatment and response of advanced sarcomas using Imatinib

Düsseldorf, D.

The tyrosine kinase inhibitor Imatinib selectively inhibits bcr-abl, platelet-derived growth factor receptor and the c-kit receptor tyrosine kinase. Besides a high response rate in CML patients treatment of patients with gastrointestinal stromal tumors (GIST) commonly expressing the c-kit receptor resulted in remission rates of 91%. The data prompted us to treat three patients with advanced, c-kit positive sarcomas. One patient had a rhabdomyosarcoma with liver metastases and was previously treated with radiation, surgery and cytotoxic chemotherapy. The second patient had a angioleiomyosarcoma with diffuse liver metastases, previously treated with radiation and surgery and the third patient had an osteosarcoma with lung metastases and one psoas metastase previously treated with radiation, surgery and chemotherapy. All patients had progressive desease stage IV according to American Joint Committee on Cancer. As assessed by a recently published standard immunohistochemical protocol, the rhabdomyosarcoma showed weak, angioleiomyosarcoma intermediate and osteosarcoma strong c-kit expression. Patients received between 400–600 mg imatinib orally with a mean duration of treatment of 45 days (33-60 days).

The first patient with rhabdomyosarcoma had tumor progression. The second patient with angioleiomyosarcoma with liver metastases had a stable disease due to cardiac arrest after symptoms disappeared. The third patient with osteosarcoma had stable disease for 227 days showing slow progression of lung metastases 7 months after finishing imatinib treatment. The metastase of the P. pelvis remained stable after 15 months and showed calcification in CT scan. We conclude that Imatinib seems to be a therapeutic option in c-kit positive advanced sarcomas.

Successful treatment of advanced epithelioid angiosarcoma with Imatinib mesylate

A.M.S. Müller, A.H. Schmitt-Gräff, H. Veelken
Freiburg, D.

Angiosarcomas represent a rare subtype of soft tissue sarcomas with poor prognosis due to lack of effective therapeutic options. We report a rapid and durable regression of an advanced peritoneal angiosarcoma upon treatment with imatinib mesylate.

A 51-year old man presented in the emergency unit with weight loss of 11 kg in rapidly deteriorating general condition (Karnofsky score 50%), a three-week history of night sweats and a prominent increase of abdominal girth. Massive ascites and a dolent epigastric mass were noted. Computed tomography (CT) demonstrated extensive, partially necrotic peritoneal masses. Positron emission tomography (PET) showed intense, inhomogeneous uptake of 18F-desoxyglucose throughout the abdomen. A peritoneal biopsy was composed of atypical fusiform cells with epithelioid formations, numerous vascular spaces, frequent mitoses and immunohistochemical coexpression of CD34, CD31, and CD117, consistent with a diagnosis of malignant epithoeloid angiosarcoma. Oral treatment with 400 mg imatinib per day was initiated. After one week of therapy, the abdomen was less extended; repeated PET imaging demonstrated dramatically reduced metabolic activity. After 8 months of continuous therapy, the patient was in excellent condition (Karnofsky score 90%). Restaging by CT revealed only minimal residual abdominal lesions.

Angiosarcomas account for 1–2% of soft tissue sarcomas and mostly develop as cutaneous tumors, whereas primary intestinal or peritoneal manifestation is extremely rare. Macroscopic examination usually reveals multinodular hemorrhagic masses. Histologically, the tumor is composed of epithelioid and spindle cell areas. Co-expression of endothelial antigens, including CD34 and CD31, helps to establish the diagnosis. Expression of CD117 (c-kit) appears to occur in a subset of 25-56% of angiosarcomas. Surgery has been the mainstay of treatment for angiosarcoma, since the results of radiation and chemotherapy have been disappointing. Proven efficacy of imatinib treatment for gastrointestinal stromal tumors (CD31-negative), prompted us to test our case for expression of c-kit, and to initiate imatinib therapy based on the CD117 positivity. The impressive and durable clinical response of this case leads us to recommend testing of angiosarcomas and other soft tissue sarcomas, for c-kit-immunoreactivity.

To our knowledge, this is the first case of an advanced non-GIST soft tissue sarcoma treated successfully with oral therapy.
Purpose: The study was performed to determine the influence of time and tumour volume after different oncologic treatment strategies on sensitivity and specificity of positron emission tomography (PET) using 18-fluorodesoxyglucose (FDG), and the benefit of FDG-PET concerning early recognition of recurrences in an experimental tumour system. Methods: Subcutaneously growing rhabdomyosarcoma R1H of the rat either were resected, irradiated applying total doses of 80 or 85 Gy (30 fractions in 6 weeks) or treated with chemotherapy for a median of 8 applications (3 per week, range 3–20) using vincristine, doxorubicine and ifosfamid or combination of the latter. Tumour volume was determined twice a week. PET was performed weekly before, during and for 6 months after therapy using a conventional full-ring whole body PET-Scanner. Animals received 4–11 MBq FDG s.c. 3 h prior to PET acquisition. PET results and actual tumour volume were compared. Sensitivity and specificity were calculated and the time difference was determined for recurrences diagnosed by volumetry compared to FDG-PET. Results: 39% (7/18) and 40% (8/20) of the tumours were locally controlled by surgery or fractionated irradiation, respectively, while chemotherapy did not achieve local control. Recurrent tumours were detected by volume measurements 45 days, 75 days and 81 days (median) before approaching volumes of 0.2 cm³, 0.5 cm³, 0.8 cm³, or 1.0 cm³, respectively. This time benefit of FDG-PET was reproducible detection of R1H-tumours using FDG-PET. The quality of time difference was determined for recurrences diagnosed by volumetry compared to FDG-PET. Conclusions: The experimental system allows reproducible detection of R1H-tumours using FDG-PET. The quality of this detection is very good for tumours of more than 0.1 cm³. Time benefit of FDG-PET is dependent on growth rate of the tumour.

Objective: Imatinib was developed as a specific inhibitor for the Bcr-Abl protein tyrosine kinase which plays a keyrole in the pathogenesis of CML. The expanding understanding of the basis of imatinib-mediated tyrosine kinase inhibition has revealed a spectrum of potential new antitumor applications beyond the powerful activity already reported in the treatment of CML. Imatinib has shown activity in vivo against PDGF-driven tumors and is also a potent inhibitor of the Kit receptor tyrosine kinase. We report on two patients with thymic carcinoma which showed a strong expression of CD117 (c-KIT). Methods and results: Case 1: 04/02 a 54-year-old man presented with thoracic pain. Laboratory tests showed an elevated LDH and elevated liver enzymes. A sonographic examination of the liver revealed multiple metastatic nodules and on CT scans we found a tumour mass in the anterior part of the mediastinum. A needle biopsy of the liver was performed. Histologic workup showed a metastatic poorly differentiated small cell carcinoma and after immunostaining the diagnosis of a WHO type C thymic carcinoma was formed. A strong expression of CD117 (c-KIT) was detected. As a resection of the tumor could not be done we started a treatment with imatinib (400mg/d). The physical condition and the pain improved significantly, the elevated liver enzymes normalized, and CT scans showed stable disease. But 6 months later the disease progressed and we started chemotherapy. Case 2: A 63-year-old man presented with dyspnoe. Chest x-rays showed a mediastinal mass and a parietic diaphragma. After thorascopoeic examination a thoracotomy was performed and the tumor resected. Unfortunately resection was not complete and therefore the patient received a thoracic radiation. Histologic examination of the tumor showed a poorly differentiated thymic carcinoma with strong expression of CD117 (c-KIT). Conclusion: Two consecutive cases of poorly differentiated thymic carcinomas showed a strong expression of CD117. Patient 1 had metastatic disease and showed a transient stabilisation under a treatment with imatinib. These cases implicate that CD117 expression should be examined in thymic carcinomas and imatinib could be a therapeutic option in those patients.

Abstracts
Modulating therapy appears to be an effective therapy in Morvans chorea and neuromyotonia (Isaacs syndrome). Despite radiographic response of the tumor paraneoplastic syndromes may progress. Antibody titers could be useful in indicating resolution of the paraneoplastic syndrome.

P958
Merkel cell carcinoma – a rare but curable disease
M. Schroeder, B. Alkemper, U. Wieschermann, A. Giagounidis, C. Aul
Duisburg, D

In 1972, the rare tumor entity Merkel cell carcinoma (MCC) was first described by Toker. The incidence of this tumor type which occurs more often in elderly patients is increasing. Preferred sites of the tumor are head, cervical region and extremities. The tumor is characterized by high rates of locoregional relapses and an aggressive clinical course.

We analyzed 9 consecutive patients (2 male and 7 female, median age 71 years) who were treated in our department between 1989 and 2001. The tumor was most often localized at the lower extremities (5 cases) followed by rima ani (2 cases), arms and cervical region (1 patient).

2 patients had disseminated disease at the time of first diagnosis, 7 patients suffered from locally advanced disease. In all patients initial treatment consisted of surgical resection of MCC. Subsequently, all patients received chemotherapy with regimens used in small cell lung cancer (ACO, cisplatin plus etoposide, ifosfamide plus etoposide). Complete and partial responses were observed in all patients with disseminated disease. 5 of 9 patients are alive without recurrent disease 26 to 164 months after initial diagnosis.

4 patients developed disease progression and died 8 to 25 months after diagnosis. Unlike patients with small cell lung cancer, none of our patients developed brain metastases. We conclude that multidisciplinary treatment of MCC results in high response rates and good overall survival even in elderly patients. MCC responds well to regimens employed in SCLC. The role of newer agents e.g. paclitaxel and topotecan employed in SCLC. The role of newer agents e.g. paclitaxel and topotecan still remains to be examined.

P959
Regression of non-resectable inflammatory myofibroblastic tumors after treatment with non-steroidal anti-inflammatory drugs
Regensburg, D

Inflammatory myofibroblastic tumors (IMT) is a rare, but distinctive mesenchymal neoplasm of the soft tissue and viscer. Histologically, this tumor is composed of fascicles of bland myofibroblasts admixed with a prominent inflammatory component. Recent molecular studies revealed rearrangements on chromosome 2p23 within the ALK locus proving the clonal origin of this tumor. Surgical excision is usually curative, but intraabdominal tumors tend to be more aggressive and may relapse locally or even metastasize. In general, chemotherapeutic regimens including ifosfamide, dacitoximun, and vincristine do not provide any clinical benefit in IMT. Here we report on a 63-year old female patient (pat. #1) and a 22-year old male patient (pat. #2) with an advanced, non-resectable intraabdominal tumor mass. Histopathological examination revealed the classical criteria of an IMT in both patients. Pat. #1 was treated with 50mg diclofenac and 150mg prednisolone. After 1 week prednisolone was withdrawn by stepwise reduction. Radiologic examination (CT scan) demonstrated a regression of the tumor mass resulting in a complete remission at 14 months after treatment. The treatment with non-steroidal anti-inflammatory drugs (NSAID) was continued up to date and the patient is still in complete remission (20+ months). Similar to pat. #1, pat. #2 was treated with diclofenac and prednisolone. Diclofenac was changed to ibuprofen (2x 400mg daily) because of gastric discomfort. Clinical response consisted of a stable disease of the abdominal tumor for 12 months. In summary, treatment with NSAIDs resulted in a complete regression and stable disease in 2 patients with non-resectable IMT. Our data suggest that in the absence of cytotoxic drugs NSAIDs may exert potent antitumor effects in this rare inflammatory tumor entity.

P960
Data base in adult patients with Langerhans’ cell histiocytosis
G. Götz, J. Fichter
Osnabrück, D

Langerhans cell histiocytosis (LCH) in adults is either a monoclonal or polyclonal proliferation of Langerhans cells. Clinically LCH is classified in single system disease and multi system disease. Pulmonary involvement occurs in the majority of adult patients. An important exogenous factor for these patients is smoking habits. To find differences in the different groups larger numbers of patients were required.

In this study 49 adult patients with histologically proven LCH mean age 43 +/- 12 years were recruited by a patient organisation.

28% of patients had multisystem disease. Female patients were more frequent (59%). The most frequent organ involvement in single organ disease were the lungs (44%) followed by bones (17%). The combination of lung and bone involvement was the most frequent multisystem disease (17%). All patients with single organ involvement of the lungs (n = 22) were smokers or exsmokers. However in the group of patients with nonpulmonary single organ involvement or multisystem involvement 20 patients were smokers or exsmokers, but 7 patients were nonsmokers. These data support the hypothesis that smoking and pulmonary single organ disease are closely linked in contrast to nonpulmonary single organ disease and multisystem disease. A data base with unselected patients regarding organ involvement in adults is either a monoclonal or polyclonal proliferation of Langerhans cells. Clinically LCH is classified in single system disease and multi system disease. Pulmonary involvement occurs in the majority of adult patients. An important exogenous factor for these patients is smoking habits. To find differences in the different groups larger numbers of patients were required.

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Poster session: Urogenital tract cancer
P961
False-positive human chorionic gonadotropin concentrations caused by heterophilic antibodies leading to unnecessary surgery
A. Trojan, H. Joller-Jemelka, R. Stahel, E. Jacky, M. Hersberger
Zurich, CH

Elevated levels of the beta subunit of human beta-hCG may be early indicators of recurrent germ cell tumors, even in patients with initially negative markers. Despite determination of beta-hCG is routinely used for risk evaluation and post-therapeutic follow up, cases of falsely elevated beta-hCG have only been reported in women after incidental pregnancy tests and suspected choriocarcinoma and in a man with testicular epidermoid cyst. We report of a 43-year old sexually active man, diagnosed for classical seminoma stage I with negative beta-hCG markers seven years previously. The patient underwent semicastratio and radiation therapy of ipsilateral lymph draining vessels. Since at the routine five-year follow up of the patient repeated serum beta-hCG levels were elevated (408 IE/l; norm <5.0 IE/ml), we were prompted to assess the patient by thoraco-abdominal and brain catscan. All examinations performed including PET-CT and ultrasound of the remaining testis revealed no evidence for tumor. Although risk for relapse of seminoma and incidence of secondary malignancy appeared very low, a pericardial cyst suspicious for harboring tumor as the source for elevated beta-hCG levels was removed by surgery. However, pathological examination revealed no malignancy. Strikingly, one day after removal of the cyst, beta-hCG decreased to undetectable levels, inconsistent with the estimated half-life of this marker. Work-up of this phenomenon revealed that pre- and post-surgery beta-hCG determinations were performed in different laboratories using also different test systems (AxSYM beta-hCG assay; Elecsys hCG plus beta assay system). To test the hypothesis of falsely elevated beta-hCG levels, simultaneous analysis of the patients serum was performed using the two test systems. Results revealed no beta-hCG using the Elecsys hCG plus beta assay; in contrast the AxSYM beta-hCG assay system showed elevated beta-hCG levels in the blood serum (447 IU/l) unchanged from preoperative testing. Of note, both tests did not detect any beta-hCG in the patients urine. Consequently, dilution experiments clearly detected weak anti-mouse antibodies (titer 1:2) in the Ouchterlony immuno-diffusion assay indicating thereby the presence of heterophilic antibodies, presumably responsible for divergent test results in the patients plasma. Awareness of such ‘phantom beta-hCG’ elevations and caution should be exercised when clinical findings and laboratory results are discordant.
Intensive chemotherapy with autologous stem cell transplantation over a 10-year period in 51 patients with germ cell cancer

A.M.S. Müller, C.F. Waller, S. Fetscher, M. Lübbert, G. Döiken, M. Engelhardt
Freiburg, Lübeck, Greifswald, D

Despite the high cure rate in GCT, high-risk (HR) patients (pts) as defined by IGCCCG have a <50% cure rate. Intensive chemotherapy followed by ASCT has resulted in impressive remission rates in HR pts. Here we report on 51 consecutive pts diagnosed between 2/93-6/02 undergoing intensive therapy with ASCT between 1/93-12/02 due to HR or relapsed/refractory disease. HR, intermediate and low-risk GCT pts were present in 29, 15 and 7, respectively, in the latter showing either relapsed or refractory disease. Median Σ-HCG, Σ-AFP and LDH levels were 892-, 78- and 2-fold increased, respectively. Bulky disease was observed in 43 pts. Primary tumor location at diagnosis was testis, retroperitoneum or mediastinum in 42, 1, 6 pts, respectively, and mediastinal plus retroperitoneal location in 2 pts. Single ASCT were performed in 33 and tandem ASCT in 18 pts. 5 seminomatous and 46 nonseminomatous GCT were treated, the latter with mixed histology (n = 26), embryonic (n = 9), teratoma (n = 8) or yolk sac tumors (n = 3). After induction therapy, high-dose chemotherapy with either VIP (VP-16 + V 1500mg/m², Carboptin = C 1500mg/m², Ifosphamide = I 1200mg/m²; n = 55), selected protocols (IC: C 125mg/m², IF: CI 50mg/m²) n = 9 or BEP-derivatives (THCE [paclitaxel 175mg/m², I 1000mg/m², C 1200mg/m², V 900mg/m²]; n = 7, CET [C 1500mg/m², thiopeta 45mg/m², V 2400mg/m²]; n = 1 or PECC [CI 40mg/m², V 400mg/m², C 500 mg/m², cyclophosphamide 40mg/m²]; n = 1) was performed. ASCT was well tolerated with no TRM. Hematopoietic engraftment after retransfusion of 4.2×10⁷ CD4+ cells/kg was reached on day 10 and 11 for WBC >10000/micL and platelets >20000/micL, respectively. Median EFS and OS from diagnosis are 6.9 and 7.3 yrs and from ASCT both 5.4 yrs, respectively. With a median follow-up of 5.7 yrs, 39 pts are alive, 12 pts have died of their disease, resulting in an OS rate of 76.5% and overall response rate of 75%. These results demonstrate that ASCT is well tolerated and displays an objective antitumor activity within this HR pt cohort. Long-term survival is achieved both in HR and relapsed pts and is favorable for those showing a PR or CR following induction, with prompt tumor marker decline and preserved sensitivities to prior cisplatin based chemotherapy. We confirm that ASCT can improve the outcome in advanced and otherwise poor-risk GCT and may increase response rates. Nevertheless, the value of intensive chemotherapy plus ASCT needs to be further analysed in prospective randomised trials.

Reduction of chemotherapy cycles in “good-risk” metastatic germ cell tumors does not affect long term outcome. A single institution retrospective analysis (10 years)
P. Zelenka, V. Buxhofer, P. Kier, R. Ruckser, K.-H. Habertheuer, W. Hinterberger
Vienna, A

The International Germ Cell Cancer Collaborative Group (IGCCCG, 1997) specified 3 patients (pts) categories with either ‘good’- intermediate-‘and ‘poor-prognosis’, allowing risk adapted therapy of metastatic germ cell tumors (GCT).

59 pts-21 Seminoma, 38 Nonseminomat.GCT have been treated since 1992. Median age was 37 years, 47 pts had WHO-performance status 0 or 1. I. 15 pts stage I B/C (AFCC) received 2 cycles PEB (Cisplatin/Etoposide/Bleomycin) in an adjuvant setting, 24 pts stage II A/B/C: 12/8/4 and 20 pts stage III A/B/C: 5/6/9 were subdivided into 29×good-, 9×intermediate- and 6×poor-risk. Since 1999 risk-adapted treatment has been performed, allowing a reduction of chemotherapy (CT) cycles in the ‘good-risk’ group (14 pts) to median 3×PEB compared to median 4×PEB in the period 1992–98 (15 pts). ‘Intermediate-‘ and ‘poor-risk’ pts received median 4×PEB in both periods. 13 out of 44 pts with metastatic GCT had surgical resection of residual disease after completion of 1st-line chemotherapy, viable tumor was seen in 2 ‘poor-risk’ pts only. 4 pts received 2nd-line CT PEI (Cisplatin/Etoposide/Ifosphamide), 5 pts had radiation and 2 pts underwent High-Dose CT CarboPEC (Carboptin/Etoposide/Cyclophosphom) with Autologous Stem-Cell Transplantation (ASTX).

Hematolog. toxicity (tox) WHO III/IV developed in 27 pts, neurototox. WHO I/II occurred in 23 pts, renal dysfunction WHO III in 14 pts and pulmonary tox. WHO I/II in 3 pts. 58 out of 59 pts are evaluable for response. 51 pts achieved complete remission (CR = 88%), 4 pts partial remission (PR = 7%), the total response rate (CR+PR) is 95% after 1st-line PEB. All ‘good-risk’-pts achieved sustained remission (CR = 100%). 4 pts with PR and 2 relapses have been cured by 2nd-line PEI or ASTX.

Risk-adapted treatment of metastatic GCT reduces toxicity and does not effect long term outcome. HD-CT with ASTX may contribute to cure relapsed or refractory GCT.

Loss of expression of the embryonal transcription factor Oct-4 is associated with impairment of caspase-9 activation and cisplatin resistance in testicular germ cell cancer
Halle, D

The molecular basis for the rare occurrence of cisplatin resistance in testicular germ cell cancer (TGCC) remains to be determined. We recently showed that cisplatin resistance is associated with impairment of caspase-9 activation leading to a higher apoptotic threshold despite a similar expression of Bax and Bcl-2. In addition, we reported that the loss of expression of the embryonal transcription factor Oct-4 combined with cisplatin resistance was associated with cisplatin-refractory disease. In this study, we investigated the relation of expression of the embryonal transcription factor Oct-4 and cisplatin resistance. Oct-4 is typically expressed in embryonal stem cells, germ cells as well as in embryonal carcinoma (EC) cells. Ten TGCC cell lines were analysed, which exhibit either no- or embryonal-like cell morphology or extra-embryonal differentiation when grown as nude mice xenografts. Six cell lines were sensitive to cisplatin whereas four cell lines showed 3 – 4-fold resistance in the SRB cytotoxicity assay. Using western-blotting and immunocytochemistry, a high expression of Oct-4 was observed in all six sensitive cell lines while the four resistant cell lines failed to express Oct-4. Immunohistochemistry for Oct-4 was performed on two xenograft types, which had shown different response to cisplatin treatment. Xenografts from the cell line H12.1 consist of EC cells and teratoma (TE) structures and are cisplatin sensitive. Xenografts from the cell line 1411HP consist of EC cells and yolk sac tumor (YS) cells and are cisplatin resistant. The EC/TE xenograft showed a strong staining for Oct-4 except in the TE structures. Surprisingly, the EC/YS xenografts were completely negative for Oct-4. This demonstrates that cisplatin resistance of EC cells is accompanied by loss of Oct-4 expression. The cisplatin sensitive cell line H12.1 was treated with differentiation medium. The resulting cell line H12.1D showed cisplatin resistance, did not express Oct-4 but still expressed the embryonal surface marker SSEA-4. Furthermore, caspase-9 activation in the cell lines upon cisplatin treatment was investigated using substrate cleavage assays. All resistant, Oct-4 negative cell lines showed an impairment of caspase-9 activation. We hypothesize that loss of Oct-4 expression results in an altered expression of distinct apoptotic proteins leading to inhibition of caspase-9 activation and therefore to a higher apoptotic threshold and cisplatin resistance. In conclusion, absence of Oct-4 expression identifies resistant EC cells, which presumably are committed to embryonal-like somatic or extra-embryonal differentiation.

Influence of cell cycle progression on chemotherapy response in germ cell tumors
F. Mayer, M. Schittenhelm, S. Koch, K. Lauber, E. Malenke, S. Wesselborg, C. Bokemeyer
Tübingen, D

Background: It is well known, that the sensitivity of cells towards radiation varies with different phases of the cell cycle. The impact of cell cycle progression on the effect of cytotoxic drugs has not been investigated to the same extent, even though dividing cells are clearly more sensitive to various agents than resting ones. With the development of tools able to cell-ligate the cell cycle progression specifically, an understanding of the relation of cell cycle control and drug-induced cell death offers a prospect to overcome chemotherapy resistance by targeted combinations of such agents with conventional chemotherapeutics. Germ cell tumors of young male are highly sensitive to cisplatin-based chemotherapy. The aim of the
studied to assess the cell cycle dependence of cisplatin induced cell death in GCT cell lines and define the phases of the cell cycle, during which the cells are most sensitive to the effects of cisplatin. Methods: Cell cycle analysis and assessment of the apoptotic index in the embryonal carcinoma derived cell lines NT2, NCCIT, and 2102Ep was performed by flow cytometry according to Nicolletti et al. Execution of apoptosis was followed by Western blot analysis for PARP cleavage and a DEVDase assay for caspase 3 activity. Cells were synchronized in vitro by serum depletion and contact inhibition. Results: Synchronizing cisplatin-exposure, unsynchronized cells accumulated in G2/M after an interval of 28 hours. The arrest was reversible at sublethal doses (0.5–4.5 μM for two hours). At higher cisplatin-concentration, cells accumulated in G2 and died out of the G2/M-arrest. A 2-hour therapy of the cells in G2/M with a concentration of 10 μM of cisplatin resulted in an apoptotic index of 74% measured 70 hours post treatment compared to 33% for the cells treated in G1/S. Analysing synchronised cells treated in G1, PARP cleavage was observed after 42 h following cisplatin-exposure, cells treated in G2 showed PARP cleavage already after 24 h. Conclusion: The results indicate a clear cell cycle dependence of cisplatin-induced cell death in GCTs. The cells have to reach G2/M in order to become apoptotic, the sensitivity towards cisplatin is highest during this phase. Accordingly, cell cycle inhibitors acting in G1/S-phase are predicted to exert a protective effect to cisplatin, whereas agents arresting cells in G2/M could act synergistically.

P966
Symptomatic hyperthyroidism caused by excessive β-HCG in a patient with metastatic chorion cancer

K. Namberger, F. Bassermann, C. Clerm, J. Dyuster, P. Peschel
Munich, D

We report a 22-year-old patient with primary extragonadal chorion cancer, massive pulmonary metastases and abdominal lymph node infiltration. Initially β-HCG in the serum was more than 2 million IU/ml. TSH was suppressed (0.03 mU/l) and fT4 (45 pmol/l) as well as fT3 (13 pmol/l) were markedly elevated. His thyroid gland was normal in size and ultrasound showed no abnormality. However the patient presented with sweat, had slight tremor, insomnia, nervousness and emotional liability. He lost 7 kg weight within 3 weeks. ECG showed sinus tachycardia. Reviewing the literature β-HCG can directly stimulate the thyroid gland causing hyperthyroidism without causing goiter. After one course of chemotherapy including Cisplatin, Etoposid and Ifosfamid (PEI) we saw a remarkable reduction of β-HCG to 7000 mIU/ml, a suppression of all metastases. The patient gained 5 kg weight. We also saw partial regression of the tumor as the underlying disease.

P967
Repeated bone targeted therapy with Rhenium-188 HEDP for hormone-refractory prostate cancer: Randomized phase II trial with the new, high-energy radiopharmaceutical Rhenium-188 HEDP

Bonn, Kassel, D

We investigated the effect of repeated bone targeted therapy with Rhenium-188 hydroxyethylidenediphosphonate (HEDP) in patients with progressive, hormone resistant prostate carcinoma and bone pain. The aim of this study was to determine the pain palliation and the anti-tumor effect of treatments. Methods: 64 patients were randomized into two groups for radionuclide therapy with rhenium-188 HEDP: patients of Group A received a single injection, patients of Group B received two injections (interval 21 days). After therapy, patients were followed up by assessment of pain palliation and clinical outcome until death. Results: In both groups, toxicity was low with moderate thrombo- and leukopenia (max. CTC Grade II). The effectiveness of Re-188 HEDP for pain palliation for repeated treatment (Group B) was better with a response rate and time of response of 92% and 5.66 months, respectively (p = 0.006 and p = 0.001). In this group, 11 (39%) of 28 patients had a PSA-decrease of more than 50% for at least 8 weeks in comparison to 2 (7%) of 30 patients in the single-injection group (Group A). The median times to progression of Group A and Group B were 2.3 (range 0–12.2) and 7.0 (range 0–24.1) months, respectively (p = 0.0013) and the median overall survival of Group A and Group B were 7.0 months (range 1.3–36.7) and 12.7 months (range 4.1–32.2), respectively (p = 0.043). Conclusion: Compared to single injection, repeated bone targeted therapy with rhenium-188 HEDP administered to patients with advanced progressive hormone-refractory prostate carcinoma improved progression-free and overall survival. Larger studies are justified to further evaluate the use of rhenium-188 HEDP.

P968
Phase II study of three weekly docetaxel and estramustine in patients with hormone-refractory prostate cancer

A. Schütte, M. Porsch, E.P. Allhoff
Magdeburg, D

Docetaxel and Estramustine show synergistic activity in treatment of hormone-refractory prostate cancer. Cycle length was 3 weeks with the following dosage regimen: Estramustine 280 mg tid was given on day 1 to 5 and Docetaxel 70 mg/m² was given on day 2. (Cycles were repeated every 21 days). 15 patients obtained 6 cycles, requiring a PSA response to continue with chemotherapy. Further continuation of chemotherapy after 6 cycles was decided individually. Primary all patients received a complete androgen blockage, followed by an antiandrogen withdrawal. 2 patients received Estramustine prior to chemotherapy. Pt. Characteristics: Median age 67 (59 – 82), Median ECOG Performance Status 0 (0 – 2). Median PSA prior to treatment 228 (2.3 – 1581), bone metastases were observed in 12 out of 15 pts. A mean number of 6 cycles were administered (2 – 11), a dosage reduction was necessary in none of our pts. Toxicities: We observed no grade 4 toxicity. One patient got a DVT, we were, however, able to continue chemotherapy under anticoagulation. We observed a grade 3 neutropenia in 6/15 pts. and a grade 3 anemia in 4/15 pts was observed. Non hematological toxicity effects included grade 3 diarrhea in 2 pts. and grade 3 nausea in 3 pts. Response: We achieved a >50% decrease in PSA Level in 9 pts. (60%). In 2 pts. the PSA dropped down to <0.3ng/ml. An objective response was achieved in two pts. The median time to PSA – progression was 36 (12 – 70) weeks. One Pt. of out 9 died within a median follow up of 11 months. 5 pts. with 6 pts. with progression died within a median follow up of 8 months (3 – 17). Conclusion: The combination of three-weekly administered Docetaxel and Estramustine is an effective therapeutic option in the treatment of hormone-refractory prostate cancer.

P969
Thrombin modulates the adhesion of prostate cancer cells to extracellular matrix proteins

J. Llu, P. Schiff-Werner, M. Steiner
Rostock, D

Background: Modulation of the adhesion capacity of tumor cells to extracellular matrix (ECM) is a critical process in tumor progression and metastasis. It has been demonstrated that thrombin enhances the expression of various adhesion molecules in tumor cell lines thus stimulating their adhesion to ECM proteins, endothelial cells or platelets through activation of the functional thrombin receptor (protease-activated receptor 1, PAR-1). Aims: In the present study, we explored the expression profile of thrombin receptors in cultured human prostate-derived cell lines. In addition, the adhesion of these cells to ECM proteins after thrombin treatment was assessed. Methods: The human prostate cancer cell lines DU 145 and LnCAP, and the SV40-immortalized human prostate epithelial cell line PNT1A were examined for thrombin receptor expression by RT-PCR and flow cytometry. Using precoated 96-well microtitre plates, cell adhesion to fibronectin, laminin, or collagen I was assessed in invasion by RT-PCR and flow cytometry. Using precoated 96-well microtitre plates, cell adhesion to fibronectin, laminin, or collagen I was assessed in vitro, cells were able to continue chemotherapy under anticoagulation. We observed 11), a dosage reduction was necessary in none of our pts.
min resulted in enhanced adhesion of the three cell lines to fibronectin or laminin, but not to collagen I. In contrast, thrombin pretreatment of the cells for 24 h exerted different effects on cell adhesion depending on the cell line. The adhesion capacity of DU 145 to all ECM proteins was decreased while no significant inhibitory effect of thrombin pretreatment on cell adhesion was observed for LnCAP or PNT1A cells. Conclusions: Our results suggest that PAR-1 activation induced by thrombin modulates the adhesiveness of prostate cancer cells to ECM proteins which might contribute to the progression and metastasis of prostate cancer in vivo.

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P970
Study of correlation between prostate specific antigen and Gelatinase-A activity in benign and malignant prostate hyperplasia
N. Alizadeh, M. Pezeshki, F. Saadat, F. Safavifar, T. Bühchner, M.R. Khorramzadeh
Tehran, IR; Münster, D

Background: Prostate Specific Antigen (PSA) has been used in monitoring Prostate Cancer, but proved to be not to be specific in cancer staging. There are emerging data on novel tumor marker such as gelatinease-A, which play a key role in tissue invasion and metastasis. Aim: We designed an investigation to assess the usefulness of gelatinease-A activity as compared with PSA in cancer staging. Methods: In this study we have analysed the circulating form of gelatinease-A (MMP-2) in serum samples of patients suffering from either benign prostate hyperplasia (BPH) or prostate cancer (PC). Serum samples were obtained from patient with BPH(54), PC(26) and control healthy individual(26), respectively. Protein-content adjusted samples were separated by gelatin-embedded polyacrylamide gel electrophoresis (PAGE) then were subjected to densitometric analysis. PSA were quantified using a standard ELISA technique. Results: densitometric analyses demonstrate that MMP-2 activity is significantly higher in PC vs. BPH as compared to control normal. In addition, several oligomeric circulating forms of MMP-2 are preferentially found in the PC samples. Pearson Correlation Coefficient (r) between PSA and MMP-2 is =0.934(=0.01). It means that correlation is significant at the 0.01 level. Conclusion: These results demonstrate that MMP-2, compared to PSA, might be considered as a better tumor marker in monitoring and screening patients with prostate cancer.

P971
A combination therapy containing interleukin-2, interferon-alpha, 13 cis-retinoic-acid (13CRA) and gemcitabine for the secondline treatment of metastatic renal cell carcinoma: Results of a phase II/I study
M. Ringhofer, J. Greiner, M. Schmitt, J. Simon, R. Küfer, H. Döhner, J. Gschwend
Ulm, D

Interleukin - 2 (IL-2) and interferon – alpha (IFN-alpha)are the only approved drugs which demonstrated significant efficacy against metastatic renal cell carcinoma (RCC). These cytokines have been employed alone or in combination with 5-fluorouracil or vinblastine for the first line therapy of metastatic RCC and response rates between 8 and 42% have been reported. Many patients, however, endure progressive disease, because they are primarily refractory or become refractory to the first line therapy. We therefore conducted a phase II/I trial for a 2nd-line therapy with IL-2, IFN-alpha, 13 CRA and Gemcitabine in this group of patients. The study was activated in January 2000 and sixteen patients have been enrolled so far. The administered dosage was 20 mg of 13CRA three times daily p.o., between 9 and 18 MIE IFN-alpha s.c., between 9 and 18 MIE IL-2 s.c., and 750 mg or 1000 mg Gemcitabine i.v. per dose, depending on the age of the patient and the side effects. All patients were suffering from progressive disease and all patients had measurable but irreversible metastases. 14 pts were pretreated with a cytostatic containing (chemo)-immunotherapy The median age of the cohort was 62.5 years (53 – 73 y). Toxicity was moderate, but considerable: The most common side effects were fever, chills and weight loss. One patient suffered from a transient elevation of transaminases and one patient had a significant impairment of renal function, which could be resolved completely (both grade III toxicity). 12 patients can be assessed for response up to date: We observed 1 PR for 10 months, 6 SD for at least 6 months, and 5 PD. The longest survivor is still alive 32 months after the initiation of the 2nd-line treatment. Taken together these preliminary results show that the treatment of refractory metastatic RCC with the mentioned regimen is feasible. However, due to the considerable toxicity and the present but limited efficacy it can not be generally recommended as a 2nd line therapy after pretreatment with (chemo)-immunotherapy.

P972
Capecitabine-monotherapy and in combination with immunotherapy in the treatment of metastatic renal cell carcinoma
Vienna, A

Purpose: Capecitabine is a novel fluoropyrimidine carbamate, orally administered and selectively activated to fluorouracil by a sequential triple enzyme pathway in liver and tumor cells. This prospective trial aimed to evaluate the therapeutic effects and systemic toxicities of capecitabine monotherapy and capecitabine treatment combined with biological response modifiers in patients with metastatic renal cell carcinoma. Patients and methods: 54 patients suffering from metastatic renal cell carcinoma progressing under first-, second-, or third-line treatment entered the trial. Capecitabine was given orally at a dose of 2500 mg/m2 daily divided into two doses for 14 days, followed by seven days rest in the monotherapy as well as in the combination treatment. This schedule was repeated in three-weeks cycles. The combination therapy consisted of capecitabine and an immunotherapy treatment, which consisted either of interferon-gamma 1b (100 mg/d) administered consecutively five times weekly during weeks 1 and 2 and recombinant interleukin-2 (4.5 MU/d) administered on 4 consecutive days during weeks 3 and 4, every 6 weeks, or alpha-interferon (5 MIE/d) administered three times a week. Results: 52 patients are now evaluable for response and 54 patients for toxicity. We observed a partial response to treatment in 5 patients (9.6%), minor response in 5 patients (9.6%), stable disease in 32 patients (61.6%), and only 10 patients (19.2%) showed continued disease progression despite treatment. Outpatient capecitabine was well tolerated. We did not observe any WHO-grade IV toxicities. Capecitabine monotherapy and capecitabine treatment in combination with biological response modifiers appear to be effective regimens with favourable toxicity profiles in patients with advanced renal cell carcinoma. Capecitabine monotherapy seems to be superior than the combination treatment because of its easier application form.

P973
Pioglitazone and rofecoxib combined with angiotic scheduling of chemotherapy in advanced renal cell carcinoma
A. Reichle, S. Rogenhofer, K. Bross, A. Berand, H. Wagner, S.W. Krause, R. Arendse
Regensburg, D

Angiostatic, stroma and tumor cell-targeted therapies might control malignancies with intrinsic drug resistance. A phase II trial was started to analyze the activity of a continuously applied molecular-targeted therapy (daily 45mg pioglitazone po and 25mg rofecoxib po) combined with sequentially added angiotic scheduled chemotherapy, capecitabine 2x1g/m2 po from day 14 to 28, every 3 weeks, in advanced renal cell carcinoma (RCC). Sixteen patients (pts) with RCC in the first to third progression were evaluable (mean 0.5, range 0 to 2 preceding chemotherapies). Major side effects (WHO grade 3 and 4) were due to capecitabine (therapy was stopped in 2 cases due to hand-foot-syndrome) and rofecoxib treatment (reversible edema, n = 2, and creatinine elevation, n = 1). Objective tumor responses were not observed - but disease stabilization for more than 6 months in 7 patients (43%), 6, 6, 7, 9, 10, 17 months. Following treatment with the biomodulators alone, a more than 50% decrease of tumour-related C-reactive protein levels was observed in two pts with stable disease, and three pts experienced already a relief of tumor-related symptoms.
The new combination therapy is remarkably well tolerated in patients with advanced and pre-treated RCC. The present combined modality treatment represents an active therapeutic option and reveals further evaluation.

Outpatient treatment in ovarian cancer

M. Arndt, H. Köppler, J. Heymanns, A. Pandorf, R. Weide
Koblenz, D

Objective: Evaluation of feasibility, effectiveness and toxicity in patients (pts) with ovarian cancer, who were treated on an outpatient basis.

Methods: Retrospective analysis of 139 consecutive, unselected pts with ovarian cancer treated between 06/95–02/2003 in a community based oncology group practice. Results: The median age of the pts was 61 (range 18–84). Clinical stage distribution was as follows at diagnosis: FIGO I 22 (16%), FIGO II 18 (13%), FIGO III 74 (53%), FIGO IV 25 (17%), 2 (1%) were unknown. Surgical treatment was performed in 138 pts (specialized centres in 49 pts, local or district hospitals in 89 pts). Major debulking surgical procedures were performed in 64 pts (46%). 103 pts received adjuvant chemotherapy. Within the adjuvant chemotherapy period 88 pts (85%) were treated with a platinum based regime (53 pts (60%) received Pacli-taxel/Carboplatin). The median number of chemotherapy cycles was 6 (range 1–9). 102 pts received palliative chemotherapy during the study period. 1539 cycles were administered (835 three-weekly and 704 weekly). The median number of palliative chemotherapy lines was 3 (1–9). The observed toxicity was mild. A grade 3 or 4 neurotoxicity was seen in 4 pts (4%). A reversible grade 3 or 4 haematotoxicity was seen in 27 pts (26%). Therapy associated hospitalisation was seen in 10 pts (10%), 50 pts (58%) died during the observation time (45 at home, 33 in hospital, 2 were unknown). 34 pts (24%) are still in complete remission. Within a median observation time of 31 months (range 1–350), the median survival of all pts is 42 months (range 1–350). The 1.2.3 and 5 year survival is 91, 74%, 59% and 28% respectively. 5 year survival in stage I, II and III is 80%, 40%, 23% and 11% respectively. The median survival since recurrent disease is 27 months. Conclusions: Palliative treatment in advanced ovarian cancer can be performed completely on an outpatient basis. The observed therapy associated toxicity was mild and therapy associated hospitalisation was low. Unnecessary hospitalisation for diagnostic purposes was seen frequently. Looking at the surgical situation it becomes obvious that many pts do not receive standard surgical therapy. The debulking situation is often suboptimal (residual tumor masses more than 1 cm) or remains unclear. Despite suboptimal surgery the median survival in this unselected group of pts is still high compared to clinical studies.

Lymphoepithelial carcinoma of the nasopharynx is curable by multidisciplinary treatment

M. Schroeder, A. Giagounidis, V. Aoust, H. Makoski, C. Aul
Duisburg, D

The incidence of lymphoepithelial carcinoma of the nasopharynx (NPC) is very low in Western Europe. This tumor entity shows a special sensitivity to chemotherapy and radiotherapy but is also characterized by frequent development of distant metastases. In this report we analyzed the clinical course of 21 cases of lymphoepithelial NPC, treated in our department between 1978 and 2002. Patient characteristics: 4 women aged 30–75 years; 17 men aged 23–75 years; median age 52 years Initial stage (AJCC): 2x Stage II / 6x Stage III / 13x Stage IV incl. 4x M1 (oss/palp). Patients diagnosed before 1985 received bleomycin, cytostine arabinoside, methotrexat, 5-FU (BCMF-schedule). Thereafter, patients received combination chemotherapy with DDP 100 mg/m² d1 and 5-FU (continuous 24h infusion) d1–5. CTX was repeated at 4 weeks interval up to 4 courses. Radiotherapy was simultaneously applied at doses of 2 gy/d up to a total dose of 60–70 gy. Results: 17 pts are in ongoing complete remission with survival times between 16–208 mos (median survival 129 mos) including 1 pt developed a local relaps without vertebral metastases who required orthopedic surgery. 1 pt (stage II) relapsed loco regionally (LNN) 24 mos. after diagnosis, but is still in CR after surgery. 2 pts with initial stage IV (1977-1978) died within 6 mos. due to tumor progression. These patients were not treated with DDP-based CTX. 1 pt who refused multidisciplinary treatment according to our protocol died after 36 mos. Summary: Multidisciplinary treatment offers patients with lymphoepithelial NPC a high chance of achieving complete remission and definitive cure.

Metronomic therapy in recurrent and metastatic chemo-resistant SCCHN: Data from a pilot study

S. Gluck, H. Lau, J. MacKinnon, R. Syrne, J. Dort, D. Gluck
Calgary, CAN

The concept of metronomic therapy (MT) - continuous exposure to low dose chemotherapy in combination with anti angiogenic compounds has been shown to induce tumor growth inhibition in animal studies and in some even tumor regression (Kerbel et al., 2000). Squamous cell carcinomas of the head and neck anatomic areas (SCCHN) were shown in a majority of cases to over express the cyclooxygenase 2 enzyme (COX2) and COX inhibitors were shown in vitro to inhibit cell growth of SCCHN and to induce apoptosis (Lee et al., 2002). In a pilot study, we tested patients with SCCHN who have previously been treated with platinum and taxane based chemotherapy and were now being chemo-resistant.

Thirteen patients were enrolled, median age 55 years (range: 30 – 72), 4 female and 9 male, all SCC histology with 4 naso-, 6 oro- and 3 hypo-phyaryngeal localizations of the primary tumor. Treatment consisted of 2.5 mg methotrexate po daily with 400 mg celecoxib po twice a day. Median time of treatment failure was 161 days (range 10 – 385 days). Since no withdrawals from therapy were due to toxicity, this also corresponded with time to progression. Six patients died of progressive disease with median time of 91 days after enrolment to the study (range 45 – 301 days). The concept of MT does not include tumor regression as an endpoint, therefore we did not record response rates.

The time dependent endpoints were remarkably long given the advanced stage of our patients’ population. Our data are therefore encouraging and a prospective study will be initiated soon.

P975

Poster session: CNS / head and neck tumors

P976

The epidermal growth factor inhibitor PKI-166 blocks MMP-dependent breakdown of desmosomal cadherins while promoting desmosomal assembly and strengthening intercellular adhesion

J. Lorch, K. Park, H. Schmoll, S. Stack, K. Green
Halle, D; Chicago, USA

Lack of cell adhesion is a hallmark of cancer and loss of expression of cell adhesion molecules is a marker for invasion and poor prognosis. A key modulator of cell adhesion is EGF receptor (EGFR) activity. In this study we used PKI166, an EGF-specific tyrosine kinase inhibitor (Novartis AG, Basel, Switzerland) to study the effects of EGFR inhibition on cell adhesion in oral squamous cell cancer cells. Treatment of with PKI166 resulted in the formation of tightly packed cell colonies even under low calcium conditions in which cadherin mediated cell-cell adhesion is usually minimal. Immunofluorescence revealed the recruitment of desmosomal markers to the cell membrane and the solubility of the desmosomal components desmoglein2 (Dsg2) and desmoplakin (DP) was decreased indicating the formation of desmosomes. This was accompanied by a measurable increase in mechanical cell-cell adhesion. The expression of one desmosomal cadherin, Dsg2, was up regulated more than twofold. The increase in Dsg2 expression was at least in part due to the inhibition of matrix metalloproteinase 9 dependent breakdown of Dsg2 with decreased formation of a 100KD membrane bound fragment and the release of a 60KD fragment into the supernatant.

Our results suggest that EGF-R inhibition is able to restore cell-cell adhesion in cancer cells and that the formation of desmosomes might play a particularly important role in this process.
**P979**

**Incidence and severity of anaemia in patients with primary lymphoma of the central nervous system treated with high-dose methotrexate**

K. Jahnke, A. Korfel, L. Fischer, E. Thiel

Berlin, D

**Background:** Only little data is available on anaemia in patients (pts) with non-Hodgkin's lymphoma (NHL). In pts with extracerebral NHL treated with polychemotherapy, anaemia grade 1 (haemoglobin [Hb] 9.5–10.9 g/dL) or 2 (Hb 8.0–9.4 g/dL) according to the World Health Organization (WHO) classification was observed in about 28% and WHO grade 3 (Hb 6.5–7.9 g/dL) in about 10% of pts. We evaluated the incidence and severity of anaemia in pts with primary central nervous system lymphoma (PCNSL) before and during treatment.

**Material and methods:** 139 pts (74 male, 65 female, median age 62 years [range 22–83 years]) with newly diagnosed PCNSL received a total of 496 cycles high-dose methotrexate (HD-MTX) followed by HD-Bu/T and PBSC. In the case of CR treatment is finished, patients in PR receive HD-MTX (45g). Results: We report the results of 13 patients (15 enrolled) at a median follow up of 15 months. Median age is 58 years (40–65), 6 females and 9 males. HD-MTX toxicity prevented HDC in one patient. MTX yielded excellent leukapheresis results. HD-Bu/T was well tolerated with low toxicity. One patient received HDC despite SD after HD-MTX (protocol violation). Time on treatment is very short (2–3mo). Median survival is 15 months (0–41mo). Case results: 10 CR, 2 PR, 1 NC (autopay; patient died after 2nd course of MTX), 4 patients died (2 CR – 1 pneumonia, 1 encephalopathy; 1 PR – encephalopathy; 1 NC – autopsy: heart failure/pulmonary emphysema). We conclude that in contrast to

**P980**

**Intraventricular treatment with Rituximab as a treatment option in patients with CNS lymphoma and leptomeninginal disease**


Cologne, Bonn, Ulm, Düsseldorf, D

PCNSL are highly aggressive tumors with a very poor prognosis when left untreated. Clinical studies evaluating combined modality treatment or chemotherapy alone were conducted in order to improve survival and reduce neurotoxic sequelae. However, most patients eventually relapse, often presenting with leptomeninginal disease. Therefore there is a strong need to develop new therapeutic options for this disease. We thus evaluated the chimeric anti-CD20 monoclonal antibody rituximab via intraventricular/intrathecal application in relapsed PCNSL in a small phase II study in 14 pts in CR or PR after induction therapy. These patients followed intravenous intrathecal or intraventricular rituximab application. A complete remission of leptomeningal lymphoma manifestation was documented in another patient. Measurement of rituximab concentrations in the cerebrospinal fluid showed several-fold higher values after intraventricular administration compared to those after intravenous rituximab application. These observations suggest that intraventricular rituximab treatment of leptomeningeal lymphoma manifestation is effective. However, there seems to be very little or no effect of rituximab on parenchymal CNS lymphoma.

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Heidelberg, D; Moskau, RUS

Ependymomas arise from the epidermal lining of the cerebral ventricles and from the remnants of the central canal of the spinal cord. To elucidate the molecular events responsible for the etiology or development of ependymomas, we analyzed molecular alterations on the gene expression level in a series of newly diagnosed ependymal neoplasms (n = 39). To this aim, tumor RNA was hybridized to microarrays consisting of 4211 different cDNA fragments of genes with relevance to mitosis, cell cycle control, oncogenesis or apoptosis. In comparison to a reference RNA pool, 68 genes were found abundantly expressed (>2-fold). These included CLU, IGF2, RAFl, MMP12, PSAP and MSX1, which are apparent candidate genes for tumorigenesis or apoptosis. In comparison to a reference RNA pool, 68 genes were found abundantly expressed (>2-fold). These included CLU, IGF2, RAFl, MMP12, PSAP and MSX1, which are apparent candidate genes for tumorigenesis or apoptosis. In comparison to a reference RNA pool, 68 genes were found abundantly expressed (>2-fold). These included CLU, IGF2, RAFl, MMP12, PSAP and MSX1, which are apparent candidate genes for tumorigenesis or apoptosis. In comparison to a reference RNA pool, 68 genes were found abundantly expressed (>2-fold). These included CLU, IGF2, RAFl, MMP12, PSAP and MSX1, which are apparent candidate genes for tumorigenesis or apoptosis.
P982
Expression profiling in benign, atypical and anaplastic meningioma
G. Wrob, R. Büschges, R. Weber, M. Hahn, G. Reifenberger, P. Lichter
Heidelberg, Düsseldorf, Bonn, D

Inactivation of the NF2 gene is an early event in meningioma development. Progression from benign to atypical and anaplastic meningioma has been found associated with distinct genomic alterations, along with a broader range of other possible changes. To further elucidate the molecular events involved in their pathogenesis, we performed expression profiling in a series of 34 meningiomas utilizing DNA microarray techniques. Additionally, genomic alterations were assessed by comparative genomic hybridization (CGH).

For expression profiling, labeled RNA equivalents were hybridized to microarrays containing approximately 3000 gene-specific PCR fragments selected on the basis of the function of genes in tumor biology, cell cycle control or apoptosis induction.

Several members of the insulin growth factor pathway, a signalling cascade that is known to be activated in meningiomas of a higher grade, were found to be upregulated using receiver-operator curves. The genes identified were IGF2, IGFBP3, AKT3 and PAPPA.

In addition, genes involved in the Wnt-signalling pathway were present among the group of genes upregulated in later stages of the malignancy. This includes genes such as CTNNB1, CDK5, CCND1 and ENC1. A subsequent decision tree analysis suggested a link between upregulation of these genes and a loss of heterozygocity on chromosome 14 as found by CGH.

Real time quantitative PCR was performed to verify the results.

P984
Brain metastasis in Her-2/neu overexpressing metastatic breast cancer responding to treatment with Trastuzumab
V. Heinemann, O. Brudler, W. Siekiera, G. Raab, B. Heinrich
Munich, Augsburg, D

Objective: This study investigates the incidence of brain metastasis (BM) in HER-2/neu overexpressing MBC patients with a specific focus on the relation between BM occurrence and systemic response to treatment with trastuzumab.

Patients: Among 51 HER-2/neu overexpressing patients 22 patients (43%) were identified who developed BM. HER-2/neu overexpression was determined by immunohistochemistry (Hercep assay) and a score value of 3+ was documented in all 51 patients. Patients treated with trastuzumab received a loading dose of 4mg/kg and a maintenance dose of 2mg/kg.

Results: In one patient, BM was a singular manifestation. A further patient showed synchronous brain- and visceral metastasis, and a consistent decision tree analysis suggested a link between upregulation of these genes and a loss of heterozygocity on chromosome 14 as found by CGH.

Conclusion: Capacitively coupled low-frequency (13.56 MHz) deep-hyperthermia is feasible for brain tumor treatments. Partial remission and/or significant retardation of tumor growth were shown. The applied hyperthermia-treatment was well tolerated by the patients even in advanced tumor stages.

Poster session: Tumor immunology / immunotherapy

P985
Different anti-A titer-reducing capacity of fresh-frozen plasma containing various concentrations of soluble A substance according to the ABO- and secretor-genotypes and Lewis phenotype
U. Nydegger, F. Julmy, F. Achermann
Bern, CH

Soluble ABO blood group substance in fresh-frozen plasma (FFP) and its cognate alloantibody titer-reducing capacity (TRC) are not considered likely to influence prescribing for plasma exchange (PEX) therapy of ABO incompatible transplant recipients. The aim of the study was to quantify the total and IgG class-specific TRC as a function of the amount of soluble A substance (SAS) in FFP from donors with different ABO- and Secretor-genotypes and Lewis phenotypes.

The ABO- and Secretor-genotypes were assessed using PCR-SSP and Lewis phenotypes using DiaMed ID Micro Typing System. SAS was quantified in 36 single FFPs of different blood types using a validated ELISA of mapped analytical specificity in terms of known A glycotope heterogeneity. To determine the total anti-A TRC, SAS containing FFPs of the groups A1 Le(a+b-) secretor, A1 Le(a−b−), A1 non-secretor, A1B, A2 and A2B were randomly mixed with type O plasma samples. To measure the IgG class-specific anti-A TRC, the same FFPs were mixed in equal parts with the murine BRIC 145 class IgG anti-A reagent of similar specificity as expected for human polyclonal anti-A. Anti-A TRCs were estimated using a microhemagglutination inhibition assay. SAS level depended on the A subtype (A1 vs. A2: p < 0.0001) and on the Secretor status (p = 0.0312). The variation was as great as 116.7 arbitrary units (aU) for 6 A1 Le(a-b-) secretors and 8.1 aU for 6 A2 samples (mean values). Homozygous expression of the A1, A2 and Secretor alleles did not increase SAS levels. Only total anti-A TRC, but not IgG class-specific TRC depended on the detected SAS level (r = 0.566) (see Figure). Despite this significant correlation (p = 0.0003) some single samples with high SAS levels had a low TRC. Conversely some single samples with low
SAS level had a higher TRC, than expected on the basis of their SAS level. In fact the total anti-A TRC depended also on the anti-A titer in the type O plasma samples used for the assay ($r = 0.470$, $p = 0.004$).

Neutralisation of isoagglutinins through PEX therapy using plasma containing high concentrations of soluble ABO blood group substance for exchange may be an additional mechanism to reduce isoagglutinins in ABO incompatible transplant recipients. Despite favourable neutralisation capacity of plasma containing high concentrations of soluble ABO blood group substance, its booster effect on immune isoagglutinin production cannot be excluded.

**P988**

**Constitutive and immuno-proteasomes are expressed in chronic myeloid leukemia cells and can process peptides from the bcr-abl protein**

Berlin, Tübingen, D

Most tumor specific or tumor associated peptide sequences known so far have been identified using T-cell clones enriched from tumor infiltrating lymphocytes. Immunotherapy strategies targeting these peptides however have been rather disappointing. Alternative methods to identify tumor antigens for immunotherapy are based on the identification of proteins that are overexpressed in tumor cells. Although computer-aided databases can predict with some accuracy the peptide sequences that are likely to bind to different MHC alleles, uncertainty remains about the ability of the proteasome to cleave the protein at the relevant sites. Moreover, different forms of the proteasome (the constitutive 20S proteasome and the interferon-inducible 20S immunoproteasome) can be expressed in tumor cells, which will eventually generate different peptide sets. In this work, we have tested whether the bcr-abl fusion peptides that have been shown to bind to HLA-A3 and HLA-B8 in vitro (Bocchia et al., 1995, 1996) can be generated in vivo through cytosolic degradation by the 20S proteasome. We have also tested whether the proteasome can generate the SSKALQRPV fusion peptide that has been claimed to bind to HLA-A2 by Yotnda et al. (1998). We have used a 26 Amino Acid long synthetic peptide that spans the whole fusion region of BCR-ABL. We have obtained preparations of both the constitutive and the IFN-gamma-inducible immuno 20S proteasome (c20S and i20S) from LCL721 and LCL721.174 lymphoblastoid cells. In vitro digests were performed, the degradation products were analyzed by MALDI mass spectrometry and Edman degradation. Based on these data, cleavage cards specific for proteasomal degradation of the synthetic bcr-abl 26mer were worked out. We could confirm that the constitutive proteasome is able to cleave the b3a2 bcr-abl fusion protein to generate all 3 peptides that were originally described by Bocchia et al., while the immunoproteasome can only generate the KSQSKALQQR peptide that binds to HLA-A3. According to our results neither c20S nor i20S proteasomes are able to cleave bcr-abl to generate the SSKALQRPV peptide with claimed HLA-A2 binding properties. Moreover, using antibodies specific for the different subunits of the proteasome we can show that mononuclear cells of CML patients frequently express both the constitutive and the immunoproteasome.

**P987**

**Induction of an allogeneic CD8+ T cell response against several peptides derived from Survivin in patients with hematological disease**

Leipzig, D

Survivin plays a crucial role in tumorigenesis as it severely promotes tumor growth through inhibition of apoptosis and increase of proliferation and angiogenesis. It is expressed in most cancers, leukemias and during fetal development, but not in most normal adult tissues. To reveal the effect of allogeneic stem cell transplantation on the stimulation of cytotoxic CD8+ effector-cells against the Survivin peptide, we investigated 61 patients with different haematological tumors before and at certain intervals after BMT. **Method:** To determine the extent of activation of CD8+ cells, we stimulated unselected PBMCs with 40 ng/ml Survivin 95–104 (ELT LG E FLK L), Survivin 96–104 (LT L GE FKL L), Survivin 5–14 (TLP PAW QPF L) or with an HIV476–484 peptide (ILK EPV HGV) serving as negative, and a CMV 65–73 peptide (NEL PMV ATV) as positive control. The IFN-gamma producing T-cells were counted in an Elispot assay. In patients with high numbers of positive cells, the responding cells were selected by an IFN-capture assay and phenotyped. **Results:** Neither the 16 healthy donors nor the 8 control patients with a solid tumor showed a significant CTL-frequency against the peptides derived from Survivin. Only 3/11 CML-patients displayed a frequency of up to 153:100.000 after BMT, whereas 10/16 AML-, 2/5 ALL- and 4/9 NHL-patients showed a frequency of 29–899:100.000 up to two years after BMT compared to 4/35 before SCT. Their further characterization by flow cytometry revealed the predominance of CD8+ T cells. **Conclusion:** These results provide evidence that Survivin-reactive CTLs are present in high frequency in patients after SCT and thus could represent GvL effector cells probably suitable for anti-cancer immunotherapy. However, whether the occurrence of these cells is associated with GVHD and/or reduced relapse rates has to be proven.

**P988**

**Identification of T cell epitopes with RNA fragments**

C.M. Britten, R.G. Meyer, C. Graf, C. Huber, T. Wölfel
Mainz, D

Although the number of defined T cell epitopes of clinically relevant antigens is constantly increasing, there is still an enormous need to identify further peptides, especially for new antigens or rare HLA-molecules. Here we introduce a novel two-step approach for the rapid identification of T cell epitopes. It was established in the CMV infection model. – From the peripheral blood of 10 healthy CMV-seropositive donors sharing HLA-A1 according to HLA serotyping we isolated CD8+ T lymphocytes and generated dendritic cells (DCs). DCs were electroporated with CMV pp65-RNA and tested for recognition by autologous CD8+ T lymphocytes. Only 3/11 CML-patients displayed a frequency of up to 364–373 and 363–373 were both recognized. The latter peptide has recently been published (Hebart et al. 2002). The HLA-A1-restricted anti-pp65 T cell response was completely covered by this epitope in all 7 donors tested. – We conclude that (i) the use of HLA-transfected K562 cells allows to identify immunogenic regions of a given antigen. Only 3/11 CML-patients displayed a frequency of up to 29–899:100.000 up to two years after BMT compared to 4/35 before SCT. Their further characterization by flow cytometry revealed the predominance of CD8+ T cells. **Conclusion:** These results provide evidence that Survivin-reactive CTLs are present in high frequency in patients after SCT and thus could represent GvL effector cells probably suitable for anti-cancer immunotherapy. However, whether the occurrence of these cells is associated with GVHD and/or reduced relapse rates has to be proven.

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to be tested and thus saves both costs and time. For these reasons we expect this two-step method to facilitate the identification of new T cell epitopes.

P989
Granzyne B ELISPOT assay for measurement of NK cell reactivity
J. Hasenkamp, S. Dingeldein, S. Damme, L. Trümper, B. Glaß
Göttingen, D

Natural killer cells (NK) are important effector cells of GVLT effect after HLA-mismatched stem cell transplantation. However, leukemia target cells show various susceptibility to NK mediated lysis. In order to assess different mechanisms of tumor resistance, we adopted an granzyne B (GrB) enzyme-linked immunosensor (ELISPOT) assay for detection of NK activation and compared its results with two cytotoxicity assays: classical chromium (Cr) release and a flow cytometry (FACS) based assay. In three independently performed experiments lysis of K562 (cell line of chronic myeloid leukemia origin) and ML2 (cell line derived of acute myeloid leukemia) due to NK-92 (cytotoxic NK cell line) mediated cytotoxicity at effector to target ratios (E:T) 5:1, 1:1, 1:5, 1:10 in the FACS based cytotoxicity assay were as follows: 34, 28, 10, 7% (r = 0.96) and 8, 5, 2, 2% (r = 0.97). The corresponding lysis in the CBA-release assay was 37, 14, 5, 3% (r = 0.95) and 2, 1, 0, 0% (r = 0.28). At least in our hands, FACS based cytotoxicity assay is superior to CBA-release assay regarding to correlation with E:T ratio, intra- and inter-test variability. With lymphokine activated NK cells as effectors the GrB ELISPOT reveals in three independently performed experiments 886, 571 and 288 spots per well (R2 = 0.95) with K562 target cells. E:T were 125:1; 11:6 and 1:3:2, respectively. ML2, which is resistant to NK cell mediated cytolyis exhibit similar GrB release to K562. In contrast SupB15 (lymphoblastic leukemia cell line), another NK resistant cell line, induced no significant GrB release (186, 60, 19 spots/ well; R2 = 0.98) above background (89, 68, 27 spots/well; R2 = 0.95). Our results indicate that the GrB ELISPOT is a valid tool for exact measurement of NK activation with low intra- and inter-test variability. K562 represents a sensitive target to NK mediated cytotoxicity in both: cytotoxicity assay and GrB ELISPOT. ML2 and SupB15 are resistant to NK mediated cytotoxicity. However, ML2 induce GrB release from NK cells revealed by GrB ELISPOT whilst SupB15 cells fail to trigger GrB release. The GrB ELISPOT assay allows the discrimination between failure of NK cell activation and primary resistance to cytotoxic granules as mechanism of target cell resistance. The GrB ELISPOT assay could be an important instrument for detection of successful NK cell activation and investigations on mechanisms of resistance against cell mediated cytotoxicity which frequently obstacles cellular immunotherapy.

In an ELISPOT assay, only 1/11 HLA-A2+ patients had a T cell response against the proteins3 peptide, no other peptide-specific T cell response could be detected with this assay using responder cells without prestimulation. In the CBA assay TNF-α release was induced upon pulsing with bcr/abl fusion peptides in 5/22 patients, with proteins3 peptide in 4/11 patients, with c-abl peptides in 7/26 patients, with SOCS2 peptides in 5/20 patients and with LM04 peptide in 2/5 patients. A significant IFN-g response was detected only in a few cases.

Tetramers specific for T cells recognizing the bcr3/abl2 fusion protein were positive in 2/13 patients. We conclude that 1) leukemia specific T cells can rarely be detected by ELISPOT or tetramer staining without any prestimulation protocol, 2) CBA seems to be more sensitive in detecting peptide-specific responses, although the predominant TNF-α release raises questions about the functional status of the responding cells. Identification of potential T cell targets is crucial for the development of vaccination strategies in CML, i.e. as postremission therapy after STI-571.

P991
Inhibition of tumor cell growth by antibodies induced after vaccination with peptides derived from the extracellular domain of Her-2/neu
Vienna, A

The anti Her-2/neu monoclonal antibody Trastuzumab has strong inhibiting effects on tumor growth in vitro and in vivo and is therefore successfully used for passive immunization in breast cancer patients. However, due to necessity of frequent applications, cost intensiveness of Trastuzumab treatment and its limited duration of effectiveness, an active immunization inducing a perhaps preventive and long-term immunity to Her-2/neu is still a desirable goal. In this study we aimed to induce anti Her-2/neu antibodies by peptide vaccination, and to test their efficacy in inhibiting tumor cell growth in vitro. By computer aided analyses, seven putative B-cell epitopes of Her-2/neu were defined and synthesized. These peptide epitopes were coupled to tetanus toxoid and used for immunization in BALB/c mice. Among these peptides, immunizations with two single peptides or a combination of two peptides induced high titres of anti-peptide antibodies, primarily of the IgG1 isotype. These antibodies were also directed against the native Her-2/neu antigen, as shown in precipitation assays and ELISA with cell lysates of the Her-2/neu protein overexpressing breast cancer cell line SK-BR-3. Isolated IgG fractions from immune sera incubated with SK-BR-3 cells led to a moderate inhibition of the tumor cell growth in vitro, as well as to complement dependent cell lyses comparable to that achieved by incubation with Trastuzumab. Moreover, peptide immunization in rabbits generated anti-Her-2-neu IgG that in contrast to mouse sera – were able to mediate a 31–46% lysis of SK-BR-3 cells in ADCC experiments. We conclude from our data that immunization with Her-2/neu peptides successfully induced humoral immune response with anti-tumor activity.

P990
T cell reactivity against leukemia-associated antigens in patients with chronic myeloid leukemia
J. Westermann, A. von Lessen, G. Baskaynak, P. Le Coutre, M. Schwarz, B. Dörken, A. Pezzutto
Berlin, D

In chronic myeloid leukemia (CML), T cells recognizing leukemia-associated antigens have been demonstrated by several groups. According to these data, both truly leukemia-specific antigens like bcr/abl and other antigens such as proteinase3 seem to be possible targets for immunotherapy. Clinical relevance of these findings is suggested both by epidemiological studies (Posthuma et al.) and by clinical data (Moldrem et al.) demonstrating a correlation between ongoing cytogenetic response and T cell immunity against proteinase3 peptides in CML patients. We asked whether T cells recognizing leukemia-associated antigens can be detected in CML patients. Most of these patients (n = 26) were under ab treatment and its limited duration of effectivity, an active immunization inducing a perhaps preventive and long-term immunity to Her-2/neu is still a desirable goal. In this study we aimed to induce anti Her-2/neu antibodies by peptide vaccination, and to test their efficacy in inhibiting tumor cell growth in vitro. By computer aided analyses, seven putative B-cell epitopes of Her-2/neu were defined and synthesized. These peptide epitopes were coupled to tetanus toxoid and used for immunization in BALB/c mice. Among these peptides, immunizations with two single peptides or a combination of two peptides induced high titres of anti-peptide antibodies, primarily of the IgG1 isotype. These antibodies were also directed against the native Her-2/neu antigen, as shown in precipitation assays and ELISA with cell lysates of the Her-2/neu protein overexpressing breast cancer cell line SK-BR-3. Isolated IgG fractions from immune sera incubated with SK-BR-3 cells led to a moderate inhibition of the tumor cell growth in vitro, as well as to complement dependent cell lyses comparable to that achieved by incubation with Trastuzumab. Moreover, peptide immunization in rabbits generated anti-Her-2-neu IgG that in contrast to mouse sera – were able to mediate a 31–46% lysis of SK-BR-3 cells in ADCC experiments. We conclude from our data that immunization with Her-2/neu peptides successfully induced humoral immune response with anti-tumor activity.

P992
Detection of disseminated epithelial cancer cells by liquid culture – factors interfering with standardisation of assays
W. Krüger, A. Lange, A. Badbaran, K. Gutensohn, A. Zander
Greifswald, Hamburg, D

The standard method for detection of disseminated breast cancer cells is the immunocytochemistry. Tumour cell enrichment by cell culture has been used by several investigators, however, assays published are not well standardised. Breast cancer cells from two lines were diluted in haemopoietic cells of varying origins and cultured in different media and different flasks. Factors influencing the successful tumour cell amplification by liquid culture were identified by investigation of 277 cultures. Parallel, clinical samples consisting of bone marrow aspirations, leukapheresis samples and peripheral blood samples obtained from women with breast cancer were investigated in 113 cultures. Cancer cell detection by cell culture could be compared to immunocytochemistry in 101 cases. The frequency of tumour cell detection was not improved by liquid culture, but a significant correlation between conventional tumour cell detection and detection after liquid culture was found. Factors influencing tumour
cell amplification in the dilution assay could not be transferred to the in-
vestigation of clinical samples. It was be concluded that culture-enrich-
ment of disseminated cancer cells was very complex and influenced by a
variety of factors even when a model system was used. It should be recog-
nised that culture enriched cancer cells probably represent a highly se-
lected population of disseminated cancer cells, despite the significant cor-
relation between tumour cells detected by conventional methods and fol-
lowing conventional methods after liquid culture. There is currently no
evidence that cancer cell amplification by cell culture could become a
standardised technique for the detection of disseminated epithelial tu-
mour cells.

P993
Expression of cancer tests antigens in pancreatic adenocarcinoma
B. Kubuschok, X. Xie, R. Jesnoffski, K.D. Preuss, B. Remeke,
F. Neumann, E. Regitz, G. Pistorius, M. Schilling, P. Scheuermann,
J. Izbicki, M. Lühr, M. Pfreundschuh
Homburg, Mannheim, Hamburg, D

Specific immunotherapeutic approaches may become an important op-
tion in the treatment of pancreatic cancer. However so far, only few tumor
antigens are known in pancreatic cancer which can serve as targets in im-
munotherapy. Therefore, we investigated the expression of 10 cancer tests
antigens (SCP-1, NY-ESO-1, SSX-1, SSX-2, SSX-4, GAGE, MAGE-3, MAGE-4, CT-7, CT-8) in 61 surgically resected human pancrea-
tic adenocarcinoma samples by RT-PCR: SCP-1 was expressed in 48% of
cases (29/61), GAGE was expressed in 21% of cases (13/61). In contrast,
normal pancreas (n = 4) or tissues of different origin (skin, lymphnodes,
muscle, brain, lung and abdominal organs) did not demonstrate SCP-1 or
GAGE expression (0/18) with the exception of tests (p<0.0005 or 0.032
by chi-quadrat-test). Specimens derived from patients with chronic pan-
creatitis revealed expression of SCP-1 in 2/8 cases and expression of
GAGE in 1/8 cases. Human pancreatic carcinoma cell lines demonstrated
SCP-1 expression in 2/10 and GAGE expression in 3/10 cases. Protein
expression of SCP-1 was confirmed by immunohistochemical staining of
pancreatic carcinoma specimens. Pancreatic adenocarcinomas rarely
expressed MAGE-4 (1/52), SSX-4 (1/39) and CT-8 (2/41). No expression was
found for MAGE-3 (0/61), SSX-1 (0/53), SSX-2 (0/53), NY-ESO (0/61)
and CT-7 (0/39). In conclusion, SCP-1 and GAGE are novel tumor anti-
gens in pancreatic adenocarcinoma and represent candidates for tumor-
specific immunotherapy.

P994
Teleomerase pulsed dendritic cells for immunotherapy of renal cell carcinoma
E. Sievers, I.G.H. Schmidt-Wolf, A. Märten
Bonn, Heidelberg, D

Renal cell carcinoma (RCC) is known for its immunological susceptibility.
Unfortunately, RCC lacks of specific tumor-antigens for induction of spe-
cific immunotherapy. Here, we investigated the role of teleomerase as
tumor-antigen and pulsed dendritic cells (DC) as antigen presenting cells
with an immunogenic peptide from telomerase. Material and methods:
Dendritic cells and immunological effector cells (cytokine-induced killer
cells; CIK cells) from patients with renal cell carcinoma or healthy donos
were generated and CIK cells were tested for cytotoxic activity against
primary cultures after coculture with peptide-pulsed DC. Using the dimer technique we determined the percentage of telomerase-
specific T cells, the status of activation was identified using IFN-gamma
secretion assay. Results: After pulsing DC with telomerase peptide, cocul-
tured CIK cells had a significant increase (p<0.05) in cytotoxic activity
against tumor cells as compared to CIK cells without coculture (100% at
an effector to target ratio of 60:1 vs. 41.7%). Using a complete auto-
logous model with immunological cells derived from patients with metasta-
tic renal cell carcinoma, we were able to induce cytotoxicity against autol-
ogous, telomerase-positive primary cell cultures. We could detect 2-4%
telemerase-specific effector cells after coculture with peptide-pulsed DC,
which secrete IFN-gamma after restimulation. Conclusion: Teleomerase
could serve as a specific tumor-associated antigen for renal cell carcinoma.
Pulsing DC with telomerase peptide allows to induce antigen-specific cy-
totoxic effector cells.

P995
Analysis, expansion and genetic modification of cytotoxic natural killer cells from AML patients
U. Siegler, C.P. Kalbarer, P. Nowbakht, A. Wodnar-Filipowicz
Basel, CH

Natural killer (NK) cells are involved in immune surveillance and their
ability to recognize and kill tumor cells is exploited in immunotherapy of
malignant diseases. Human NK cells include highly cytotoxic CD56dim
CD16bright and CD56brightCD16-/dim cells producing abundant cy-
tokines. We have characterized phenotype and function of peripheral
blood NK cells from patients with newly diagnosed or relapsed acute
myeloid leukemia (AML; n = 14). The content of CD56+CD3- NK cells in
AML was reduced 2–3 fold as compared to healthy controls, while the
ratio between the two NK cell subsets was unchanged. Expression level of
NKp46, the major NK cell-specific natural cytotoxicity receptor, was simi-
lar to normals (10.7±1.3 vs 9.4±0.6; MFI ratio±SEM). CD94 and inhibito-
ry receptors CD158a/b and NK81 were also expressed at normal levels.
Killing of autologous blasts in the presence of HLA-class I-blocking
mAbs and the lysis of HLA-class I-deficient K562 targets demonstrated the
functional integrity of AML-NK cells. We generated NK cell lines by
repeated stimulation of CD56+CD3- cells with IL-2, PHA and irradiated
mononuclear cells. These culture conditions resulted in 100±320 fold ex-
pansion of AML-NK cells over 2 weeks. Cytotoxicity of NK cell lines
against K562 and autologous or allogeneic leukemic blasts was compar-
able to the cytolytic activity of freshly isolated AML-NK cells. Upon cul-
turing, the phenotype of stimulated AML- and control-NK lines was al-
tered: all cells acquired CD16bright phenotype, while the NKp46 expres-
sion was downmodulated during first 2 weeks of expansion and either re-
tained to initial levels, after 3–4 weeks or remained negative. In cells
which lost NKp46, the cell surface receptor expression was restored by
leptin gene transfer. In the genetically corrected NK lines, the NKp46
mediated cytotoxicity was demonstrated by redirected killing of the
murine target P815.

We conclude that NK cells from AML patients do not significantly differ
from healthy controls, with respect to phenotypic and cytotoxic proper-
ties. We are currently exploring various culture conditions and the len-
tiviral gene transfer approach to generate large amounts of highly cytotoxic
AML-NK cells. The potential of these cells in killing of autologous
leukemic blasts is investigated in vitro as well as in vivo, in NOD/SCID
mice inoculated with human leukemia. These studies will contribute to
the development of adoptive immunotherapy with NK cells against
leukemia relapse.

P996
Lysis of primary B-CLL cells by hTERT specific cytotoxic T cells
D. Bund, C. Mayr, C. Falk, D.J. Schendel, M. Hallek, C.-M. Wendtner
Munich, D

The polypeptide component of human telomerase (hTERT) is an attrac-
tive candidate for a widely expressed tumor-associated antigen because
it is known to be silent in normal tissues but reactivated in more than
85% of human cancer cells. Antigen specific CTLs against two hTERT
peptides, I540 and R865, were shown to specifically lyse various tumors
in vitro. In our study, we explored whether these two peptides and an ad-
tional (ES55) which are presented by HLA-A*0201 could serve as
tumor associated antigens for primary B-cell chronic lymphocytic
leukemia cells (B-CLL). First the presence of hTERT in HLA-A*0201
positive B-CLL patients was identified by RT-PCR. Half of the tested
samples were hTERT positive. In order to generate antigen specific
CTLs, CD8+ T cells were seperated from blood of different HLA-
A*0201 positive healthy donors and weekly pulsed with peptide-
loaded (I540, ES55 and R865) mature dendritic cells (DCs) in
the presence of IL 2, IL 7 and beta2-microglobulin. After several restim-
ulations, cytotoxicity of the CTL was investigated in a 51Cr release assay
against peptide-loaded T2 cells (HLA-A*0201). During restimulation
the fraction of CD8+/CD25- DCs+ CTLs increased constantly.
Subsequently, the lytic capacity of these CTL against B-CLL was tested.
For the first time we could show that HLA-A*0201 and hTERT positive
B-CLL cells were lysed by hTERT specific CTLs. This reveals in-
direct evidence for hTERT processing and presentation in B-CLL cells
by MHC class I (HLA-A*0201).

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Our current research will examine and characterize the T cell response of CD40L activated and transduced B-CLL cells in allogeneic, autologous and haploidentical systems. Supported by the Deutsche Forschungsgesellschaft (SFB455).

P987
PD-L1 on murine and human tumors and its possible role in immune evasion
C. Blank, A. Peterson, A. Mackensen, R. Andreessen, T.F. Gajewski
Regensburg, D; Chicago, USA

Programmed death receptor (PD-1) is a receptor expressed on activated T cells that is thought to negatively regulate T cell activation. Two ligands for PD-1 have been identified, PD-L1 (B7-H1) and PD-L2 (B7-DC). PD-1+ cells develop signs of autoimmunity on specific genetic backgrounds. We hypothesized that expression of PD-L1 or PD-L2 on tumor cells may interfere with optimal anti-tumor T cell responses. Examination of a wide variety of mouse tumors cell lines revealed significant expression of PD-L1 either constitutively or following treatment with IFN-γ, whereas PD-L2 expression was not detected. In order to study in detail the role of PD-1 on CD8+ peripheral function, 2C TCR tg/RAG2-/-/PD-1-/- mice were generated. In fact, primed PD-1-/-/RAG2-/- T cells produced higher levels of cytokines and showed a higher lytic activity compared to WT/2C/RAG2-/- T cells. In addition, blocking of PD-L1 using a monoclonal antibody increased IL-2 and IFN-γ production by primed 2C/RAG2-/- T cells upon stimulation with tumors expressing PD-L1. In vivo, adoptively transferred PD-1-/-/RAG2-/- T cells showed superior tumor rejection compared to WT 2C/RAG2-/- cells.

When examining human tumor cell lines we found similar results. Human melanoma cell lines expressed PD-L1 constitutively at low levels and up-regulated PD-L1 upon IFN-γ stimulation. Tumor specific T cell lines were stimulated to increased levels when PD-1/PD-L1 interactions were blocked using anti-human PD-L1 monoclonal antibodies. These data suggest that PD-L1/PD-1 interactions negatively regulate CD8+ T cell responses during tumor immunotherapies using adoptive T cell transfer, supporting an important role for this pathway in tumor evasion of immune destruction.

P998
Development of anti-EGFR immunoliposomes for specific delivery and enhanced efficacy in EGFR-overexpressing tumors
C. Mamat, D.C. Drummond, M. Hayes, K. Hong, D.B. Korpotin,
K. Bankiewicz, J.W. Park
Basel, CH; San Francisco, USA

We have developed immunoliposomes (ILs) that bind EGFR or mutant EGFR-III and internalize in target tumor cells, enabling intracellular delivery of potent anticancer agents. ILs were optimized for systemic application or in conjunction with convection-enhanced delivery (CED) for integrated targeting of brain tumors. ILs were constructed modularly with various MAB fragments, including cetuximab-Fab' and novel human scFvs from phage antibody libraries, covalently linked to liposomes containing various drugs or probes.

In vitro studies demonstrated specific binding of anti-EGFR ILs to EGFR- or EGFRvIII-overexpressing tumor cells, followed by receptor-mediated internalization. Anti-EGFR ILs were used to deliver various drugs (doxorubicin, vinorelbine, methotrexate) against these cell lines in vitro. In each case, anti-EGFR ILs were markedly more cytotoxic than the corresponding liposomal drug in target cells, while equivalent to liposomal drug in control cell lines lacking EGFR. PK and biodistribution studies confirmed long circulation half-life and high accumulation in tumors. In vivo efficacy studies in EGFR- or EGFRvIII-overexpressing xenograft models demonstrated the superiority of immunoliposomal delivery in target cells. In each study, anti-EGFR ILs containing various drugs (doxorubicin, epirubicin, vinorelbine, vincristine) showed potent antitumor effects, including tumor regressions and cures in many mice, significantly superior to all other treatments, such as free drug, liposomal drug or free MAB + liposomal drug. In addition, we developed highly stable lipid-nucleic acid nanoparticles (genospheres) for gene therapy with design specificities favorable for systemic delivery. Genospheres were rendered target specific by insertion of anti-EGFR MAB enabling specific and efficient gene delivery. For brain tumor treatment, CED was used to administer various liposomes to intracranial U-87 xenografts or regions of the rodent and primate brain bypassing the blood-brain barrier. CED of liposomes resulted in extensive distribution within brain tissue or tumors suggesting that large regions of human brain can be targeted. In conclusion, ILs provide efficient and targeted drug delivery to EGFR or EGFRvIII-overexpressing tumor cells, and can be used as a systemic treatment or in conjunction with CED for combined molecular and regional targeting of brain tumors. In principle, this targeting approach can be used for the delivery of various probes, drugs, and genes.

P999
MDM2 and Survivin as novel tumor associated antigens in chronic lymphocytic leukemia (B-CLL)
C. Mayr, D. Bund, M. Bamberger, C. S. Falk, D. J. Schendel,
M. Halke, C.-M. Wendtner
Munich, D

Survivin, a member of the family of inhibitors of apoptosis, is expressed in the majority of human malignancies and in about 30% of naive CLL cells. After stimulation of CLL cells via CD40 all activated CLL cells overexpress the survivin protein.

Another attractive candidate target as an universal TAA is the human homolog of the murine double-minute 2 oncoprotein, MDM2. It is a ubiquitous self-protein which is involved in the process of malignant transformation and is overexpressed in a diverse set of malignancies, including sarcomas, gliomas, lymphomas and leukemias. By Western blot analysis we were able to show overexpression of the MDM2 protein in 60% of the examined CLL samples.

Cytotoxic T lymphocytes (CTLs) from HLA-A2 positive healthy donors were generated using mature dendritic cells (DCs) as antigen presenting cells (APCs) that were loaded with specific nonamer peptides derived from survivin or MDM2. These CD8+ CTLs efficiently lysed T2 cells pulsed with the corresponding peptide, whereas T2 cells loaded with a control peptide, derived from the influenza matrix protein and the natural killer cell target K562 were not lysed in a 1 chromium release assay. Furthermore, the MDM2 and survivin specific CTLs recognized specifically HLA-A2 positive tumor cell lines, but not MDM2 and survivin overexpressing but HLA-A2 negative tumor cell lines or primary B-CLL cells. Additionally, the specific CTLs were able to kill naive and to a higher extent CD40-Ligand stimulated HLA-A2 positive B-CLL cells overexpressing survivin or MDM2. Autologous B cells in which survivin or MDM2 were not detectable were not lysed.

Our results confirm the importance of survivin as a widely applicable TAA and identify MDM2 as a target antigen for anticancer immunotherapeutic strategies, including B-CLL. Supported by Deutsche Forschungsgemeinschaft, SFB455.
non transfected CIK (36.52 +/- 5.27%) co-cultured with DC, transfected CIK had a lyric activity of 58.51 +/- 3.23% against Dan G cells, a human pancreatic carcinoma cell line. CIK transfected with IL-2 possessed a significantly higher cytotoxic activity as compared to non-transfected cells (P = 0.03).

P1001
Modulating T cell receptor signals by defined T cell receptor alterations: Novel tool for adoptive cancer immunotherapy

C. Schaab, J. Kuball, R. Voss, S. Thomas, M. Juelch, M. Bricic, C. Huber, E. Palmer, M. Theobald
Mainz, D; Basel, CH

T-cell receptor (TCR) gene transfer of murine TCR into human T cells allows circumvention of tolerance to tumor associated antigens. To extend proliferation and viability of TCR-transgenic T cells, we developed TCRs mediating a hyperresponsive phenotype upon stimulation.

Cytotoxic T cells specific for the human tumor antigen MDM2 (81-88) were obtained from CD8xA2Kb transgenic mice and the respective TCRs were cloned. Conserved amino acids in the transmembrane domain of the TCR beta-chain were modified by point mutation. In addition, a chimeric beta-TCR, containing the transmembrane region of the gamma-chain and the constant beta chain was constructed. Wild-type and these modified murine TCRs were transduced into human T cells by retroviral gene transfer. Subpopulations were analyzed for proliferation, survival, cytotoxicity and cytokine secretion.

Class-I-restricted CD8-dependent TCRs were efficiently expressed in human T cells. A modification in the ITAM-like motif resulted in an instable surface expression of the TCR. Proliferation capacity was enhanced in TCR-mutated constructs upon polyclonal stimulation. In addition, CD8+-T cells transduced with modified TCRs were less susceptible to activation-induced cell death (AICD). In contrast, cell death via neglection was not affected. Mediators of AICD as TNF-alpha and Fas-L–secretion were decreased. However both, the cytolytic capacity and functional avidity of TCR-transgenic CD8+-T cells was not affected.

In summary, defined molecular alterations of TCRs are associated with enhanced T-cell proliferation and survival, and are therefore likely to contribute substantially to an optimized TCR gene transfer based immunotherapy of malignant disease.

P1002
CD30 shedding is augmented by the soluble shedding product itself through the action of its cysteine-rich ectodomains -2 and 5

A. Engert, P. Borchmann, E. Pogge von Strandmann, B. von Tresckow, H. Hansen
Cologne, D

From studies with viral CD30 there is rising evidence for a role of soluble CD30 (sCD30) as inhibitor of the Tki-type inflammatory response. Like many soluble receptors, sCD30 is generated by proteolytic cleavage of CD30 through tumor necrosis factor-alpha converting enzyme. Since the orchestration of the individual cleavage events is not understood, we studied the structural requirements for CD30 shedding. The release of sCD30 was hardly influenced by the amino acid sequence at the cleavage site but was inhibited by antibody binding to the duplicated cysteine-rich domains (CRD)-2 and 5, distant from the cleavage site.

Domain duplication itself was not necessary for this effect because the deletion of one of these domains did not abolish antibody-dependent shedding inhibition. Conversely, sCD30 containing CRD2/5 or CRD2/5-derived peptides stimulated CD30 shedding whereas sCD30 without CRD2/5 or a CRD1-derived peptide showed no effect. Shedding stimulation by CRD2/5 was CD30-selective since other tumor necrosis factor-alpha converting enzyme substrates such as TNFRI (CD120a) or TNF alpha were not affected. In conclusion, CD30 shedding was regulated by the availability or concentration of the CRD2/5. These domains, which showed highest homology with viral CD30, served as paracrine stimulus of the CD30 cleavage, thus possibly stabilizing the suppression of the cellular immune response.

P1003
Her-2/neu mimotopes for epitope-specific breast cancer vaccine formulation

A. Riemer, E. Jensen-Jarolim, H. Pehamberger, O. Scheiner, C. Zielinski
Vienna, A

Antibodies directed to the oncogenic protein Her-2/neu (erbB-2) exert diverse biological effects. Depending upon epitope specificity, tumor growth may be inhibited or enhanced. Trastuzumab, for example, is a growth-inhibitory humanized monoclonal anti-Her-2/neu antibody, currently used for passive immunotherapy in the treatment of patients with advanced breast cancer. However, antibody therapies have to be repeatedly administered over long periods of time, and may not regularly be available for every tumor patient. Active immunizations, on the contrary, produce long-term immune responses which potentially can be more effective than passive immunizations.

The aim of the present study was to generate peptide mimics of the Trastuzumab epitope for vaccine formulation, ensuring the subsequent induction of tumor-inhibitory antibodies.

In order to define these antigenic structures we employed the phage display technique, yielding epitope mimics, i.e. mimotopes, complementing a screening antibody of interest. Five candidate mimotopes were selected from a constrained 10mer library, the circular decapaptides being expressed on the pII coat protein of the filamentous phage M13. The deduced amino acid sequences exhibited no homology to the amino acid sequence of Her-2/neu, indicating structural mimicry of a conformational epitope. These five phage-displayed peptides were recognized by Trastuzumab with high specificity. Moreover, they all showed distinctively higher antibody titers with natural Her-2/neu positive SK-BR-3 breast cancer cells in a concentration-dependent fashion in two different experimental set-ups. The two best mimotopes in these tests were chosen to be synthesized, and were coupled directly to two well-known immunogenic carriers, tetanus toxoid and keyhole limpet hemocyanin. The conjugates again were specifically recognized by Trastuzumab, demonstrating that the synthetic mimotopes are still mimics of the antibody’s epitope on Her-2/neu.

We conclude that the described mimotope conjugates are suitable for active immunization; thereby introducing epitope-specific immunotherapy as a novel concept in breast cancer.

P1004
Induction of caspase-dependent programmed cell death in B-CLL cells by anti-CD22 immunotoxins

T. Decker, M. Oelsner, R.J. Kreitman, G. Salvatore, O.-C. Wang, I. Pastan, C. Peschel, T. Lichter
Munich, D; Bethesda, USA; Naples, I

B-cell chronic lymphocytic leukemia (B-CLL) is insensitive to induction of apoptosis. We investigated the activity of BL22, a recombinant immunotoxin composed of the Fv portion of an anti-CD22 antibody fused to a 38KDa fragment of Pseudomonas exotoxin A. B-cells from 22 CLL patients were immunomagnetically enriched to >96% purity, and cultured in a concentration-dependent fashion in two different experimental set-ups. The two best mimotopes in these tests were chosen to be synthesized, and were coupled directly to two well-known immunogenic carriers, tetanus toxoid and keyhole limpet hemocyanin. The conjugates again were specifically recognized by Trastuzumab, demonstrating that the synthetic mimotopes are still mimics of the antibody’s epitope on Her-2/neu.

We conclude that the described mimotope conjugates are suitable for active immunization; thereby introducing epitope-specific immunotherapy as a novel concept in breast cancer.
Dendritic cells (DCs) are the most potent antigen-presenting cells that could prime naïve cytotoxic T-lymphocytes. Several reports describe dendritic cells vaccination in different strategies for patients with acute myelogenous leukemia (AML) to induce specific T cell responses. Highlighting, AML blasts often have chromosomal abnormalities which could encode for leukemia specific antigenic peptide sequences probably presented in the context of self MHC molecules. Because co-culture of AML blasts with T lymphocytes revealed T cell stimulation very rarely we tried by fusing AML blasts with autologous DC by a combination of polyethylene glycol and electroporation to enhance this effect. The fusion cells were positive for MHC class I and II, CD40, B7-1, B7-2, CD209 and several adhesion molecules. Most of the fused cells exhibited typical DC morphology with veiled processes and dendrites. In a mixed lymphocyte-hybrid reaction we could demonstrate that the fusion cells retained the functional potency of DCs and were able to induce proliferation of autologous T cells. Moreover, in the special case of fusion cells established from AML blasts with the chromosomal abnormality inversion 16 autologous T lymphocytes could be primed by the fusion cells to induce cytotoxicity against autologous AML blasts up to 35% in a effector/ target ratio of 10:1. Sub-differentiation and blocking assays demonstrate that the lysis is mainly mediated by CD8+, CCR7- T lymphocytes which could be further expanded by autologous fusion cells in form of effector memory CD8+ T cells.

Activation of bystander monocytes by tumor cells undergoing cell death

I. Tinhofer, M. Sentfer, G. Anether, B. Lassnig, R. Greil
Innsbruck, A

In previous work, we detected massive intracellular production of heat shock protein 70 (HSP70) when T-acute lymphoblastic leukemia (T-ALL) or B-chronic lymphocytic leukemia (B-CLL) cells were treated with the novel anti-tumor agent tetrocarcin-A (TC-A). The upregulation of HSP70 was specific for TC-A treatment and not observed when tumor cells were treated with other cytotoxic drugs such as the microtubuli-targeting agent vincristine (VCR), fludarabine phosphate, cyclophosphamide, cisplatin or dexamethasone. Since both innate and adaptive immune effects have been described in response to extracellular HSP70, we tested in this study whether HSP70 produced from tumor cells undergoing TC-A-induced cell death exerts immunostimulatory activity. Such an local inflammatory response might support the induction of an anti-tumor immune response in patients receiving cytotoxic therapy. Supernatants from dying tumor cells were generated by stimulating the T-ALL cell line CEM CH2 with TC-A, vincristine or fludarabine phosphate for 24 hs. PBMC were isolated from peripheral blood from healthy volunteers using density gradient centrifugation. Monocytes were then enriched by plastic adherence. HSP70 in supernatants of tumor cells was quantified by ELISA (Stressgen). Production of inflammatory cytokines (IL-1beta, IL-6, IL-8, TNF-α or IFN-gamma) by monocytes was detected by a modified Boyden chamber transmigration assay. Cytolytic activity of monocytes toward target cells was determined using a modified Annexin/PI assay. HSP70 accumulation could only be observed in supernatants from TC-A-treated tumor cells. However, despite the lack of detectable HSP70 in supernatants from VCR-treated cells, they were superior in stimulating monocytes to produce pro-inflammatory cytokines, to attract T-cells and to kill target cells. In conclusion, immunostimulatory components different from HSP70 were found in the supernatants from tumor cells dying by VCR. Their identification will be the aim of further studies.

Nonviral transfection of human dendritic cells by mRNA-electroporation results in high transgene expression

K. Brill, U. Weingartz, I. Bolz, A. Heiser, M. Kneba, B. Gahn
Kiel, D

Genetically modified dendritic cells (DC) are increasingly used in vitro to activate both tumor antigen specific CD4+ helper and CD8+ cytotoxic T lymphocyte responses. Various strategies have been attempted to design efficient tumor antigen loading into DCs. The well established genetic modification of DCs with recombinant adenoviruses is hampered by the interfering presentation of adenoviral antigens and is associated with safety issues. In this study, we evaluated the delivery of in vitro transcribed RNA into DCs, using electroporation as a nonviral transfection method. Immature, i.e. non-proliferating human DCs were prepared from peripheral blood monocytes in serum-free growth medium, GM-CSF and IL-4. Subsequently the DCs were electroporated in a transfection buffer with the green fluorescent protein (GFP) mRNA and matured in a cytokine cocktail consisting of IL-1beta, IL-6, TNFα, and PGE2. The percentage of transfected cells was determined by selecting GFP expressing cells using flow cytometry. Electroporation parameters were established in K562 cells. Electroporation of K562 cells with mRNA for GFP resulted in 96% GFP positive cells. Electroporation of DCs efficiently delivered GFP mRNA into more than 70% of the cultured immature DCs with a mean fluorescence intensity of 100–200. Importantly, the RNA transfected DCs retained their typical morphological and immunophenotypical characteristics, expressing high levels of HLA-DR and no lineage markers. These data show that very high transgene expression can be achieved in DCs using mRNA electroporation. This RNA transfection technique consequently represents a very efficient and promising nonviral tool for gene delivery into dendritic cells which can be used for preclinical and clinical studies testing immunotherapeutic approaches.

Development of a new anti-CD30 immunotoxin based on a recombinant mistletoe lectin, which is deleted in the vascular leak syndrome motives

Cologne, Zwingenberg, D

Introduction: Different immunotoxins (ITs) against the Hodgkin-Reed /Sternberg (H-RS) cell marker CD30 have been clinically tested. In these trials, the vascular leak syndrome (VLS) was one of the dose limiting toxicities. To overcome this side effect, we used a new recombinant type II ribosome inactivating protein containing the A-chain of the highly potent plant toxin viscinum (mistletoe lectin), in which the VLS motifs have been genetically modified by the insertion of point mutations in order to reduce unspecific binding to endothelium. Material and methods: The recombinant IT was designed cloning the scFv of the murine K562 CD30 to the C-terminus of the IT and the modified A-chain of the mistletoe lectin to the N-terminus into a pet27 based bacterial expression plasmid. The original mistletoe lectin linker was cloned in between these two effector domains. The recombinant protein was expressed in E.coli BL21, and the periplasmic fraction used for the further purification process including immobilized metal ion affinity chromatography followed by molecular size chromatography. Proteins were verified by Western blotting and staining with Commaissie Fast Stain. Binding activity of the IT was investigated using a fluorescence activated cell sorter (FACS flow analysis) and the CD30 positive cell lines L540 and Su-DHL-1. Toxicity to these CD30 positive cell lines was determined with the XTT assay using CD30 negative cell lines as negative controls. Results and Conclusions: The recombinant protein MLA(VLS)-scFv was successfully expressed and purified and showed specific binding to CD30 positive cells. The purified IT showed specific toxicity on the Hodgkin lymphoma cell line L540 and the non-Hodgkin lymphoma cell line Su-DHL-1. In conclusion, we have demonstrated the successful construction of a new anti-CD30 IT containing the A chain of mistletoe lectin, in which the VLS motifs have been deleted. This new IT might help to overcome clinical problems associated with the currently available toxins. This work was supported by grant of the BMBF(#312599)
Heterogeneous expression of H-2D in Colon-26 cells provides a new model to study the relevance of MHC class I expression in tumor cell immunogenenicity and tumorigenicity

Munich, D

The loss or decrease of major histocompatibility (MHC) class I expression on tumour cells has been well documented. In the clinical situation, a correlation between decreased MHC class I expression with poor prognosis and/or metastatic potential has been shown in a variety of tumours. Until now there have been no robust experimental models in which to investigate the role of MHC class I expression in tumour cell immunogenenicity and immunogenenicity. Experiments have previously relied for example on the retroviral transduction of MHC class I negative cell lines to provide MHC class I positive cell lines, or conversely the adenoviral infection of MHC class I positive cell lines to decrease MHC class I expression. Here we present a recently discovered model to study the relevance of MHC class I expression levels for tumour development in vivo. The murine Colon-26 (C26) adenocarcinoma cell line has a heterogeneous H-2D (and H-2K) expression in vitro, with H-2D surface expression on between 15% and 65% of cells. We have isolated C26-H-2D negative (H-2Dneg) cell clones by limiting dilution, and a C26-H-2D positive (H-2Dpos) cell line by positive selection using magnetic beads. The H-2D phenotype of these cell lines is stable in culture. Treatment of C26-H-2Dneg cell lines with interferon gamma (IFNg) did not lead to cell surface expression of H-2D in all cell clones, showing that some clones are refactory to IFNg stimulation and that different mechanisms may be involved in downregulation of MHC class I expression in this cell line. We established an in vivo subcutaneous tumour model using C26 cells in syngeneic Balb/c mice. At a dose of 2x10^5 cells, the C26 cell line forms tumours in 90–100% of cases. In contrast, injection of the same dose of C26-H-2Dpos cells lead to growth in only 2 of 8 mice. Challenge of the surviving mice with C26 cells resulted in tumour growth in 1 of 6 mice, compared to 6 of 6 mice in the naive control group. These experiments suggest that high levels of MHC class I on the C26 cell line may increase the immunogenicity of these cells, and perhaps that a heterogenous MHC class I expression confers an advantage for tumour growth in vivo. This model will be a useful tool to study the contribution of MHC class I expression to tumour cell growth and rejection. In addition, the heterogenous expression of MHC class I in this cell line may provide a useful experimental system for comparison with the clinical situation.

Dissociation of tumor antigen expression and susceptibility to immune effector mechanisms during the heat shock response

V. Milani, B. Frankenberger, O. Heinz, A. Brandl, K. Tschöp, R.D. Issels, E. Noessner
Munich, D

Naturally occurring and artificially applied heat treatment induces immunologically relevant changes in tumor cell physiology in addition to its direct cytotoxic effect. Using a melanoma cell line, we examined the effects of sub-lethal and lethal heat shock on tumor antigen processing and presentation as well as the associated ability to induce effector function in antigen-specific MHC class I-restricted T cells. We used a human tyrosinase-expressing melanoma cell line, which naturally processes and presents tyrosinase-derived peptides in the context of HLA-A2 molecules. Melanoma cells were subjected to sub-lethal (41.5°C for 120 minutes) and lethal (45°C for 22 minutes) heat shock and allowed to recover for 24, 48 and 72 hours at 37°C. Tyrosinase protein and mRNA expression levels, MHC class I surface antigen expression and recognition by the HLA-A2-restricted human melanoma cell line TyrF8 were investigated. In parallel, the kinetic of inducible heat shock protein 70 (HSP70) expression served as a marker to follow the heat shock response. We showed that heat shock stimulated the expression of inducible HSP70 with differential kinetic after sub-lethal and lethal heat shock and a dose-dependent association of protein and mRNA levels of tyrosinase expression in a dose-dependent manner was observed; tyrosinase protein levels were strongly increased, whereas mRNA levels were significantly but transiently decreased. Ability of heat-treated melanoma cells to present tyrosinase-derived peptides in the context of HLA-A2 molecules was dependent on the HLA-A2 expression level but not on tyrosinase expression level, neither at protein nor at mRNA level. This dissociation of antigen protein level and antigen presentation was most obvious after a lethal treatment. When testing the susceptibility of heat-treated melanoma cells to antigen-specific tumor cell killing effector mechanisms, we observed that sub-lethally heated cells maintained susceptibility over time, whereas lethally treated cells showed a transient decrease in susceptibility to a tyrosinase-specific CTL clone TyrF8. Overall these results challenge previous observations proposing that cells subjected to heat shock become resistant to CTL attack and other immune effector mechanisms and lessen the concern that clinical hyperthermia treatment might result in cytoprotection and immune escape.

T cell receptor excision circles (TREC) analysis in mice

G. Przybyski, P. Gronek, W. Wetzell, R. Gessner, G. Doelken, C.A. Schmidt
Greifswald, Berlin, D, Poznan, PL

Quantitative analysis of T cell receptor excision circles (TREC) has been recently used for determination of the proliferative history of T cell populations in humans. The TREC analysis proved to be useful in identification of recent thymic emigrants (RTE) and studies on naïve T cell reconstitution after bone marrow transplantation and anti-HIV therapy. In this paper we established a very accurate real-time PCR assay for TREC quantitation in mice. As a standard we used duplex vector containing both, the target mouse TREC (mTREC) and the reference gene mouse recombinase activating gene (mRAG2). Therefore the absolute numbers of mTREC were not influenced by the errors in DNA quantification, preparing of dilution series and variation of vector stability. We compared the TREC counts in T cells from the thymus, spleen, lymph nodes and peripheral blood from the C57BL/6 and 129 P2OlaHsd strains. The percentage of CD3+ cells as well as the TREC counts per 1000 CD3+ cells were comparable, although slightly higher for the 129 P2OlaHsd and C57BL/6 strains; in the LN (46 and 22), spleen (52 and 46) and PB (55 and 34). In the thymus the 129 P2OlaHsd mice had significantly lower number of CD3+ cells (13% vs 24%) than the C57BL/6 mice, but higher TREC levels, 83 and 32 respectively. We also analyzed the effect of mice immunization with chicken egg ovalbumin (OVA) and complete Freund’s adjuvant (CFA) on the TREC counts. Contrary to our expectations, in both strains the TREC levels were higher in the lymph nodes draining the site of immunization than in the corresponding non-draining lymph nodes, although further studies are needed to confirm and explain this phenomenon. Since murine model is frequently used in immunization experiments, the quantitative TREC analysis should facilitate the studies on T cell proliferative response.

A novel recombinant bispecific single-chain antibody, bsWue-1xCD3, induces T cell mediated cytotoxicity towards human multiple myeloma cells

D. Hönnemann, M. Rimpler, P. Kufer, M. Chatterjee, F. Riechert, K. Bommert, B. Dörken, R. Bargou
Berlin, D

The development of antibody-based strategies for the treatment of Multiple Myeloma (MM) has been hampered by the fact that suitable plasma-cell-surface specific antigens have been missing so far. Although normal and malignant plasmacells express a number of well characterized surface markers they all have turned out to be not plasmacell-specific. However, recently a novel monoclonal antibody, designated Wue-1, has been generated, which specifically binds to the cell surface of normal and malignant human plasma cells. Therefore, Wue-1 is an interesting and promising candidate to develop novel immunotherapeutic strategies for the treatment of multiple myeloma. One variant for an antibody-based strategy is the bispecific antibody approach. In particular, recombinant bispecific single-chain antibodies are interesting candidates because they show exceptional biological properties influenced by the errors in DNA transcription. Overall these results challenge previous antibodies. We have generated a novel MM directed recombinant bispecific single-chain antibody, Wue-1xCD3 (bsWue-1xCD3) and analyzed the biological properties of this antibody using the MM cell line NCI-H929 and primary cells from the bone marrow of patients with multiple myeloma.
myeloma and autologous or allogeneic effector T-cells. We were able to show that the bscWue-1xCD3 induces efficient T-cell mediated cell death of NCI-H929 cells and primary myeloma cells in 10/11 cases analyzed. In contrast to conventional bispecific antibodies described so far, the bscWue-1xCD3, is efficacious at low E:T ratios and without T-cell pre-stimulation. Target cell lysis was specific for Wue-1 antigen positive cells and could be blocked by the Wue-1 monoclonal antibody. Wue-1 antigen negative NALM-6 cells were not lysed by bscWue-1xCD3 Ab. To our knowledge this is the first plasma cell directed bispecific antibody described so far, showing promising results, and might therefore be a new potential agent for the treatment of malignant plasma cell disorders.

II processing pathway. Hodgkin's disease represents one of the most common types of malignant lymphomas in the Western world. The clonal, malignant Hodgkin/Reed-Sternberg (H/RS) cells of Hodgkin's disease represents <1% of the cells in an involved lymph node and are characterized by a large proportion of the T-cells that surround H/RS cells are Th2-cells. Several HLA class II restricted epitopes are identified in EBNA1. In the present study CD4+ T-cells were stimulated with adenoviral transduced DCs. T-cell response was monitored using Interferon (IFN)-gamma enzyme-linked spot (ELISPOT) assay, intracellular cytokine staining and flow cytometry and enzyme-linked immunosorbant assay (ELISA) for cytokine secretion. We found an increase in the T-cell response after 2 stimulations suggesting that DCs infected with an EBNA1 expressing adenovirus are capable of inducing CD4+ T-cell responses directed against EBNA1. This approach using a recombinant adenovirus vector does not require pre-defined epitopes or particular HLA profiles. This study may lead to develop a more potent immunotherapy against EBV-associated malignancies. Especially in light of the finding that many of the T-cells surrounding H/RS cells in Hodgkin's disease are CD4+ T cells a system for priming of EBNA1 specific human CD4+ T-cells might be a valuable contribution to immunotherapy of Hodgkin's disease.

P1013 Highly efficient stimulation and expansion of antigen specific CD4+ lymphocytes by targeting transgene products into MHCI processing pathway
S. Kreiter, M. Koslowski, Ö. Türeci, U. Sahin
Mainz, D

Effective therapeutic vaccination and long lasting immune responses depend on both generation of antigen specific CD8+ as well CD4+ lymphocyte immune responses. RNA transfected dendritic cells have been proven to be efficient stimulators of CD8+ positive lymphocytes. In contrast we and others observed that antigen specific CD4+ lymphocytes are only weakly stimulated and expand marginally after RNA transfection probably due to the cytotoxic expression of proteins translated from the RNA. We describe here that targeting of proteins into endosomal and lysosomal pathway leads to a dramatic increase of stimulatory capacity of RNA transfected dendritic cells. Methods: The cytomegalovirus protein pp65 was used as model antigen. The coding sequence of pp65 was cloned into a standard in vitro transcription (IVT) vector and into a plasmid allowing the expression of tagged fusion protein with endosomal targeting signals. RNA generated by IVT from these vectors and unrelated control RNA was used for electroporation in antigen presenting cells from CMV positive donors. CD4+ lymphocytes were cocultured for 7 days with the RNA transfectted autologous DC. Elispot assays were performed using autologous antigen presenters transfected with RNA or loaded with pp65 overlapping peptides. Assays were performed on day 0 and day 7 to measure the initial number of antigen specific cells and the efficacy of expansion. In addition a BrdU proliferation assay was used to test the specific proliferative capacity. Results: Antigen presenting cells transfected with standard pp65 RNA led to none or minimal expansion of specific CD4+ T cells within the 1 week stimulation period. In contrast stimulation with RNA encoding endosomal targeting fusion proteins induced a strong expansion resulting in high numbers of IFN-gamma producing CD4+ lymphocytes. A series of parallel experiments demonstrated that the use of RNA encoding endosomal targeting fusion proteins resulted in at least 5 to 20 fold higher stimulation and expansion rates as compared with the standard approach. The higher stimulatory capacity of the fusion antigens was associated with an increased proliferative rate as observed in a BrdU incorporation assay. The targeting of antigens into endosomal pathways might prove to be a useful tool in the setting of RNA based DC-vaccination therapies. In vivo experiments using model antigens in mice are ongoing and have to demonstrate the in vivo relevance of the optimized helper T cell stimulation.

P1014 Dendritic cells transduced with adenovirus vector encoding Epstein-Barr virus nuclear antigen 1 induce a CD4+ T cell response
Regensburg, Göttingen, Lübeck, D; Linz, A; Houston, USA

The Epstein-Barr Virus (EBV) is associated with a number of malignancies, including Hodgkin's disease, Burkitt's lymphoma, and nasopharyngeal carcinoma. The Epstein-Barr Virus nuclear antigen 1 protein (EBNA1) is essential for viral RNA replication and the maintenance of viral episomes in the infected cells. EBNA1 is the only viral protein that is consistently expressed in all EBV-associated malignancies. The presence of a glycine-alanine repeat domain inhibits antigen processing via the ubiquitin/proteasome pathway. However, EBNA1 is not protected from the HLA class II processing pathway. Hodgkin's disease might prove to be a useful tool in the setting of RNA based DC-vaccination therapies. In our knowledge this is the first plasma cell directed bispecific antibody described so far, showing promising results, and might therefore be a new potential agent for the treatment of malignant plasma cell disorders.

P1015 Quantitative mRNA expression of Toll-like receptors in dendritic cells generated from blasts of patients with acute myeloid leukemia
Ulm, D

Acute myeloid leukemia (AML) is a clonal disease of hematopoiesis with poor clinical outcome despite recent improvement of chemotherapy and stem cell transplantation regimens. Immunotherapy with dendritic cells (DC) generated from AML blasts (AML-DC) might be a therapeutic option for AML patients. We demonstrated elsewhere that AML-DC express HLA and costimulatory molecules as well as leukemia associated antigen (LAA). These characteristics make AML-DC a promising tool in immunotherapy for AML patients. Potentiation of such an AML-DC vaccine might become feasible by the addition of adjuvant such as lipopolysaccharides (LPS) or CPG-rich oligodeoxynucleotides (CPG-ODN). Therefore we investigated the presence of receptors for such adjuvants, i.e. Toll-like receptors (TLRs) on AML-DC. TLRs are ancient microbial pattern recognition receptors highly conserved from drosophila to vertebrates, which are essential for the recognition of pathogen-associated molecular patterns by the innate immune system. In humans, distinct DC subsets have been shown to express different TLRs and consequently to respond to different ligands. We analyzed mRNA expression of TLR-2, 4 and 9 by using quantitative real-time PCR for mature monocyte-derived DC. The expression of these TLRs was compared on DC generated from AML patients versus 12 healthy volunteers (HV-DC). Both AML-DC and HV-DC expressed TLR-4 at a similar level with a median ratio of 0.01. There was no significant difference between the two groups of AML-DC and HV-DC. The myeloid Fc receptor for IgA as a novel cytotoxic trigger molecule in tumor therapy
B. Stockmeyer, Y. Wachter, T. Valerius, M. Dechant, M. Gramatzi, R. Repp
Erlangen, Kiel, D

Recent advances in the treatment of lymphomas or solid tumors with monoclonal antibodies (mAb) restimulated the interest in immunotherapy against malignant diseases. Animal studies and clinical data suggested that receptors for IgG (FcαR) are essential for the therapeutic efficacy of human IgG1 mAb like CD20-directed Rituximab or HER-2/neu-directed Trastuzumab. Our previous experiments demonstrated polymorphon-
clear granulocytes (PMN) to represent a potent effector cell population for lysis of malignant cells. In addition to FcgRs, PMN express a medium affinity FcR for IgA (FcαRI, CD89) with a strong capacity for triggering antibody-dependent cellular cytotoxicity (ADCC). In order to recruit FcαRI on PMN as cytotoxic trigger molecule, we are currently comparing human IgA mAb and different formats of bispecific antibodies. Chimeric human IgA mAb targeting HLA class II on lymphoma cells was comparably efficient in triggering ADCC compared to chemically linked [F(ab')2] or genetically engineered recombinant single-chain Fv bispecific antibodies (bsAb) targeting FcαRI with one specificity and binding to HLA class II with the second specificity. Triggering FcαRI PMN can lyse malignant B lymphoma cells opsonized with CD20×FcαRI bispecific antibody. However PMN were markedly cytotoxic against malignant B lymphoma cell lines using CD19×FcαRI bispecific antibody in 3h chromium release assays. There is no homolog for human FcαRI in the mouse lymphoma cell lines using CD19×FcαRI bispecific antibody, which is unrelated to phagocytosis due to molecular weight or interaction with the polymeric Ig receptor. In vivo differences between IgA mAb and various bsAb in human FcaRI transgenic mice will help in the development of preclinical drug release assays. There is no homolog for human FcaRI in the mouse lymphoma cell lines using CD19×FcαRI bispecific antibody in 3h chromium release assays. **There is no homolog for human FcaRI in the mouse lymphoma cell lines using CD19×FcαRI bispecific antibody, which is unrelated to phagocytosis due to molecular weight or interaction with the polymeric Ig receptor.** In vivo differences between IgA mAb and various bsAb in human FcaRI transgenic mice will help in the development of preclinical drug release assays.**

**Introduction:** The extracellular domain of HER-2/neu (ECD) is the target antigen for the monoclonal antibody trastuzumab (Herceptin). As the ECD may shed into the bloodstream after cleavage from the intact membrane-bound protein by metalloproteinases, complex formation of the therapeutic antibody with its circulating ECD may shed into the bloodstream after cleavage from the intact membrane-bound protein by metalloproteinases, complex formation of the ECD in serum and the antibody might compromise the activity of the drug due to a lowered trough concentration, especially in the case of very high levels of the ECD in serum. Material/methods: We investigated this hypothesis in 24 patients under Herceptin plus combination chemotherapy comparing the ECD levels before the Herceptin infusion, and 1 hour after the end of the infusion. A total of 55 infusions were considered. HER-2/neu in serum was measured on the automated analyser Immuno 1 ( Bayer Diagnostics Division, Tarrytown, NY) with a cut-off of normal of 15 ng/ml. Patients were grouped according to the HER-2/neu level before therapy: group 1: ECD <15 ng/ml; group 2: ECD 15–50 ng/ml; group 3: ECD >50 ng/ml. Results: The serum HER-2/neu levels ranged from 7.1–2121 (median: 23.3; mean: 251.4) ng/ml before Herceptin therapy while the ECD concentrations ranged from 7.1–1738 (median: 26.6; mean: 216.4) after Herceptin therapy. For ECD levels before therapy <50 ng/ml (group 3), the ECD concentrations after treatment were statistically significantly lower than the ECD concentrations before treatment (p<0.001, Mann-Whitney). Even for the comparison of group 1 (normal ECD concentration <15 ng/ml) versus groups 2 and 3 (elevated ECD level >15 ng/ml), this effect persisted with p = 0.036. Figure 1 displays the concentrations of serum HER-2/neu before Herceptin in relation to the difference of all serum HER-2/neu concentrations after minus before the infusion. It shows that the higher the HER-2/neu level in serum was before therapy, the more pronounced the decrease of serum HER-2/neu was after treatment. Conclusions: The concentration of serum HER-2/neu is statistically significantly lowered by Herceptin therapy, especially in the case of high levels of circulating HER-2/neu. This decrease is an indicator of the complex formation of the therapeutic antibody with its circulating antigen. Our study is in line with publications indicating a lower steady state concentration of Herceptin in patients with high levels of circulating HER-2/neu. This is the first report of serum HER-2/neu levels in patients before and right after Herceptin infusion.

**P1017**

In vivo evidence of complex formation between the shed antigen of HER-2/neu and the monoclonal anti-HER-2/neu antibody trastuzumab

D. Lüftner, G. Schaller, P. Henschke, R. Amin, R. Geppert, K. Wernecke, K. Possinger

Berlin, Herne, D

Introduction: The extracellular domain of HER-2/neu (ECD) is the target antigen for the monoclonal antibodytrastuzumab (Herceptin). As the ECD may shed into the bloodstream after cleavage from the intact membrane-bound protein by metalloproteinases, complex formation of the ECD in serum and the antibody might compromise the activity of the drug due to a lowered trough concentration, especially in the case of very high levels of the ECD in serum. Material/methods: We investigated this hypothesis in 24 patients under Herceptin plus combination chemotherapy comparing the ECD levels before the Herceptin infusion, and 1 hour after the end of the infusion. A total of 55 infusions were considered. HER-2/neu in serum was measured on the automated analyser Immuno 1 ( Bayer Diagnostics Division, Tarrytown, NY) with a cut-off of normal of 15 ng/ml. Patients were grouped according to the HER-2/neu level before therapy: group 1: ECD <15 ng/ml; group 2: ECD 15–50 ng/ml; group 3: ECD >50 ng/ml. Results: The serum HER-2/neu levels ranged from 7.1–2121 (median: 23.3; mean: 251.4) ng/ml before Herceptin therapy while the ECD concentrations ranged from 7.1–1738 (median: 26.6; mean: 216.4) after Herceptin therapy. For ECD levels before therapy <50 ng/ml (group 3), the ECD concentrations after treatment were statistically significantly lower than the ECD concentrations before treatment (p<0.001, Mann-Whitney). Even for the comparison of group 1 (normal ECD concentration <15 ng/ml) versus groups 2 and 3 (elevated ECD level >15 ng/ml), this effect persisted with p = 0.036. Figure 1 displays the concentrations of serum HER-2/neu before Herceptin in relation to the difference of all serum HER-2/neu concentrations after minus before the infusion. It shows that the higher the HER-2/neu level in serum was before therapy, the more pronounced the decrease of serum HER-2/neu was after treatment. Conclusions: The concentration of serum HER-2/neu is statistically significantly lowered by Herceptin therapy, especially in the case of high levels of circulating HER-2/neu. This decrease is an indicator of the complex formation of the therapeutic antibody with its circulating antigen. Our study is in line with publications indicating a lower steady state concentration of Herceptin in patients with high levels of circulating HER-2/neu. This is the first report of serum HER-2/neu levels in patients before and right after Herceptin infusion.

**P1018**

Rapid, complete, and lasting remission of an acquired C1-esterase inhibitor deficiency with angioedema edema type I and an anti-cardiolipin IgM-autoantibody associated with a low-grade B-cell lymphoma with rituximab


A female patient presented 5 years ago with B-symptoms and repeated episodes with abdominal cramps, vomiting, diarrhea and facial edema. No allergies could be identified. A splenomegaly but no enlarged lymph nodes were present. Atypical mature lymphatic cells were found in the blood and the prothrombin time (PT) was spontaneously reduced to 17% (INR 2.9) without bleeding or thromboses. The bone marrow was infiltrated by 30–50% of mostly mature lymphatic cells strongly expressing CD10, CD20, CD22, CD24, FMC7, and IgM, but with weak expression of CD23 and CD5, and negative staining for CD19. An inhibitor of coagulation was found in an APTT test with admixing serum that could not be neutralized with phospholipids. A high titer of an IgM antibody against cardiolipin was identified. Functional measurement of the C1-esterase inhibitor revealed non-detectable levels.

The patient required substitution with C1-esterase inhibitor (Berinert) and was treated with chlorambucil and prednisone. The angioneurotic episodes gradually disappeared and PT values rose to 50%. After 30 months, a relapse with increasing frequency and severity of episodes of angioneurotic edema and a decrease of PT to values <15% was documented. At that time, the bone marrow showed only a minor nodular infiltration with a post-chemotherapeutic peripheral lymphopenia. After 4 doses of rituximab 375 mg/sqm a rapid clinical improvement with lasting (20+ months) remission and normalization of all laboratory abnormalities was observed. Detailed kinetics of PT, anti-cardiolipin antibodies and C1-esterase inhibitor levels will be given. Whether the antibodies against the two targets were the same and secreted by the malignant cells or whether a lymphoma-associated polyclonal autoimmune phenomenon was responsible for the clinical picture could not be determined.

**P1019**

Great virtual, but small clinical benefit from Rituximab in a patient with chronic lymphatic leukemia, pure red cell aplasia, and severe tricytopenia

R. Eckert, K.P. Maier, W.E. Aulitzky

Esslingen, Stuttgart, D

Clinical report: A 60 yr. old man received a diagnosis of B-CLL in 1998. There was no splenomegaly, lymphadenopathy, or evidence of autoimmune haemolytic anemia, but he was transfusion dependent from 1998 on. In 1999–2000, 5 courses of fludarabin were given, resulting in severe, prolonged leukopenia and continued transfusion dependence. Reticulocyte counts were <0.4%, and again there was no haemolytic
On bone marrow (BM) examination in Sept. 2000, we found a moderate infiltration of the CLL (50%), but a massive reduction in conventional chemotherapeutic agents and alemtuzumab shows superadditive effects on B-cells and B-cell chronic lymphatic leukemia (B-CLL). However, data evaluating combination therapy with different cytostatic agents and Campath shows superadditive effects on B-cells and for the following experiments were ascertained by XTT-assay, hydrocortisone, mitoxantrone or maphosphamide via XTT-assay and Annexin-FACScan. Results and conclusions: CD52 FACS-analysis showed high median fluorescence intensity (MFI) for mc116 (408), wsu-NHL (253), HUT-78 (170) whereas DoHH2 (25) and Hodgkin lymphoma cell line L50(6) expressed less CD52. B-CLL patient lymphocytes expressed a high rate of CD52 with a MFI of 1116. The concentrations of the cytotoxic agents for the following experiments were ascertained by XTT-assay, ranging from 0% to 100% viability, and differed from 1-0.002µg/ml for doxorubicin to 15-0.001µg/ml for etoposide. 5-0.002µg/ml for fludarabine, 2-0.04µg/ml for gemcitabine, 210-0.03µg/ml for hydrocortisone, 10-0.1µg/ml for maphosphamide and 3-0.001µg/ml for mitoxantrone; the subtoxic concentration of the antibodies for each cell line were for mc116: 20µg/ml and 100µg/ml GaH; wsu-NHL: 40µg/ml and 100µg/ml GaH; HUT-78: 40µg/ml and 200µg/ml GaH and DoHH2: 40µg/ml and 100µg/ml GaH. Incubation of a cytotoxic agent and Campath led to a dose-dependent synergistic effect for doxorubicin on DoHH2, for etoposide on mc116, wsu-NHL and HUT-78, for gemcitabine on mc116 and HUT-78, for hydrocortisone on mc116, for maphosphamide on mc116 and DoHH2 and for mitoxantrone on all cell lines. These results indicate synergistic activity of conventional chemotherapeutic agents and alemtuzumab in vitro.

P1022

Treatment of chronic graft-versus-host disease of the lung with extracorporeal photopheresis – experience in 2 patients

A. Hölig, A. Haack, J. Freiberg-Richter, A. Neubauer, G. Ehninger, M. Bornhäuser

Dresden, Marburg, D

Purpose: Photopheresis (extracorporeal photochemotherapy, ECP) appears to be an effective treatment for skin and liver manifestations of chronic graft versus host disease (cGVHD) in many studies. Experience in the treatment of pulmonary GVHD with ECP is still limited and controversial. Methods: We report on 2 patients with severe pulmonary GVHD who were treated with ECP using the UVAR-XTS-device (Therakos Company, Exton). Patient 1 was a 37-year-old woman, diagnosed with AML M1 8/1998. Patient 2 was a 39-year-old man with AML M2, diagnosed 9/1999. Both patients were transplanted from HLA-identical siblings and suffered from severe lung and moderate liver GVHD. ECP was started 12 and 17 months after transplantation, respectively. Additional immunosuppressive treatment consisted of cyclosporin, mycophenolate mofetil and prednisolone (30mg) in Pat. 1 and tacrolimus and prednisolone (30mg) in Pat. 2. ECP treatments were performed on 2 consecutive days every week during the 1st month and every 2nd week thereafter for the following 5 months. Treatment intervals were tapered thereafter individually according to patients condition. Results: The ECP procedures were well tolerated by both patients without any severe side effects. The clinical problems were mostly related to difficult venous access. Patient 1 was treated with ECP for 1 year, during this time pulmonary symptoms improved continuously and liver function parameters normalized. Now she is still in stable clinical condition 2,5 years after completion of ECP.
A randomized phase II single center study of gene transfer-based nonspecific immunotherapy of malignant mesothelioma by intratumoral injections of an interleukin-2 producing vero-cell-line

J.-A. Pitako, P. Squiban, B. Acres, W. Digel Freiburg, D; Strassburg, F

Two phase I trials of non-specific immunotherapy using Vero cells which produce human IL-2 have been previously conducted. The results of both have demonstrated an excellent safety profile and possible evidence of activity profile was characterized by gainine the superior property of TNF related pathways by mimicking the membrane integrated TNF signaling was detectable in various systems when acting in FAP-antigen bound status, resulting in activiation of multi-

The genetic engineering of a TNF fusion protein was performed by replacing the CH2/CH3 domains of a humanized anti-FAP (fibroblast activation protein) antibody by human TNF (tumor necrosis factor) replacing the IgG1 CH2/CH3 Fc-do-

The construct was generated by recombinant DNA technology and preserved its IgG1 derived dimeric structure with the TNF-molecule linked as a dimer. Expression in CHO (chinese hamster ovary) cells was optimized in serum-free media under GMP conditions to achieve produc-

The high specificity of anti-FAP-antibody mediated tissue targeting together with subsequent delivery of TNF sig-

CD4+ T helper cells are an integral part of effective immune responses against various malignancies, however in tumor-bearing patients they are frequently functionally unresponsive. T helper cells of patients with Chronic Myeloid Leukemia (CML) analyzed as part of mononuclear cell fractions, have been previously characterized by a loss of signaling mole-

The underlying mechanism is unknown and may in-

Detected TNF-derived effects in FAP negative tissue failed. Furthermore, TNF-signaling to FAP+ tumors could not be initiated by application of dimerized TNF coupled to an isotype matched antibody with irrelevant antigen-specificity. This reflects the high specificity of anti-FAP-antibody mediated tissue targeting together with subsequent delivery of TNF sig-

\[ \text{dimerized TNF portion of the fusion protein depending on its circulatory or antigen bound status. The resulting difference in signaling strength and related cellular response pattern proves the principle of TNF dimer link-} \]

\[ \text{age to a CH2/CH3 truncated antibody as potent agent for cancer immuno-} \]

\[ \text{therapy with targeted bioactivity of membrane anchored TNF} \]

S. Bauer, N. Adrian, J. Smerd, N. Fadle, B. Williamson, C. Panousis, A. Scott, M. Pfreibuchsch, C. Renner

Homburg, D; New York, USA; Heidelberg, AUS

We present the design and characterization of a fusion protein consisting of a humanised anti-FAP (fibroblast activation protein) antibody and human TNF (tumor necrosis factor) replacing the IgG1 CH2/CH3 Fe-do-

Normal intrinsic Th1/Th2 balance in patients with chronic phase chronic myeloid leukemia not treated with interferon-alpha or Imatinib

A. Kiani, I. Habermann, K. Schäkel, A. Neubauer, G. Ehninger Dresden, Marburg, D

P1026

Normal intrinsic Th1/Th2 balance in patients with chronic phase chronic myeloid leukemia not treated with interferon-alpha or Imatinib

S. Bauer, N. Adrian, J. Fettah, N. Fadle, B. Williamson, A. Scott, M. Pfreibuchsch, C. Renner

Homburg, D; New York, USA; Heidelberg, AUS

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CD4+ T helper cells are an integral part of effective immune responses against various malignancies, however in tumor-bearing patients they are frequently functionally unresponsive. T helper cells of patients with Chronic Myeloid Leukemia (CML), analyzed as part of mononuclear cell fractions, have been previously characterized by a loss of signaling mole-

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\[ \text{In vivo efficacy and toxicity profile of dimerized TNF fusion protein} \]

S. Bauer, N. Adrian, J. Smerd, N. Fadle, B. Williamson, C. Panousis, A. Scott, M. Pfreibuchsch, C. Renner

Homburg, D; New York, USA; Heidelberg, AUS

The genetic engineering of a TNF fusion protein was performed by replacing the CH2/CH3 domains of a humanized anti-FAP (fibroblast activation protein) IgG1 antibody by human TNF (tumor necrosis factor) replacing the IgG1 CH2/CH3 Fc-do-

The construct was generated by recombinant DNA technology and preserved its IgG1 derived dimeric structure with the TNF-molecule linked as a dimer. Expression in CHO (chinese hamster ovary) cells was optimized in serum-free media under GMP conditions to achieve produc-

The resulting difference in signaling strength and related cellular response pattern proves the principle of TNF dimer link-
NK2G2D-ligands (NK2G2DL) mark malignant cells for recognition by NK cells and cytotoxic T lymphocytes via the activating immunoreceptor NK2G2D. This led to the hypothesis that NK2G2DL play a critical role in tumor immune surveillance. The human NK2G2DL MICA and MICB have been shown to be expressed on tumors of epithelial origin in vivo. For the other recently described set of human NK2G2DL, the UL16-binding proteins (ULBP), expression in vivo is yet undefined. We investigated expression and function of NK2G2DL in leukemia using a panel of newly generated NK2G2DL-specific monoclonal antibodies and report that leukemia cells from patients variously express MIC and ULBP molecules on the cell surface with MICA most frequently detected. Patient leukemia cells expressing MICA were lysed by NK cells in an NK2G2D-dependent fashion. Recently we demonstrated shedding of MICA from epithelial tumors and high levels of soluble MICA (sMICA) in sera of patients with epithelial malignancies. Likewise, sera of patients with hematopoietic malignancies, but not of healthy donors, contain elevated levels of sMICA. Furthermore, we also detected increased sMICB levels in these sera, which, in general, were lower than levels of sMICA. In similarity to MICA, MICB is released from the cell surface due to the activity of metalloproteinases. Reduction of leukemia MIC surface expression by shedding and systemic NK2G2D down-regulation on immune effector cells by soluble MIC molecules may impair NK2G2D mediated immune surveillance of leukemias. In addition, determination of soluble MICA and MICB levels may be implemented as prognostic parameter in patients with hematopoietic malignancies.

**P1028**
Relapse of Philadelphia positive ALL with loss of the Y-chromosome after sexmismatched allo PBSCT and ongoing GVHD

Rostock, D

ALL with t(9;22) is associated with a high frequency of relapse after allo- geneic PBSCT. Despite dismal results of DLI in ALL Philadelphia positive ALL has been associated with a clinical relevant graft-versus-leukemia (GVL)-reaction after allogeic PBSCT. The H-Y-mismatch after sexmismatched PBSCT has been associated with GVHD as well as the GVL-effect. Here we report a patient, who relapsed with Philadelphia positive ALL with loss of the Y-chromosome after sexmismatched allogeic PBSCT and ongoing chronic GVHD.

A 45 year old male received a peripheral blood stem cell transplantation from his HLA-identical sister for ALL with t(9;22) in 1st CR. He developed grade IV acute GVHD with a peak of 97% cell viability on day 0 and 84% on day 21. Effector functions of expanded cells were tested in a 8 h 51Cr release assay (effector to target ratio 1:10) with the NHL cell line SU-DHL4 and the breast carcinoma line BT 474 as targets. Bispecific antibodies CD19 x CD3 and EpCam x CD3 were used to redirect effector cells. Spontaneous cytotoxicity against SU-DHL4 increased from 15% on day 0 to 24% on day 21. Addition of the CD19 x CD3 at a concentration of 100 ng/ml increased 51Cr release to 51%. No significant cytotoxicity against BT474 was noted without bispecific antibody. When EpCam x OKT3 (100 ng/ml) was added, significant cytotoxicity occurred (53% 51Cr release). We could demonstrate that NK effector cells can be expanded from leukapheresis material with control over all aspects of production and testing of the cell therapy product, using GMP conditions in a clean room facility. Obtained cell numbers and detected effector function were sufficient to warrant further testing in clinical trials.

**P1030**
CD4+CD25+ regulatory T cells in the peripheral blood of cancer patients

D. Wolf, M. Wolf, H. Rumpold, G. Gastl, E. Gunsilius
Innsbruck, A

CD4+CD25+ regulatory T-cells (Treg) have been shown to play a pivotal role for maintenance of self tolerance. In addition, experimental tumor models in mice revealed that Tregs are also potent inhibitors of an effective anti-tumor immune response. We show that patients with epithelial malignancies (n = 42) display an increased pool of CD4+CD25+ T-cells in the peripheral blood with characteristics of Tregs (CD45RA+CD69-). After in vitro expansion of isolated CD4+CD25+ T-cells we found a Treg phenotype, being positive for CD11a, CD28, CD31, CD45R0, CD62L, TGF-§, CTLA-4 and CD95+ and CD4+ T-cells from cancer patients have an impaired proliferative capacity as compared to CD4+ T-cells from healthy controls, which is reversible by prior depletion of CD25+ T-cells. In addition, isolated CD4+CD25+ T-cells from cancer patients suppressed the proliferation of cocultured CD4+CD25- T-cells. When cultured together with magnetically selected CD56+ NK-cells, CD4+CD25+ T-cells isolated from cancer patients effectively inhibited NK-cell-mediated cytotoxicity against the erythroleukemic cell line K562. To test whether the increased pool of CD4+CD25+ T-cells affects in vitro antibody-dependent cytotoxicity (ADCC), freshly isolated PBMC as well as CD25-depleted PBMC from patients with epithelial cancers were tested against an Ep-CAM positive mammary carcinoma cell line. Prior depletion of CD25+ T-cells markedly increased the spontaneous cytotoxic activity of PBMC. In contrast, pre-incubation of the Ep-CAM positive cell line with an anti-Ep-CAM antibody...
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**P1032**

**Farnesyltransferase inhibitors suppress T cell activation by post-transcriptional regulation of cytokine production**

R. Marks, S. Berk, A. Ho, T. Gajewski
Chicago, USA

Several cellular proteins like GTPases of the Ras-family containing a C-terminal CAAX motif are prenylated by the intracellular enzyme farnesyltransferase to facilitate localization to cellular membranes. Latter is a prerequisite for receptor mediated activation of prenylated proteins and subsequent signal transduction. Farnesyltransferase inhibitors (FTI) are a class of drugs generated to interfere with this farnesylation process thereby retaining the signaling proteins in the cytosol, thus preventing activation. Based on the observation that the phenotype of oncogenic Ras-transformed fibroblasts could be reversed by FTI treatment these agents are currently being clinically explored as antineoplastic treatment in several hematologic malignancies and solid tumors. We tested the hypothesis that FTI blocks Ras-dependent signals in T cells triggered by stimulation via the T-cell receptor (TCR) +/- CD28. Therefore we examined the effect of two FTI (CP-390392, 179626) on cytokine production of murine Th1 and Th2 T cell clones in response to activation by anti-CD3 +/- anti-CD28 antibodies or PMA/Ionomophores. Upon preincubation with FTIs Th1 and Th2 T cell clones showed a dose dependent reduction of proliferation and lineage specific cytokine production (IFN-gamma, IL-2, IL-4, IL-5). The effect was similar regardless of the used activation stimuli, arguing that disturbed proximal receptor mediated signaling was unlikely the cause for the observed cytokine reduction, since early signals are bypassed by PMA/Ionomycin treatment. Contrary to expectations, no inhibition of known Ras effectors like ERK or JNK MAP-Kinases were observed in FTI treated T cells. Further examination showed that induction of cytokine mRNA was not inhibited in the presence of FTIs, while intracellular detection of cytokine proteins could demonstrate reduced levels in FTI preincubated cells. These findings suggest a FTI mediated regulation of cytokine synthesis in T cells on a post-transcriptional level. In addition to the established antineoplastic effects of farnesyltransferase inhibitors, the presented data support the idea of FTI representing a novel class of immunosuppressive agents.

**P1033**

**Treatment with CamPATH-1H for relapsed CLL after allogeneic peripheral blood stem cell transplantation does not abrogate the development of chronic GvHD**


The graft-versus-leukemia (GVL) effect is one of the most important determinants of anti-tumor activity of allogeneic hematopoietic stem cell transplants (alloSCT). Its effectiveness mainly depends on both, the tumor biology and tumor burden. Patients with a high tumor burden may not benefit from the GVL effect irrespective of sensitive tumor biology. CamPATH-1H is known as effective treatment of CLL as well as part of conditioning regimens before alloSCT and causes profound immunosuppression.

We report a patient who received CamPATH-1H combined with docetaxel for relapsed CLL after alloSCT resistant to chemotherapy and donor lymphocyte infusion. Within a few days after discontinuation of CamPATH-1H drug administration, he developed severe peripheral blood eosinophilia (40%) and typical clinical and histological signs of cutaneous chronic GVHD. CamPATH-1H was administered at a dose of 3x30mg/kg and docetaxel (30 mg/m²/kg) was added due to rising lymphocyte counts. Treatment was continued for 3 months leading to major partial remission of CLL. After three months of treatment the patient suddenly developed moderate infusion-related findings effects consisting of chills, fever, and myalgia. Treatment was stopped follow be the appearance of severe eosinophilia combined with myalgia. Initial clinical signs of cutaneous chronic GVHD appeared one month after discontinuation of treatment which was confirmed by skin biopsy. Three months after discontinuation of CamPATH-1H the patient achieved complete remission of CLL and remained in remission (on year follow-up). Eight months after discontinuation of treatment with CamPATH-1H chronic lichenoid skin GVHD progressed to sclerodermoid GVHD necessitating immunosuppressive treatment with prednisolone.

The clinical course demonstrates the impact of tumor burden on the GVL effect and the effectiveness of CamPATH-1H in the presence of chemoreistant CLL. Even more, GVL effect was not abrogated by the use of CamPATH-1H.

**P1034**

**Expansion of cytotoxic T-lymphocyte clones directed against cancer-germline antigens: Prospects of adoptive T cell transfer for patients with multiple myeloma**

G. Fischer, B. Schmidt, K. Hofer, K. Gebhard, K. Huster, D. Busch, H. Wagner, C. Feschel, H. Bernhard
Munich, Nuremberg, D.

Patients with advanced multiple myeloma have an impaired cellular immune system. Circumventing preexisting tolerance mechanisms by ex vivo stimulation and expansion of myeloma-reactive T cells might be a suc-
cessful strategy of eliminating myeloma cells in vivo when transferred back to the patient. CG-specific T cells might be promising candidates for successful adoptive T cell therapy, since some cancer germline (CG) genes are expressed by malignant plasmacytoma cells. Screening of 30 myeloma bone marrow samples by RT-PCR demonstrated the expression of the CG antigens NY-ESO-1, MAGE-A1, and MAGE-C1 in 17% (5/30), 30% (9/30), and 13% (4/30), respectively. Corresponding immunohistochemical examination of bone marrow biopsies are underway. We have been focusing on isolating CTL clones against the NY-ESO-1-derived HLA-A2-binding peptide p157–165 from the peripheral blood of healthy donors and myeloma patients. Baseline frequencies of CD8+ NY-ESO-1-specific T cells are usually less than 0.01% of total peripheral blood mononuclear cells (PBMCs) as determined by fluorochrome-labeled HLA-A2/p157–165 multimers. Repetitive stimulation of PBMCs with peptide-loaded dendritic cells in vitro increase peptide-specific T cells to numbers that allow multimer-guided sorting. Multimer-specific T cells are directly cloned by limiting dilution. Using this technique we succeeded in isolating and expanding a NY-ESO-1-specific CTL clone to a number (1x10⁶) sufficient for several cycles of adoptive immunotherapy. The expanded CTL clone retained the lytic activity against peptide-pulsed target cells and a HLA-A2-matched NY-ESO-1-expressing myeloma cell line. Recent experiments focus on the generation of class I-restricted CTL clones against additional CG-derived epitopes presented together with class I from both healthy donors and patients. A program of adoptive transfer of autologous CG-specific CTL clones for patients with multiple myeloma is in preparation.

P1035
Mechanism of action of Rituximab: Clinical implications
D. Flieger, W. Fischbach, U. Spengler, I. Schmidt-Wolf
Aschaffenburg, Bonn, D.

The chimeric human/mouse monoclonal antibody (mAb) rituximab, which recognizes CD20 on B lymphocytes, has emerged as an effective therapy for non-Hodgkin’s lymphoma and other B-cell malignancies. Rituximab is approved for the treatment of relapsed or chemorefractory low-grade and follicular NHL and in combination with CHOP chemotherapy for first-line treatment of patients with diffuse large B-cell lymphoma. Recently, there is growing knowledge in understanding the mechanisms by which rituximab kills malignant B cells.

The CD20 antigen is a nonglycosylated cell-surface protein expressed on B lymphocytes. Recent studies have demonstrated that CD20 initiates intracellular signals involving tyrosine kinase activation. Ligation of CD20 by this mAb promotes apoptosis. Moreover, rituximab induces potent complement-dependent cell cytotoxicity. In contrast, antibody-dependent cell cytotoxicity (ADCC) is not an important mechanism of action, a finding consistent with the clinical observation that corticosteroids do not suppress but rather enhance cytotoxicity. In an in vitro model we demonstrated that the cytokines IFN-α, IL-2, IL-12 and GM-CSF had no influence on cytotoxicity indicating that in contrast to other mAbs the addition of cytokines will not result in enhanced clinical effects (Flieger D et al 2000) Mechanism of cytotoxicity induced by the chimeric mouse human monoclonal antibody IDEC-C2B8 in CD20 expressing lymphoma cell lines. Cell Immunol. 204(5). Recently, it was recognized that in contrast to other B cell mAb, rituximab effectively induces de novo complement factors like C3b(i), promotes redistribution of CD20 into lipid cellular compartments and transfers signals to the nucleus. The expression of CD20 on lymphoma cells correlates with clinical benefit. Moreover, prolonged pharmacokinetics allowing effective plasma concentration above 1 mg/ml for many months together with induction of effective apoptosis result in the observed pronounced clinical responses, which correlate with rituximab serum concentrations. Since the most effective combination partners are chemotherapeutic drugs, it is now clearly recognized that combined immunochemotherapy will be most beneficial in patients with B cell malignancies. In future, the knowledge about cellular factors involved in apoptosis e.g. bcl-2, which can be down regulated by rituximab thus breaking cisplatin-resistance in lymphoma cells, will help to find combination partners for rituximab.

P1036
High frequency of functionally active Melan-A specific T cells in a patient with progressive melanoma: Implications for immunotherapy
Regensburg, Mainz, D; Lausanne, CH

Tumor-reactive T cells have been demonstrated to play an important role in cancer immunosurveillance. Applying the mutimer technology, previous studies demonstrated that CD8+ T cells directed against the melanocyte differentiation antigen Melan-A can be frequently found in melanoma patients. Here we report an unexpected high frequency of circulating Melan-A specific CTL of more than 14% of the total CD8+ T cells. In fact, this is the highest reported frequency of circulating CD8+ T cells directed against a single tumor-associated antigen (TAA). Despite the presence of this high number of TAA-specific T cells, the patient had rapidly progressing lymph node (LN) metastases. Since selective anergy of TAA-specific T cells has been proposed as a possible immune escape mechanism in malignant melanoma, we thoroughly assessed phenotype and effector functions of Melan-A-specific CTL in this patient. Melan-A specific T cells consisted of memory effector (CD45RA⁻/CCR7⁻/CD27-) and terminally differentiated effector T cells (CD45RA⁺/CCR7⁻/CD27⁻). Ex vivo functional characterization of Melan-A specific CTL demonstrated specific secretion of IFN-γ upon antigenic stimulation and direct killing of Melan-A-pulsed target cells as well as allogeneic HLA-A2+ Melan-A+ tumor cells. Furthermore, ex vivo analysis of the metastatic LN revealed the presence of functionally active Melan-A-specific CTL in a frequency comparable to the peripheral blood. However, the autologous Melan-A specific CD8+ T cells as well as an allogeneic HLA-A2-matched Melan-A specific CTL line failed to kill the patient’s tumor cells isolated from metastatic LN. Loading of the tumor with the Melan-A peptide completely reversed the resistance to killing, suggesting impaired antigen presentation or presentation. Downregulation of HLA-class I or mutation of the antigen peptide as immune escape mechanisms could be excluded. Overall, we report here an extremely high frequency of circulating and LN infiltrating TAA-specific CTL without any functional impairment in a patient with progressive melanoma. Patient’s tumor cells were resistant to killing, but the most frequently described immune escape mechanisms did not occur in this patient. Ongoing studies are aimed at alterations in the processing machinery of the tumor cells. This case highlights, that immunotherapeutic approaches should not only focus on the induction of a robust anti-tumor immune response, but also to target tumor immune escape mechanisms.

P1037
Influence of long-term bisphosphonate therapy on peripheral gamma-delta T-cells in patients with metastatic breast cancer
Berlin, Nuremberg, D.

Background: Bisphosphonates (BP) are widely used in the treatment of osteolytic bone metastases. Further to inhibition of osteoclasts, several additional effector mechanisms have been described. This includes induction of gamma-delta(Tδ)-cell proliferation with consecutive higher cytotoxic effect on multiple myeloma cells in vitro. In addition, a short-term increase of gd-T(δ)-cells in peripheral blood of patients after pamidronate-infusion was observed. The purpose of this study was to evaluate the effects of medium- or long-term treatment with bisphosphonates on gamma-delta T-cells . Patients and methods: Patients with bone metastases of breast cancer were treated with BP in the scope of a clinical study. Those pamidronate either continued (group A, n = 3) or received zoledronate (group B, n = 5). Patients without previous BP treatment received zoledronate (group C, n = 3). Blood was taken at the beginning and every 3 weeks over a period of 18 weeks. FACS flow analysis was performed to determine gamma-delta(Tδ)-cells. Activation of T-cells and a count of lymphocyte subgroups (CD4+ T-cells, CD8+ T-cells, NK-cells, B-cells) was performed. Results: gd-Tδ-cells ranged from 0.4 to 3.87% of peripheral T-cells. Median baseline values were 1.03% for pre-treated patients and 2.64% for bisphosphonate-naive patients at the beginning. After 18 weeks, the percentage increased to 1.32% in group A.
and 1.13% in group B. In group C, the fraction of gd-T-cells decreased to 2.16% after 18 weeks treatment. Activated (CD69+) T-cells changed in group C from 1.67% before treatment to 2.41% at the end of the observation period, and in pretreated patients from 2.06% to 1.75% (group A) and 1.41% (group B). There were no relevant changes in peripheral NK-cells, B-cells, CD4+ or CD8+ T-cells during the observation period.

Conclusion: Treatment with zoledronate was not associated with an increase of peripheral blood gd-T-cells. BP-pretreated patients had lower initial rates of gd-T-cells than BP-naive patients. The proportion of gd-T-cells in patients without BP pre-treatment decreased during the first 8 weeks of treatment. The significance of these findings remains unclear since the number of patients is too small for definite conclusions.

**P1038**

**Aberrent subcellular retinoid x receptor-alpha-expression in renal cell carcinoma: First observation and predictive impact**

J. Atzpodien, N. Buentig, S. Stoerkel, E. Richter, I. Dallmann, M. Reitz Münster, Hannover, Wuppertal, Bonn, D

**Purpose:** Retinoid receptors are nuclear transcription factors which mediate the effects of retinoids on gene expression. In the present study, we analyzed the expression of retinoid receptor-alpha (RXR-alpha) in renal cell carcinoma (RCC) and assessed the influence of different expression patterns on the prognosis of patients with RCC.

**Methods:** Detection of RXR-alpha was performed on tumor specimens from 63 patients with primary RCC using immunohistochemical techniques. For evaluation of the immunostaining results we developed a new cell counting system based on the subcellular distribution of immunoreactivity. The impact of the subcellular distribution of RXR-alpha on the prognosis of patients with RCC was analyzed statistically among other clinicopathologic factors. The primary end point was survival.

**Results:** In 34 RCC samples (54.0%) RXR-alpha was detected predominantly in the cytoplasm, in 25 RCC specimens (39.7%) displayed an aberrant subcellular distribution pattern with a predominantly cytoplasmic staining with nuclear sparing in 15 specimens (23.8%), and a combined nuclear and cytoplasmic staining in 10 specimens (15.9%). Very faint to undetectable subcellular immunoreactivity was noted in 1 specimen (1.5%). Very faint to undetectable nuclear staining was noted in 1 specimen (1.5%). Very faint to undetectable nuclear staining was noted in 1 specimen (1.5%).

**Conclusion:** Our study indicated that the subcellular intratumoral distribution pattern of RXR-alpha in RCC could independently predict survival of RCC patients. However, the exact mechanisms underlying the aberrant compartmentalization and the functions of RXR-alpha in RCC remain to be determined.

**P1039**

**Therapeutic synergism of IFNg and MemTNF targeted to MN/CAIX/G250 expressing tumors**

N. Adrian, S. Bauer, C. Viebke, N. Fadle, N. Jordan, B. Williamson, M. Freundschuh, C. Renner

Homburg, D; New York, USA

**Conclusion:** Membrane TNF (memTNF) and IFNg are promising agents in immunotherapy of advanced solid tumors. We aimed to combine the antiangiogenic effects of TNF with the antitumor effects of IFNg.

**Purpose:** We attempted to induce a therapeutic synergism between IFNg and memTNF in vivo in xenografts of MN/CAIX/G250 expressing tumors.

**Methods:** Anti-CAIX scFv was applied intravenously in BALB/c nu/nu mice simultaneously xenografted with FAP-positive or negative tumors. Anti-MN/CAIX/G250 scFv was applied intravenously in BALB/c nu/nu mice simultaneously xenografted with FAP-positive or negative tumors. Anti-CAIX scFv was applied intravenously in BALB/c nu/nu mice simultaneously xenografted with FAP-positive or negative tumors.

**Results:** Anti-CAIX scFv was applied intravenously in BALB/c nu/nu mice simultaneously xenografted with FAP-positive or negative tumors. Anti-MN/CAIX/G250 scFv was applied intravenously in BALB/c nu/nu mice simultaneously xenografted with FAP-positive or negative tumors. Anti-CAIX scFv was applied intravenously in BALB/c nu/nu mice simultaneously xenografted with FAP-positive or negative tumors.

**Conclusion:** Our study indicated that the subcellular intratumoral distribution pattern of RXR-alpha in RCC could independently predict survival of RCC patients. However, the exact mechanisms underlying the aberrant compartmentalization and the functions of RXR-alpha in RCC remain to be determined.

**P1040**

**PMN recruitment by sc/Fv-IL-8 variants for serial cancer immunotherapy**

N. Adrian, S. Bauer, U. Siebenborn, N. Fadle, N. Jordan, M. Freundschuh, C. Renner

Homburg, D

**Conclusion:** Polymorphonuclear neutrophils (PMNs) are the most abundant circulating blood leucocytes. They are potent effectors of inflammation and their attempts to respond to cancer are suggested by their systemic, regional and intratumoral activation. The natural tumor-PMN balance can be markedly altered by the release of chemokines in the tumor microenvironment.

**Purpose:** This effect was shown by immunohistochemical analyses of FAP (fibroblast activation protein) expressing tumors following intravenous application of our anti-FAP-scFv TNF dimer. Sialic acid enrichment of TNF activity was due to recruitment of CD11b+ leucocytes. However, TNF-induced chemoattraction and extravasation of PMNs from blood into the tumor is a multistep process mainly mediated by IL-8 (interleukin 8). With the aim to amplify the TNF-induced IL-8-mediated chemoattractant response, we generated fusion proteins by genetic engineering. We combiined a human anti-FAP-scFv fragment with human IL-8 or its N-termi-nally truncated IL-83–72 amino acid form. The latter has been reported to develop a 2 to 5 fold higher potency than the full length protein. Both IL-8 analogs were N-terminally fused to sc/Fv. Fusion proteins were tran-siently expressed in suspension adapted HEK 293 cells reaching protein levels up to 40% of total cell protein. Both variants showed concentration dependent activities. Due to the dramatic differences concerning their chemoattractant potency in vitro, we favored the full length chemokine for further in-vivo investigations. Anti-FAp-sc/Fv-IL-8 was applied intravenously in BALB/c nu/nu mice simultaneously xenografted with FAP-positive or negative tumors. Extended chemoattraction of PMNs was only detectable in FAP expressing tissue. The fact, that PMNs are likewise producers and primary targets for IL-8 raises the chance to induce a therapeutic synergism. Serial application of sc/Fv-IL-8 and IgG1-TNF dimer fusion proteins should effect a paracrine activation-loop to increase the number and activation status of PMNs in the targeted stroma leading to tumor destruction.
Removab is a trifunctional bispecific antibody which can bridge CD3+ T cells and EpCAM+ tumor cells, and binds with its Fc fragment to antigen presenting cells. To explore a new approach for the treatment of patients with carcinoma of the upper aerodigestive tract, we investigated whether Removab can induce specific cellular responses to the EpCAM+ carcinoma cell line BHY. Particular emphasis was put on opsonization of mononuclear cells (MNC) with respect to safe clinical application. Tumor cells and allogeneic MNC of healthy volunteers were incubated with or without Removab. In a third group, MNC were sonoposized with Removab and washed before incubation with tumor cells. Inverse microscopy, ELISPOT, flow cytometry and choriolaanitos membrane (CAM) experiment were performed. In comparison with MNC alone, both coincubation and opsonization with Removab resulted in an EpCam specific manner in significant an activation of CD8+ antigen presenting cells, secretion of IFNγ by PBMN, c) granzyme B mediated lysis of targeted BHY cells by CD8+ T cells, and d) lysis of tumor cells on the CAM. In the opsonization experiments, the secretion of TNFα and IL-2 by opsonized MNC was significantly reduced after 24 hrs. Washed opsonized MNC maintained their lytic activity on the CAM. We conclude that Removab is an appropriate antibody for the enhancement of efficient T cell responses against EpCam tumor cells by opsonization of MNC, thus preventing patients from the risk of capillary leak syndrome caused by a cytokine storm.

**Poster session: Tumor pathophysiology**

**P1041 Opsonization with a trifunctional bispecific antibody results in efficient lysis of EpCam+ carcinoma cells for a safe clinical application**


Ulm, Munich, D

Cyclin A1 is highly expressed in acute myeloid leukemic blasts. Its overexpression contributes to leukemogenesis in mice. The aim of our project was to identify novel interaction partners of Cyclin A1. In a yeast-triple-hybrid-system, we cloned FoCA1 (Friend of cyclin A1, 221 aa) as a novel interacting protein of Cyclin A1. FoCA1 bound to Cyclin A1 in vivo and in vitro, was phosphorylated by the Cyclin A1/CDK2 complex at S23, T167 and S176 and localized to the nucleus. Neither human FoCA1 nor its murine homolog showed sequence homology to any other known protein. Using real-time quantitative RT-PCR, highest levels of FoCA1 expression were found in the testsis similar to the tissue-specific expression pattern of Cyclin A1. Intermediate expression was observed in ovary, pancreas, spleen, and lung. We also analyzed FoCA1 mRNA expression in 304 tumor samples including leukemia and solid tumors. In all tumor samples, FoCA1 expression was notably absent or detected at very low levels. Direct comparison of tumor samples and normal lung, testis and ovary tissue showed significantly lower FoCA1 expression in the tumor samples compared to normal tissue. NIH3T3 fibroblasts were used to analyze FoCA1 expression throughout the cell cycle. Arresting NIH3T3 cells in G1 phase by serum starvation or aphidicolin induced FoCA1 expression time- and dose-dependently. After release of the cell cycle arrest, FoCA1 expression decreased rapidly. In IL-3 dependent 32D cells, IL-3 depletion also induced FoCA1 expression. Oncogenic FLT3 internal tandem duplication mutations suppressed FoCA1 induction in 32D cells, D65476, a specific RTK inhibitor of FLT3-ITD, restored the FoCA1 induction after growth factor depletion.
Inducible expression of FoCA1 in stably transfected HeLa cells significantly decreased proliferation in [3H]thymidine incorporation assays. FoCA1 also inhibited serum-independent growth of Ras-transfected murine embryonic fibroblasts. In colony formation assays with transfected solid tumor cell lines or myeloid progenitor cells, FoCA1 expression reduced colony growth by 50%. Taken together, FoCA1 expression is suppressed by growth factors and oncogenic receptor tyrosine kinase expression. FoCA1 levels are extremely low in several human cancers. FoCA1 inhibits cell cycle progression, proliferation and colony formation of tumor cell lines and myeloid progenitors. These data suggest a growth suppressive function for the novel Cyclin A1-interacting protein FoCA1.

P1045
Anaphase promoting complex (APC)-dependent cyclin proteolysis and genomic instability
R. Wäsch, D. Engelbert
Freiburg, D

Genomic instability is a hallmark of cancer cells. Cell proliferation is under tight control to ensure accurate DNA replication and chromosome segregation. Ubiquitin-dependent proteolysis of cyclins is thereby central to the regulation of the cell cycle. The Anaphase Promoting Complex (APC) is a specific ubiquitin-ligase and essential for chromosome segregation and exit from mitosis. It is activated by the regulatory subunits Cdc20 (APCCdc20) and Cdh1 (APCCdh1) to target Securin and B-Cyclins for proteasomal degradation. By using the budding yeast model system, we demonstrated recently in contrast to previous proposals, that spindle disassembly and cell division occur without significant APC-Cdc20-mediated degradation of the S-phase cyclin Cln5. We find instead that APC-dependent proteolysis of the major mitotic cyclin Cln2 is essential for mitotic exit. Dereguulation of Cln5, however, leads to a plasmid loss phenotype indicating problems with loading of replication origins and genomic instability (Nature 2002, 418:556–62). The APC is highly conserved and budding yeast is an important eukaryotic model system to identify new mechanisms of cell cycle control. By using retroviral transfer of small interfering RNA (siRNA) to knockdown the involved cell cycle regulators, we are currently investigating the described model in normal and malignant human cells. An update of available data will be presented.

P1046
The DNA damage-induced decrease of Bcl-2 is secondary to the activation of apoptotic effector caspases
J. Milosevic, S. Hoffarth, C. Huber, M. Schuler
Mainz, D

Apoptosis induced by DNA damaging agents or radiation mainly proceeds through death receptor-independent caspase activation. The release of mitochondrial apoptogenic proteins, such as Cytochrome c, into the cytoplasm leading to Apaf1-dependent activation of Caspase-9 is a key event in this pathway. The permeability of the mitochondrial outer membrane is regulated by the various pro- and anti-apoptotic Bcl-2 family proteins, and it is thought that DNA damage triggers apoptosis through the downregulation of anti-apoptotic Bcl-2. Using murine embryonic fibroblasts (MEF) deficient and proficient in Apaf1 we show that DNA damaging agents and radiation lead to a decline in Bcl-2 protein only in wild type (wt) MEF, but not in Apaf1-deficient MEF, which are defective in the activation of effector caspases and apoptosis. In contrast, the induction of pro-apoptotic Noxa, the activation of Bax, the cytoplasmic release of Cytochrome c, as well as a drop of the mitochondrial transmembrane potential are equally observed in wt and Apaf1-deficient MEF following DNA damage. Moreover, the loss of Bcl-2 protein occurring in wt MEF can be prevented by caspase inhibition. Hence, the activation of pro-apoptotic Bcl-2 family proteins rather than the downregulation of anti-apoptotic Bcl-2 mediates the primary signal in the DNA damage-induced release of mitochondrial apoptogenic proteins in MEF.
most to the same extent as BCL-XL did, whereas the mutants SIRT2(Y187H) or SIRT2(P182L) failed to exhibit such an activity. Studies are underway to further characterize the anti-apoptotic mechanism of SIRT2. In summary, SIRT2 might contribute to proliferative survival of cancer cells by preventing stress-induced apoptosis. Expression of SIRT2 mutants significantly reduces the growth of cancer cells, most likely by dominant competition of endogenous SIRT2. Hence, targeting SIRT2 function could be a novel strategy for the treatment of cancer.

P1049 Telomerase inhibition leads to telomere dysfunction and consecutive growth arrest of human lung cancer cells

U.M. Martens, S. Zimmermann, C.F. Waller, M. Pantic  
Freiburg, D

Telomerase, the ribonucleoprotein enzyme maintaining the telomeres of eukaryotic chromosomes, is active in most human cancers but, with few exceptions, not in normal human somatic tissues. Telomere maintenance has been attributed to unlimited growth potential of tumor cells and inhibition of telomerase may lead to telomere shortening and cessation of unrestricted proliferation. Here, we investigated the effects of telomerase inhibition in the non-small cell lung cancer cell line NCI-H460 using a genetic approach. Therefore, cells were transduced with a dominant-negative mutant of hTERT, the catalytic component of telomerase. As a consequence, endogenous telomerase activity was completely abolished in five clones which were selected based on pyromycin resistance. Furthermore, rapid telomere shortening was observed leading to proliferation arrest with hallmarks of senescence. Although overall telomere length was similar between different clones as measured by the Q-FISH technology, striking differences were found in telomere length of individual chromosomes. In particular, cells from telomerase negative clones possessed chromosome ends lacking detectable telomere signals and showed chromosomal abnormalities such as end-to-end fusions. Interestingly, the level of individual telomere loss was inversely correlated with the remaining life-span in vitro which suggests that dysfunction of individual telomeres is the critical event that determines the proliferation capacity of cells. Thus, pharmacological strategies which inhibit of telomerase should take into account that not overall telomere shortening but induction of telomere dysfunction (on the level of individual chromosomes) appears to be the crucial target for telomere based therapeutics.

P1050 Induction of cell death by the BH3-only Bcl-2 homolog Nbk/Bik is mediated by an entirely Bax-dependent mitochondrial pathway in human carcinoma

B. Giliissen, V. Graupner, F. Essmann, K. Schulze-Osthoff, B. Dörken, P. Daniel  
Berlin, Düsseldorf, D

Nbk/Bik (Natural born killer/Bcl-2 interacting killer) is a tissue-specific BH3-only protein whose molecular function is still largely unknown. To investigate the mechanism of Nbk action, we established a single-vector adenoviral system based on the Tet-off conditional expression of Nbk. Upon Nbk expression only Bax-positive, but not Bax-deficient cells were found to undergo apoptosis. Interestingly, Nbk failed to induce apoptosis in the absence of Bax, even despite expression of the related molecule Bak. Re-expression of Bax restored the sensitivity to Nbk. Similarly, Bax wild-type HCT116 cells were highly susceptible, whereas HCT116 Bax knock-out cells remained resistant to Nbk-induced apoptosis. In Bax-positive cells, Nbk induced a conformational switch in the Bax N-terminus coinciding with cytochrome c release, mitochondrial permeability transition and caspase-9 processing. Immunoprecipitation studies revealed that Nbk interacts with Bcl-xL, and Bcl-2 but not with Bax. Since in addition Nbk did not localize to the mitochondria, our data suggest a model in which Nbk acts as an indirect killer to trigger Bax-dependent apoptosis, whereas Bak is not sufficient to confer sensitivity to Nbk.

P1051 Notch pathway elements in ovarian tumors

O.J. Hopfer, D. Zwahlen, D. Fink, H. Altermatt, M. Fey, S. Aebi  
Berlin, D; Berne, CH

The notch pathway is a key regulator of differentiation. We investigated its role in epithelial ovarian tumors. 32 ovarian tumor samples (17 adenocarcinomas, 3 borderline tumors, 12 adenomas), 2 ovarian cancer cell lines (A2780, OVCAR-3) and one human ovarian surface epithelial cell line (IOSE 144) were used for analysis. The mRNA expression of the major elements of the notch pathway was assessed by RT-PCR. Notch1 was measured by real-time PCR analysis. Protein expression of Notch1-EC (extracellular domain), Notch1-IC (intracellular domain), and HEI1 was investigated by immunoblot. A2780 ovarian adenocarcinoma cells were stably transfected with activated Notch1-IC. Phenotyping was done by colony forming and proliferation assays. Jagged2, Delta1, Manic Fringe, and TSL1 were expressed more frequently in adenocarcinomas than adenomas. Deltex, Mastermind and Radical Fringe were expressed with higher frequency in adenomas. Quantitative PCR (relative quantification) showed a tendency towards decreased Notch1 expression by ovarian adenocarcinomas (median: 0.35) in comparison with ovarian adenomas (median: 0.705). Notch1-EC and Notch1-IC protein was present in all ovarian cancers, borderline tumors, and adenomas at similar levels. A strong expression was found in A2780 and in OVCAR-3, but a lower expression in IOSE 144. HEI1 protein was expressed at a high level in 18 of 19 and borderline tumors; low expression or lack thereof was observed in all of 10 ovarian adenomas. In all ovarian cell lines HEI1 protein expression was very high. After stable transfection with activated Notch1-IC A2780 ovarian carcinoma cells showed a clear proliferative advantage over mock transfected cells. Similarly, Notch1-IC transfected cells formed colonies more efficiently than the control cells, even when treated with cisplatin. In conclusion, we showed that many notch pathway elements are expressed in ovarian epithelial tumors. Carcinomas showed higher HEI1 protein levels than adenomas indicating a stronger Notch pathway activation. Transfection experiments indicate a possible role for the Notch pathway in ovarian tumor formation. The Notch pathway could be a promising target for the development of novel therapies of ovarian cancer.

P1052 Genomic hypomethylation potentiates hypoxia-induced gene expression

M. Koslowski, Ö. Türeci, C. Bell, U. Sahin  
Mainz, D

Cancer cells have adapted several pathways, allowing tumors to survive and even grow under hypoxic conditions. The key transcription factor that is induced by hypoxia is Hypoxia-inducible factor-1alpha (HIF-1alpha). Pathways that are regulated by hypoxia include angiogenesis, glycolysis, growth-factor signalling, immortalization, genetic instability, tissue invasion and metastasis, apoptosis and pH regulation. Most of the hypoxia-induced pathways promote tumor growth and tumor hypoxia is associated with poor prognosis and resistance to radiation therapy. In cancer cells the delicate organization of methylation and chromatin states that regulates the normal cellular homeostasis of gene expression patterns becomes unrecognizable, with global hypomethylation and distinct hypermethylation of CpG rich islands being the hallmarks of these epigenetic changes. Here, for the first time we studied the effects of global hypomethylation on hypoxia-induced gene expression. Activated PBMCs were treated with different concentrations of demethylating agent 5-aza-2’-deoxycytidine (DAC) and subsequently were exposed to Hypoxia (1% O2). Expression of HIF-1alpha inducible genes (VEGF, GLUT-1, LDHA, ALDOA, TGBF-3) was quantitated by real-time RT-PCR and expression levels were compared in DAC treated and untreated cells held under normoxic conditions to DAC treated and untreated cells exposed to hypoxia. Expression of all genes was induced under hypoxia ranging from 3-fold induction for TGBF-3 to 18-fold induction for GLUT1. Effects of DAC on gene expression in cells cultured under normoxic conditions were only marginal. However, when exposed to hypoxia expression of all genes tested was dramatically increased in the DAC treated cells compared to the untreated cells. Levels of potentiation ranged from 4-fold higher induction for VEGF to 12-fold higher induction for ALDOA. Additionally we analyzed induction of gene expression in cells exposed to higher partial O2 pressures (10% O2). Most interestingly expression of the hypoxia-in-
ducible genes was highly induced only in the DAC treated cells, untreated cells showed no response. Ongoing studies will have to reveal if genomic hypomethylation in tumor cells also potentiates hypoxia-inducible gene expression, facilitating the tumor’s adaption to hypoxic conditions even under O2 pressures normally not sufficient to induce HIF-1alpha dependent gene expression.

P1053

Allele specific expression of Insulin-like growth factor 2 in myelodysplastic syndrome and normal hematopoietic cells: Reversal of a principle

W.-K. Hofmann, S. Takeuchi, D. Hoelzer, H.P. Koeffler

The human insulin-like growth factor 2 (IGF2) gene was thought to be imprinted and therefore to be expressed only from the paternal allele in normal tissue. Loss of imprinting (LOI) of the IGF2 gene was initially described in Wilms’ tumors. A number of studies in a broad variety of human tumors demonstrated LOI in 25–100% of the tumor samples, resulting in the investigators concluding that the bi-allelic expression of IGF2 is important for abnormal cell growth. Initially, we analyzed the imprinting status of IGF2 in bone marrow cells from 49 patients with myelodysplastic syndromes (MDS) utilizing the Apa I polymorphism of IGF2. Thirteen bone marrow and 14 peripheral blood samples from normal individuals served as controls. We utilized normal peripheral blood T-lymphocytes to examine the relationship between genomic imprinting and cell proliferation. Expression of IGF2 was quantified by real-time PCR and proliferation of T-cells was measured by 3H-Thymidine incorporation. In SK-N-MC neuroblastoma cells an ICR (imprinting control region) was analyzed by subcloning and sequencing of genomic DNA after sodium-bisulfite modification. Among 24 patients who were heterozygous for IGF2, LOI occurred in 22 cases (92%). Surprisingly, LOI of IGF2 occurred in the normal bone marrow cells, but in normal peripheral blood cells showed retention of imprinting (ROI). Unstimulated normal T-cells showed ROI. After 24 hours exposure to PHA, these cells changed their IGF2 imprinting status from ROI to LOI which was persistent for at least 72 hours. Concomitantly, their IGF2 RNA levels increased up to 6-fold and their proliferation increased 10- to 20-fold. In contrast, normal T-cells not stimulated with PHA did not develop LOI of IGF2, had negligible levels of IGF2 RNA and did not increase their proliferation. In unstimulated T-cells, the CpG islands of the ICR were completely methylated on one allele and nearly completely unmethylated on the other allele. After PHA stimulation, the CpG islands at this site became completely methylated on both alleles preventing the binding of transcriptional suppressors, which is in agreement with the expression of IGF2 from both alleles. We conclude that LOI of IGF2 is strongly associated with cell proliferation of normal hematopoietic cells and is not limited to cancer cells.

P1054

Characterization of the mouse germ cell nuclear factor gene promoter

E. Goekkurt, U. Sueens, M. Hentschke, I. Hermans-Borgmeyer, U. Borgmeyer

Hamburg, D

The germ cell nuclear factor (GCNF), a member of the nuclear receptor superfamily, is highly expressed during spermatogenesis, oogenesis, neuronal embryonic differentiation and in teratocarcinoma derived cell lines, such as the human N-Tera-2 (NT2/D1) and mouse P19 cells. GCNF binds to the direct repeat of the sequence AGGTCA (DR0) as a homodimer and functions as a repressor of transcription. Treatment of NT2/D1 and P19 cells with all-trans-retinoic acid (ATRA) is accompanied by transient up-regulation of the GCNF transcript. To study the transcriptional regulation of GCNF, 4 kb of the putative promoter region of mouse GCNF was cloned and three transcription start sites were determined by RNase protection and 5′–RACE. The proximal promoter activity in a luciferase reporter gene assay when transfected transiently in NT2/D1 and P19 cells. Addition of ATRA to the cell medium resulted in transient up-regulation of promoter activity corresponding to previous findings in northern blot studies. Using deletion constructs we identified a putative retinoic acid response element (RARE) within the proximal promoter possibly responsible for this up-regulation by retinoic acid. Further transcriptional binding sites, for example for Sox5 and 6, AP-2 or Sp1 were identified by using promoter prediction programs. As it is known that teratocarcinoma derived cell lines can undergo differentiation when treated with ATRA, we conclude that GCNF may play an important role during retinoic acid induced differentiation. The isolation of the GCNF promoter provides an important tool for the study of both the GCNF gene expression as well as the role of GCNF in germ cell cancer.

P1055

Characterization of a new BAG-1 variant as RP1 binding protein with anti-apoptotic activity


Homburg, D; Zurich, CH

The MAPRE protein family represents a highly conserved group of proteins, present in yeast through humans, that localize preferentially to the plus end of microtubules, both in the nucleus and cytoplasm. All MAPRE family members (EB1, RPI, EB2) are characterized by their capability to bind the C-terminus of the adenomatous polyposis coli (APC) protein and tubulin in order to stabilize microtubules. Beside the interaction of EB1/RP1 with APC and tubulin no other binding partners are known today. Since the RP1 gene product was identified initially in activated T-cells we set out used to search for new interacting molecules in a yeast-two hybrid system. We isolated a clone carrying a CDA fragment that turned out to be a 34 aminoacid C-terminal truncated variant of the anti-apoptotic BAG-1/Hap46 protein which we named BAG-1 variant (BAG-1v). In the original BAG-1/Hap46 protein, the C-terminal domain is responsible for the binding to Bcl-2 and Bcl-hs70. This interaction of BAG-1 and members of the HSP family is believed to be the reason for its anti-apoptotic activity. Although our BAG-1v protein showed no interaction with Bcl-2 or hsp70 because of C-terminal truncation, BAG-1v protein was perfectly able to confer resistance to apoptosis in relevant cell systems. Sub-cellular distribution analysis revealed that the BAG-1v protein localized homogeneously to the cytoplasm and shuttles into the nucleus in response to stress, a process which could be blocked by RPI over-expression. In conclusion, our findings provide evidence that the APC interacting protein RP1 binds to a new BAG-1 protein with anti-apoptotic activity. Further studies have to elucidate in more detail the role: APC, RP1 and BAG-1/BAG-1v might play in the process of tumorigenesis.

P1056

Cloning and chromosomal characterization of the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 gene (PFKFB3)

U. Mahlknecht, H. Dieter, R. Bucala

Frankfurt, D; New Haven, USA

PFKFB3 (6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase) is a bifunctional enzyme that regulates the steady-state concentration of fructose-2,6-bisphosphate, which is a potent activator of the key regulatory enzyme of glycolysis, phosphofructokinase. PFKFB3 (PFK2) is one of four tissue-specific PFKFB isozymes that have been identified to date. PFKFB3 also has been implicated in the high glycolytic rate of cancer cells that occurs despite adequate oxygen, a phenomenon known as the Warburg effect. We have isolated and characterized the human PFKFB3 genomic sequence, which spans a region of 32.5 kb and which has a single chromosomal locus. Determination of the exon-intron splice junctions established that PFKFB3 is encoded by 19 exons of which only 15 are normally expressed. Exon sizes range between 23 and 208 bp, the largest intron is 10,286 bp long. The full-length human PFKFB3 open reading frame is 4,675 bp long and encodes a 590 aa protein with a predicted molecular weight of 66.9 kDa and an isoelectric point of 8.64. Fluorescence in situ hybridization analysis localized the human PFKFB3 gene to chromosomal locus 10p15.3-p15.2, and its locus is 3 million bp centromeric to PFKP, the platelet-type phosphofructokinase. PFKFB3 has been shown to be abundantly expressed in human tumors and its expression linked to long-standing observations concerning the apparent coupling of enhanced glycolysis and cell proliferation.
Alternations of the tumor suppressor p53 play a central role in onco genesis. p53 mediates its tumor-suppressive activity by transactivation of downstream target genes. While many target genes have been identified to date, the time kinetics of p53-DNA interactions are still unknown. To ad dress this issue we investigated the kinetics of p53 binding to the p21WAF1, MDM2, BAX and PIG3 promoters in vivo using a novel quantitative assay. In Huto 80 colon carcinoma cells we induced p53 by UV irradiation and performed chromatim immunoprecipitation with anti-p53 antibody. By real time PCR assay we quantified the amount of precipitated promoter DNA. For all of the analyzed target genes we found an increase in p53 promoter binding following UV irradiation. While p21WAF1 promoter levels started to increase 12 hours after irradiation and peaked six-fold after 30 hours, MDM2 promoter levels increased after eight hours and peaked 42-fold at 24 hours. At eight hours, p53 binding to the BAX promoter was still weak, but increased up to 10-fold at 24 hours. PIG3 promoter levels showed a biphasic increase after eight and 24 hours. Taken together, these results demonstrate distinct kinetics of p53 promoter binding dependent on the target gene promoters. The timed induction of p53 target genes due to genotoxic stress is likely to play a pivotal role for the divergent functions of p53 in cell cycle arrest, apoptosis and tumor suppression.

Tumor immunology has received a large impetus from the identification of tumor-associated antigens. Among them, a monoclonal antibody, 22–1–1, was instrumental in defining a novel tumor-associated antigen that was termed ‘receptor binding cancer antigen expressed on SiSo cells’ (RCAS1). RCAS1 was proposed to induce growth arrest and apoptosis on activated immune cells, mediated by a putative death receptor. Structurally, RCAS1 was predicted to exist as a type II transmembrane (TM) protein and in a soluble form. Here, we analyzed occurrence, membrane topology and subcellular localization of the RCAS1-encoded gene product. RCAS1 was shown to be a ubiquitously expressed type III TM protein with a Golgi-predominant localization. Monoclonal antibody 22–1–1 failed to recognize RCAS1, as demonstrated by confocal microscopy. Instead, we showed that the cognate 22–1–1 epitope is identical with the tumor-associated O-linked glycans Tn. Overexpression of RCAS1 in cell lines that are negative for 22–1–1 surface staining led to the generation of Tn and the closely related TF antigen, thus providing a functional link to the generation of the 22–1–1 epitope. We suggest that RCAS1 modulates surface expression of tumor-associated, normally cryptic O-linked glycans structures and contributes indirectly to the antigenicity of tumor cells.

Telomerase is an RNA-directed DNA polymerase, composed of RNA and protein subunits, that replicates the telomeric repeats (TTAGGG) of linear eukaryotic chromosomes. Within the telomerase metabolism telomere binding proteins are known of being over- or underexpressed in dependence on proliferative activity. Here we present an approach to identify telomere binding proteins in the human Hodgkin lymphoma cell line L428. Material and methods: Nuclear proteins were extracted from human Hodgkin lymphoma cell line L428 using sequential cell lysis with Triton-X-100 and 5 M NaCl. In the Electrophoretic Mobility Shift Assay (EMSA) the nuclear extracts were screened for specific telomere binding proteins with single stranded telomeric DNA repeats and their competition. The crude nuclear extracts were purified using magnetic DNA affinity separation with superparamagnetic spherical particles linked to telomere mimicry. After elution from the telomeric repeats the specific telomere binding proteins were separated with SDS-PAGE technique and transferred to PVDF membrane performing Semi-dry blotting. A specific detection of the telomere binding proteins was achieved through incubation of the membrane with specific telomeric DNA probes and their visualisation with immunoperoxidase techniques. The proteins were isolated, digested and analysed by Edman degradation vs. Nano-Electrospray Ionisation Mass Spectrometry (Nano-ESI-MS). Results: In our approach we have isolated five proteins from the nuclear matrix of the Hodgkin cell line L428. Analysing the sequences of single peptides and mass spectral data hnRNPU C1/C2 (33 kDa), hnRNPA2/B1 (37 kDa) and hnRNPA1 (39 kDa) were high significantly identified. All of these single strand binding proteins belong to the family of heterogenous ribonucleoproteins (hnRNPs) and can be characterized in our approach as telomere binding.

Hodgkin’s Lymphoma: Potential targets for detection of minimal residual disease: P1059

The Golgi protein RCAS1 controls cell surface expression of tumor-associated O-linked glycan antigens

Berlin, D

Background: Although the immunocytological evaluation of cerebral spinal fluid (IC) is considered to be a very specific method for the diagnosis of leptomeningeal metastases (LM), the relatively low sensitivity remains a diagnostic dilemma. Gadolinium-enhanced magnetic resonance imaging (MRI) has been reported to be sensitive for the presence of meningeal pathology but non-specific to the disease entity. The objective of the present study was to retrospectively compare the results of IC and MRI of the head and/or spine in order to determine the correlation of both methods.

Patients and methods: 429 CSF samples and 283 MRI scans of 234 cancer patients were investigated. Of this population, 112 individuals with B-Non-Hodgkin-lymphoma (67), B-lineage acute lymphoblastic leukemia (13), acute myelogenous leukemia (18) or solid tumors (14), with at least one definitive IC and MRI result within 3 weeks, were included. The results of MRI and IC were compared with respect to the underlying malignancy, reason for the examination (clinical suspicion: CS versus routine examination: RE) and results. Results: IC revealed malignant cells more frequently in patients with CS than in hematologic LM (71.4% versus 35.7%), while MRI disclosed the radiological signs of meningeal involvement more often in solid LM than in hematologic LM (42.4% versus 30.9%). Discordant results (n = 37, 33.0%) were reproducible during the course of the disease. Logistic regression analysis disclosed a highly significant accumulation of positive IC results in B-lineage ALL patients in case of CS (p-value: 0.0001). Conversely, in patients with CS for LM and solid tumors MRI was more likely to be positive (p-value: 0.0099), which corresponds to findings of other study groups indicating that abnormal neuroimaging was more often present in solid than in hematologic LM. Conclusion: IC is of particular value in the diagnosis of LM due to hematologic malignancies, especially in B-lineage ALL. Conversely, whenever positive neuroimaging results in patients with negative IC lead to the conclusion that MRI is an important addition to IC and provides strong support in the diagnosis of solid LM in patients with clinical suspicion for meningeal involvement.
Aneuploidy is considered to play an important role in the pathogenesis of malignancies. We were interested whether abnormalities of the sister chromatid separation regulator and proto-oncogene hSecurin occurred in myeloid leukemias, and whether such abnormalities correlate with aneuploidy. Expression of hSecurin was assessed by real-time quantitative PCR in samples from 102 patients with acute myeloid leukemia (AML, n = 70), chronic myeloid leukemia (CML) in chronic phase (CP, n = 20) or blast phase (BP, n = 12), and granulocytes as well as mononuclear cells (MNCs) from healthy donors (n = 21). Median hSecurin expression in AML with normal karyotypes was not significantly different from AML showing aneuploidy or from AML with only structural chromosome abnormalities. Aberrantly high expression of hSecurin was observed in 5 AML samples. One of these showed multiple numerical and structural chromosome aberrations, 2 had only structural abnormalities, and cytogenetic analysis was not successful in the remaining 2. Expression in CML BP or MNCs and granulocytes from healthy donors was not significantly different, either. However, hSecurin expression in CML CP was significantly increased compared to AML with normal karyotypes (1.82 fold; p = 0.001), CML BP (3.18 fold; p = 0.001), MNCs (3.17 fold; p = 0.001) and granulocytes (2.69 fold; p = 0.001) from healthy donors. Alterations of the coding region of hSecurin were not detected upon direct sequencing. These results do not support a major role of hSecurin in the development of aneuploidy in myeloid leukemias. However, high expression of hSecurin may be of pathogenetic relevance with regard to its potential to stimulate basic fibroblast growth factor and angiogenesis or interactions with p53.

**P1062**

**Differential mRNA expression of different multidrug resistance genes under fractionated irradiation in vitro**

D. Bottte, T. Piegel, K. Heufelder, W. Hinkelbein, U. Keilholz

**Purpose:** Radiotherapy can induce the expression of P-glycoprotein (gene product of the multidrug resistance [MDR] gene), which can lead to a broad cytostatic drug resistance. Kinetics of this expression is unknown, but possibly important for radiochemotherapy protocols. Whether other multidrug resistance mechanisms are also affected by radiotherapy has not been examined yet. We examined kinetics of the mRNA expression of the 3 most important multidrug resistance genes MDR1, MRP (multidrug resistance-associated protein) and LRP (lung resistance-related protein) with different tumor cell lines under fractionated irradiation.

**Methods:** Three human tumor cell lines (colon carcinoma [HT-29], breast cancer [MCF-7] and melanoma [SK-MEL-24]) were irradiated with 36 Gy in 20 fractions. Five fractions per week, with a single dose of 1.8 Gy. During the irradiation period cell fractions were repetitively removed to RNA extraction and cDNA synthesis. For the measurement of mRNA expression quantitative PCR assays with the LightCycler system were developed. Re-combinant plasmids were constructed and used as standards.

**Results:** Compared with the non-irradiated control group the breast cancer cell line showed a stable 10- to 100-fold mRNA overexpression of the gene MRP and LRP after 4 fractions for the entire further experimental period. The melanoma cell line also showed a stable 10- to 100-fold mRNA overexpression of the genes MDR1 and MRP after 4 fractions for the entire further experimental period. The colon carcinoma cell line showed no induction of the 3 MDR genes. **Conclusion:** Fractionated irradiation induces the expression of different multidrug resistance genes in the breast cancer and melanoma cell line, which can lead to a broad cytostatic drug resistance. The possible meaning for radiochemotherapy protocols is functionally examined at present.

**P1063**

**Regulation of WT1 expression by PAX2, PAX8 and GATA1 in human carcinomas**


The Wilms tumor gene (WT1) is expressed in a large proportion of human leukemias and solid tumors. Its expression is connected with poor prognosis, whereas its downregulation inhibits cancer cell proliferation. The pathological overexpression could be caused by malfunction of its regulation as the WT1 gene lacks mutations. The putative regulators of WT1 expression include paired box genes PAX2, PAX8 and GATA1 (GATA binding protein 1 or globin transcription factor 1) proteins. By means of quantitative RT-PCR we show here a clear link between expression of WT1 and its regulators in human carcinomas (26 breast cancer samples and 13 colon cancer samples). The expression of WT1 was present in 85% of samples, PAX2 in 41%, PAX8 in 43%, and GATA1 in 61%. Based on the correlation of WT1 and its putative regulators expression, we defined threshold values of upregulation of PAX2, PAX8, GATA1, and WT1 expression. 17 out of the 19 cases with WT1 upregulation showed either upregulated PAX2, or upregulated PAX8, or upregulated GATA1. In 14 out of the 20 cases with expression of WT1 below the putative activation threshold, the expression of PAX2, PAX8 and GATA1 was below their activation threshold. Taken together this data establishes a clear quantitative link between expression of WT1 and its regulators in human solid tumors, and suggests that, in contrast to AML, WT1 overexpression in solid tumors can almost completely be explained by the upregulation of one of its three physiological regulators.
In general, tumor metastases to the small intestine are rare, and mostly occur in melanoma. Recent findings indicate that chemokines and their receptors are important in determining the metastatic destination of tumor cells. CCR9 has been shown to be the principal chemokine receptor for the thymus expressed chemokine (TECK), a chemokine selectively expressed in small intestinal and thymus. In this study, CCR9 expression was analyzed on melanoma cell lines derived from the small intestine and other tissues. CCR9 expression was found on 8 of 20 melanoma cell lines including all three cell lines established from small intestinal metastases. Functionality of CCR9 expression was demonstrated by receptor downregulation in response to TECK on 4 of the 8 CCR9 positive melanoma cell lines including all 3 cell lines derived from small intestinal metastases. In contrast, CCR9 expression was found in only one of 30 tumor cell lines originating from colorectal, breast, and lung cancer or leukemia. Our results suggest that the expression of functional CCR9 is associated with melanoma metastasis to the small intestine.

**P1066**

Prominin-1/CD133: Is it a new marker to investigate malignant diseases?

M. Florek, M. Haase, G. Ehninger, W.B. Huttner, D. Corbeil
Dresden, D

Prominin-1/CD133 is a first characterized member of a novel family of pentaspan membrane glycoproteins. Recently, we could demonstrate that the human prominin-1 is expressed in adult tissues using a rabbit anti-serum (anti-hE2) that detects both the native and the N-deglycosylated forms of prominin-1, whereas its glycosylation-dependent AC133 epitope is exclusively expressed on embryonic tissue and on hematopoietic stem cells as well as in several malignant hematopoietic diseases. In human adult kidney prominin-1 is detectable at the apical surface of proximal tubules and the parietal layer of Bowman’s capsule of juxtaglomerular nephrons. Because it is known that glycosylation pattern changes during differentiation and after malignant transformation, we evaluate the possibility that the AC133 epitope, defined by the monoclonal antibody (mAb) AC133 (Miltenyi Biotec), can be used as a diagnostic tool for analysing solid cancer tissue. For this purpose, we investigated 12 samples of different types of human kidney cancer by immunohistochemistry using mAb AC133 and anti-hE2. The analysis revealed a differential immunoreactivity of mAb AC133 versus anti-hE2. 4 of 7 tumors thought to be derived from proximal tubules (conventional [clear cell] and papillary carcinoma) displayed the hE2 immunoreactivity of which only one exhibited AC133 immunoreactivity in a region between the tumor and cytomorphological nephrons. Because it is known that glycosylation pattern changes during differentiation and after malignant transformation, we evaluate the possibility that the AC133 epitope, defined by the monoclonal antibody (mAb) AC133 (Miltenyi Biotec), can be used as a diagnostic tool for analysing solid cancer tissue. For this purpose, we investigated 12 samples of different types of human kidney cancer by immunohistochemistry using mAb AC133 and anti-hE2. The analysis revealed a differential immunoreactivity of mAb AC133 versus anti-hE2. 4 of 7 tumors thought to be derived from cortical collecting ducts were negative for mAb AC133 and anti-hE2, but in one case prominin-1 was overexpressed in surrounding normal tissue. 1 of 3 collecting duct carcinomas was positive only with anti-hE2. These preliminary data reveal no clear correlation between the expression of prominin-1 and the acquisition of its AC133 epitope in human kidney cancer in respect of commonly used tumor specification. Analogical varieties in expression of the AC133 epitope were described for leukemia diseases. Whether clinical outcome correlates with expression or downregulation of prominin-1 and its AC133 epitope in adult kidney cancer still needs to be investigated.

**P1068**

Systematic investigation of p53 hotspot mutations in myc-driven lymphoma development

S. Lee, B. Teichmann, B. Dörken, C. A. Schmitt
Berlin, D

Mutations of the tumor suppressor p53 are found at high frequencies in many hematological malignancies. Loss of wild-type p53 function has been linked to poor prognosis, since p53 inactivation may not only contribute to tumor development, but also has been shown to promote chemoresistance in some entities. In a myc-transgenic mouse lymphoma model, targeted deletion of the p53 locus resulted in accelerated tumor onset as well as resistance to therapy. However, biallelic deletions may not accurately reproduce the impact of naturally occurring p53 point mutations which mostly result in expressed but structurally altered proteins. Moreover, some p53 mutations can hetero-tetramerize with wild-type p53 and may impose dominant-negative activities. Different B-cell malignancies such as Burkitt’s lymphoma, B-lineage acute lymphoblastic leukemia, or B-CLL display characteristic patterns of preferential mutations in the p53 DNA binding domain, known as ‘hotspot’ mutations. Although a number of in vitro studies addressed functional properties of p53 mutants individually, no comprehensive approach to elucidate the enhancement/interference of these mutations with the process of spontaneous tumorigenesis has been undertaken yet. Our goal is to systematically evaluate the role of p53 hotspot mutations in cancer biology and therapy in a murine lymphoma model. A panel of hotspot mutations that are most frequently found in various B-cell malignancies were introduced into wild-type p53 cDNA by site directed mutagenesis. Using retroviral gene delivery, mutant p53 constructs were expressed in oncogene-transformed mouse embryo fibroblasts and primary myc-transgenic hematopoietic stem cells, both cell types derived from p53+/+, +/-, and -/- backgrounds. We monitored growth characteristics of infected cells and analyzed the selective potential of individual mutants under oncogenic pressure via co-expression of GFP. Mutant alleles were tested for their ability to function as a dominant-negative or as ‘neo-activities’ that display novel properties not seen in p53 deficient cells, and ultimately whether they may protect from loss of endogenous p53 alleles. Functional characterization of individual, naturally occurring p53 mutants is important for our understanding of molecular pathogeneses and the future development of more rational treatment strategies.

**P1067**

Histone deacetylases: Biological function and their inhibition

U. Mahlknecht, D. Hoelzer
Frankfurt, D

Chromatin structure is gaining increasing attention as a potential target in the treatment of cancer. Relaxation of the chromatin fiber facilitates transcription and is regulated by two competing enzymatic activities, histone acetyltransferases (HATs) and histone deacetylases (HDACs), which modify the acetylation state of histone proteins and other promoter-bound transcription factors. While HATs, which are frequently part of multisubunit coactivator complexes, lead to the relaxation of chromatin structure and transcriptional activation, HDACs tend to associate with multisubunit corepressor complexes, which result in chromatin condensation and transcriptional repression of specific target genes. HATs and HDACs are known to be involved both in the pathogenesis as well as in the suppression of cancer. Some of the genes encoding these enzymes have been shown to be rearranged in the context of chromosomal translocations in human acute leukemias and solid tumors, where fusions of regulatory and coding regions of a variety of transcription factor genes result in completely new gene products, which may interfere with regulatory cascades that control cell growth and differentiation. On the other hand, some histone acetylation modifying enzymes have been located within chromosomal regions that are particularly prone to chromosomal breaks. In these cases gains and losses of chromosomal material may affect the availability of functionally active HATs and HDACs, which in turn disturbs the tightly controlled equilibrium of histone acetylation. We review herein the recent achievements, which further help to elucidate the biological role of histone acetylation modifying enzymes and their potential impact on our current understanding of the molecular changes involved in the development of solid tumors and leukemias.
Impact of Ataxia telangiectasia mutated gene inactivation on MYC-induced lymphomagenesis and chemosensitivity in a transgenic mouse model

M. Reimann, I. Schildhauer, B. Dörken, C.A. Schmitt
Berlin, D

Understanding the molecular basis for resistance to anticancer therapy is of central importance to develop novel strategies that may circumvent the problem of treatment failure.

One key player that determines chemosensitivity of cancer cells following DNA damaging therapies is p53. Functional p53 converts genotoxic stress or signals from activated oncogenes into cellular fail-safe responses, such as apoptosis or senescence. In tumor cells the p53 pathway is often dysregulated mainly due to mutations within the p53 gene. However, defects in genes that modulate p53 activity may serve as an alternative mechanism to compromise p53 function.

The ATM kinase identified to be mutated in patients suffering from Ataxia telangiectasia is one of key mediators that link the DNA damage signalling cascade to the p53 pathway. Upon DNA double-strand breaks ATM induces the phosphorylation of crucial p53 residues. The importance of ATM on tumorigenesis is underscored by the fact that allelic ATM deletions have frequently been observed in B-CLL and B-ALL. Clinical investigations unveiled a correlation between ATM and p53 defects and poor outcome in B-CLL patients. However, the molecular mechanisms by which ATM deficiency can produce hypersensitivity to DNA damaging treatments on one hand but increased tumor susceptibility and ultimately chemoresistance on the other hand remain largely unclear.

In order to generate B cell lymphomas with defined ATM lesions, ATM knockout mice were crossed to Eu-myc transgenic mice in which lymphomagenesis is triggered by constitutive expression of the myc oncogene in the B cell compartment.

Interestingly, heterozygous ATM loss did not accelerate myc-driven lymphoma onset, and no evidence for inactivation of the remaining wild-type allele has been found so far. This is consistent with the view that oncogenic signalling to cellular fail-safe networks is independent of ATM. Furthermore, tumor onset data based on allelic deletion of the ATM locus will be discussed.

Since data from ATM-deficient mouse embryo fibroblasts indicate a role for ATM signalling in response to DNA double strand breaks, myc-driven lymphomas were examined regarding the impact of ATM loss on phosphorylation of p53-serine 15 and availability of p53 effector functions following irradiation and anticancer drug treatment in vitro. Moreover, compiled treatment response data of mice harbouring lymphomas with and without defined ATM lesions will be presented.

Patients undergoing primary resection of gastrointestinal cancers show increased intraoperative fibrin generation when compared to patients with low risk of postoperative venous thromboembolism

W. Korte, K. Jung, K. Gabi
St. Gallen, CH

Background: Patients with gastrointestinal (gi) cancers have high fibrinogen concentrations and a high incidence of postoperative venous thromboembolism. However, it is unknown whether these patients have increased intraoperative fibrin generation (which might contribute to the increased incidence of postoperative venous thromboembolism). Objectives: To evaluate whether gi cancer patients have increased perioperative fibrinogen levels; have higher intraoperative fibrinogen consumption; and postoperatively and patient as well as anaesthesiological data were monitored and prothrombin fragment F1+2 were measured pre- intra- and postoperatively and patient as well as anaesthesiological data were monitored and prothrombin fragment F1+2 were measured pre- intra- and postoperatively.

Result: Both groups had similar gender distribution, age, body mass index, anaesthesiologic risk classification, duration of surgery, support with blood products and intraoperative infusion volume. Patients with gi cancers have significantly higher fibrinogen concentrations before, during and after surgery (delta fibrinogen ~ 0.6 g/l, p = 0.002 to 0.006); they show an early increase in intraoperative thrombin generation, while fibrin generation is delayed. However, gi cancer patients generate more soluble fibrin per gram fibrinogen (median 1.51 vs. 3.19 µg/g, p = 0.039) or per nmol F1+2 (median 3.18 vs. 1.75 µg/nmol) than non cancer patients early during surgery. Conclusions: Displaying increased fibrinogen levels at any time, gi cancer patients generate more soluble fibrin per unit thrombin and per gram fibrinogen early during surgery as compared to non-cancer patients. These data suggest that pre-operative reduction of fibrinogen concentrations might be a worthwhile strategy to test in order to reduce the risk of postoperative thromboembolism in gi cancer patients.
werden. Dabei ist die fachliche Ausrichtung frei wählbar. Jedes Modul ist in sich geschlossen. Die Reihenfolge der verschiedenen Module können die TeilnehmerInnen innerhalb eines bestimmten Rahmens frei kombinieren und wählen. Die ganze HoFa I umfasst 60 Unterrichtstage die for- gendermassen verteilt sind: Grundmodul 24 Tage Fachmodul 30 – 36 Tage Einzelmöglich bis 6 Tage Individuelle Studienzeit ca. 30 Tage Voraussetzungen für TeilnehmerInnen sind ein Diplom in Gesundheits- und Krankenpflege, mindestens 2 Jahre Berufserfahrung und ein Anstel- lungsverhältnis von mind. 60% im gewählten Schwerpunkt. Der Pilotkurs mit Schwerpunkt Onkologie wurde mit Erfolg abgeschlossen und ein neuer Kurs begann im Mai 2003. Ziele: Auseinandersetzung mit den theoretischen Grundlagen und Beurteilung des Praxistransfer Kompetenzerweiterung Berufsrolle entwickeln und reflektieren – Professionalisierung Konzept der HoFa I Praxisbezug – Handlungsorientierung Vertiefung und Spezialisierung im Fachbereich Förderung des fachlichen Austausches Förderung des Theorie – Praxis Transfer Förderung der Eigenverantwortung Weiterbildung nach Mass. Der Pilotkurs mit Schwerpunkt Onkologie wurde mit Erfolg abgeschlos- sen und evaluiert und ein neuer Kurs hat im Mai 2003 begonnen. 1075 Qualitätssicherung bei der Anwendung von Chemotherapien in Spandau, Berlin J. Potenberg, G. Sproßman-Günther Berlin, D In letzter Zeit hat in Deutschland und Europa der Stellenwert standardi- sierten Arbeitsabläufe (standard operating procedures = SOP) im klini- sch-medizinischen Alltag erheblich zugenommen. Um ein gleichblei- bend hohes Qualitätsniveau der täglichen Arbeit zu garantieren und zu belegen, sollten diese Arbeitsabläufe und die dazugehörigen Dokumenta- tionen ständig weiter verbessert werden. Aus diesem Grund haben wir Anweisungen, Regeln und Richtlinien in der Internistischen Onkologie für unser Krankenhaus entwickelt. Diese dienen als Werkzeuge und sollen die Sicherheit der Therapie für unsere Patienten gewährleisten und garantieren. Gerade in Zeiten beschränkter personeller und finanzieller Mittel erscheint dies wichtig, um unsere tägliche Arbeit gut zu verrichten. Das Ev. Waldkrankenhaus Spandau ist ein akademisches Lehrkrankenhaus. Im Jahr 2002 wurden in unserem Haus bei 624 Patienten eine bösar- tige Erkrankung diagnostiziert. Bei 197 Patienten wurde eine Chemothera- pie durchgeführt. Im Durchschnitt wurden diese Patienten fünfmal zur Durchführung einer solchen Therapie aufgenommen. Die Mehrheit unse- rer Patienten war an soliden Tumoren erkrankt (59 Mammakarzinome, 29 Ovarialkarzinome, 29 Endometrium- und Zervixkarzinome, 31 kolorrektale Karzinome, 8 Magenkarzinome, 8 Pankreaskarzinome, 14 Bronchialkarzi- nome, 5 Sarkome, 16 andere solide Tumoren), die Minderheit hatte hä- matologische Systemerkrankungen (6 Plasmozytome, 7 Lymphome, 6 Leukämien). Zum Beispiel: Patienten mit einem Mammakarzinom können mit ver- schiedenen Kombinationen von Zytostatika behandelt werden (CMF, EC, Taxane). Unterschiedliche Zytostatika verursachen in unterschiedlichem Ausmaß Übelkeit und Erbrechen. Deshalb erscheint es sinnvoll einer be- stimmten Zytostatika-Kombination eine auf diese Kombination speziell abgestimmte antiemetische Behandlung primär zuzuordnen. Erfahrt der Patient darüber hinaus Übelkeit oder Erbrechen, kann dies antiemetische Behandlung entsprechend der Richtlinie verändert werden. Für jede Verordnung einer zytostatischen Therapie existiert in unserem Haus ein eigenes Rezeptformular. In diesem Rezept werden notwendige Bedingungen und Voraussetzungen zum Beginn einer Chemotherapie genannt (z.B. minimale Leukozytenkonzentration, maximale Höhe des Kreatininwertes). Darüber hinaus enthält das Rezept zum Beispiel die Dosis der Medikamente und die Applikationszeit. Bei bestimmten Medi- kamenten werden Nebenwirkungen genannt und Informationen für diese individuelle Therapie gegeben. Schnelle und effektive Behandlung von Paravasaten gemäß den erarbeiteten hausüblichen Richtlinien kann Schäden minimieren. Darüber hinaus erlaubt die Dokumentation die exakte Nachbeobachtung. Das konsequente Einhalten, Entwickeln und Weiterentwickeln von Richtlinien und Regeln durch Ärzte, Pflegekräfte, MTA’s und Apothe- kern als gesamtes onkologisches Team sichert die Qualität der Behand- lung während der Chemotherapie für die Sicherheit der Patienten. Quality assurance in the application of chemotherapy in Spandau, Berlin J. Potenberg, G. Sproßman-Günther Berlin, D The demand for standard operating procedures and standardized working every day has recently become an important issue in Germany and Eu- rope. The documentation of this standardized working every day must be increased as it assures the quality of daily work. That is why we developed directives, rules and guidelines in the oncolo- gical section of our hospital. These tools ensure and guarantee therapeutic safety for our patients. In times of less financial and personal resources it means an important way of daily work of doing it well. The Ev. Waldkrankenhaus Spandau is an academic hospital. In 2002 624 malignant diseases have been diagnosed. 197 of these patients made a chemotherapy. On average the patients came to our hospital for a chemotherapy five times. The majority of the diseases were malignant solid tumors (59 breast, 29 ovary, 8 uterus or cervix cancer, 31 colorectal, 8 stomach, 8 pancreas, 14 lung, 5 sarcoma, 16 other solid cancer), the mi- nority were hematologic diseases (6 multiple myeloma, 7 lymphoma, 6 leukemia). For example a patient with breast cancer can be treated with different therapeutic regiments (CMF, EC, Taxane). Different cytotoxic agents mean different emetogenic potentials. Therefore it is reasonable to assign a specific therapeutic regiment to a specific antiemetogenic treatment. It is the patient unexpected and never- theless attacked by nausea or emesis the antiemetogenic treatment can be increased according to the guideline. Every single cytotoxic treatment has its own prescription form, including the individual single antiemetogenic therapy and including the conditions to allow to start therapy at all. The prescription contains the pharmacologic dates like dosis, timing, side- effects and other informations for this individual therapy. Chemotherapies are able to induce negative side effects, for example par- avasates. When side effects like this happens the acting according to guidelines can keep damages to a minimum. On top of it the documenta- tion allows an exact observation of this patient. Acting towards the directives and rules by the different protagonists (physicians, nurses, assistsants, pharmacists) assures the quality treatment during the chemotherapy as an act of safety for the patients. 1076 Die Aufgaben der Pflegenden auf einer Beratungsstelle für familiäre Tumorerkrankungen – ein Pilotprojekt H. Stoll, W. Weber, R. Herrmann Basel, CH Hintergrund: 40% der Krebspatienten haben einen oder mehrere nahe Verwandte mit Krebs. In einer kleinen Zahl ist ein Mendelserher Erbgang nachvollziehbar und genetische Tests werden zunehmend kommerziell erhältlich(1). Methode: Während eines halben Tages pro Woche sehen ein Onkologe und ein Onkologiepfleger Menschen in der Beratungsstelle für familiäre Tumorerkrankungen. Der Ablauf der Betreuung folgt dem Algo- rithmus des Schweizerischen Netzwerks für Gentests und -beratung (2). Im Vordergrund steht die verifizierte Familienanamnese. Resultate: 50 Personen wurden in den ersten 5 1/2 Jahren zugewiesen. 39 Frauen, 11 Männer, mediana Alter 40 (range: 21 – 77); 29 (58%) mit 1 oder 2 Tumo- ren, 19 (38%) waren gesund und 2 (4%) hatten Krebs aber eine negative Familiengeschichte. Die Familiengeschichte zeigte: 15 (30%) Brust-Brust, 10 (20%) Brust-Andere, 8 (16%) Kolorektal-Kolorektal, 4 (9%) Magen- Magen/Darm, 3 (7%) Brust-Ovar, 8 (18%) andere Kombinationen. Gen- tests wurden in 10 (20%) Familien (5 BRCA1+2, 3 hLMH1+hMSH2 und 2 p53).
3 Germutations were identified (2 hMSH2 and 1 hMLH1). Mehe-
re families were studied. All probands and their families were identified. Conspicuous cancer families were identified. The role of the Pfolgen in the Org.

Cancer predisposition testing is moving into mainstream healthcare (1). Methods: A half a day per week oncology family consultation in a uni-

versity medical oncology division is offered to the medical community with minimal advertising. The clinic follows the procedures of the Swiss Network for Cancer Predisposition Testing and Counselling (2).

Conclusion: This pilot oncology family clinic project demonstrates that conspicuous cancer families with high risk individuals are identified. Counselling and mutation testing is facilitated within a research oriented environment.

(1)Calzone K.A. and Biesecker B.B.: Genetic testing for cancer predispo-


Oncology family clinic in a university hospital – a pilot demonstration project

H. Stoll, W. Weber, R. Herrmann

Basel, CH

Background: Forty percent of cancer patients have one or more near rela-
tive with cancer. In a minor proportion there is Mendelian determination. Cancer predisposition testing is moving into mainstream healthcare (1).

Methods: A half a day per week oncology family consultation in a uni-

versity medical oncology division is offered to the medical community with minimal advertising. The clinic follows the procedures of the Swiss Network for Cancer Predisposition Testing and Counselling (2).

Results: 50 individuals have been referred consecutively during the first 5 1/2 years: 39 female, 11 male, median age 40 years (range: 21y – 77y); 29 (58%) had one or two neoplasias, 19 (38%) were unaffected and 2 (4%) had cancer with a negative family history. The family cancer patterns were: 15 (30%) breast-breast, 10 (20%) breast-others, 8 (16%) colorectal-colorectal, 4 (9%) gastric-gastrointestinal, 3 (7%) breast-ovary, 8 (18%) other combinations. Gene testing was done in 10 (20%) families (5 colorectal, 4 gastric, 1 breast-ovary). 3 pathogenic mutations have been identified (2 hMSH2 and 1 hMLH1). Several families are studied by a research consortia. All probands and their families are counselled for cancer prevention and early detection in general as well as relating to their family history and ev. mutation testing results. Conclusion: This pilot oncology family clinic project demonstrates that conspicuous cancer families with high risk individuals are identified. Counselling and mutation testing is facilitated within a research oriented environment.

(1) Calzone K.A. and Biesecker B.B.: Genetic testing for cancer predispo-


Symptomkontrolle

1078 Entwicklung eines Qualitätsstandards für das pflegerische Erstgespräch zur Chemotherapie bei ambulanten onkologischen Patienten

U. Neumann

Aarau, CH

It has been shown by a study of patients with different types of cancer during the past 20 years that venous access devices (VADs) are a major source of infection in patients undergoing chemotherapy. The incidence of catheter-related bloodstream infection (CRBSI) remains high, even though various strategies have been implemented to reduce the incidence. Despite the efforts to minimize CRBSI, patients still experience the complications associated with VADs, such as pain, discomfort, and decreased quality of life. Therefore, it is essential to develop and implement strategies to prevent CRBSI effectively. The aim of this study was to evaluate the effectiveness of a newly developed protocol that focuses on a comprehensive approach to VAD management. The protocol includes education, hand hygiene, and the use of prophylactic antibiotics. The protocol was implemented in a large medical center, and the results were compared with the previous practice. The incidence of CRBSI decreased significantly after the implementation of the protocol. This study highlights the importance of developing evidence-based protocols for the prevention of CRBSI in patients undergoing chemotherapy. Further research is needed to evaluate the long-term effectiveness of these protocols.
Punktionskomplikationen und die korrekte Behandlung von Paravasaten können aber schwere Folgen wie chirurgisches Debridement last immer verhindernd sein. Man unterscheidet bei der Therapie zwischen Paravasaten durch Vinalkaloids (Hyaluronidase) und solche durch Anthrazykline und verwandte Substanzen (Dimethylsulfoxide).

Im Referat werden die Daten einer grossen Literaturrecherche vorgestellt und daraus ein Standard zur Behandlung von Paravasaten abgeleitet.

1081 Role of parenteral nutrition support in patients with advanced pancreatic cancer
Berlin, D
N utritional deterioration and progressive weight loss are commonly found in patients (pts) with APC. Decreased nutritional status arises from anorexia, insufficiency of pancreas, as well as treatment toxicity and obstruction of the gastrointestinal tract. Is parenteral nutrition support (PNS) in patients with APC necessary, if the patient is unable to balance his calorie consumption by oral nutrition? Methods: Pts with APC treated with chemotherapy were prospectively evaluated for their nutritional status (NS) by investigation of body mass index (BMI) and bioelectrical impedance analysis (BIA). Common parameters for BIA are: ECM (extracellular mass), BCM (body cell mass) and phase angle. NS assessment performed at the beginning and every 4-6 weeks during PNS.Pts had an improvement of their NS if they accomplish at least two of the following parameters: BMI > +5%; ECM/BCM index > -5%; phase angle > +5%. Stable NS is defined as: BMI +/- 5%; ECM/BCM +/- 5%; phase angle +/- 5%. Declined NS is defined as: BMI > -5%; ECM/BCM index > -5%; phase angle > -5%. Results: 32 patients (pts) with a deterioration of NS received PNS. 27 patients benefit from PNS. 15 pts showed an improvement and 12 pts showed a stable NS with PNS. 5 pts could not improve or hold their NS despite PNS. Considering BMI alone, 28 pts benefitted from PNS (BMI > +5% = 16pts; BMI > +10% = 10pts). Regarding ECM/BCM index alone, 25 pts benefit from PNS (ECM/BCM > +5% = 15pts; ECM/BCM > -10% = 10pts). Observing phase angle alone, 28 pts benefit from PNS ( > +5% = 15pts; > +10% = 13pts). Only 4 pts showed declined phase angle on PNS. Conclusion: Nutritional deterioration and progressive weight loss are commonly found in patient with APC. The majority of pts (84%) with APC benefit from PNS in terms of BMI and BIA, whereas 16% could not benefit from PNS. Patients who are unable to balance their calorie consumption by oral nutrition due to obstruction/dysfunction of the gastrointestinal tract or treatment toxicity during multimodal chemotherapy, may therefore benefit from PNS.

1082 Strukturierte Pflegeintervention zum selbständigen Management der Übelkeit vom Chemotherapie-Patienten in stationär-ambulanten Setting
M. Landenberger
Halle, D
Fragestellung: Es wird über erste Ergebnisse einer pflegewissenschaftlichen Studie aus der Hämatologie-Onkologie berichtet. Es ist eine randomisierte kontrollierte Multizentristudie, die öffentlich gefördert und an zwei onkologischen Zentren durchgeführt wurde.

1083 Inkontinenz – eine pflegerische Herausforderung bei Patienten mit einem Prostatakarzinom
C. Wiedmer
St. Gallen, CH

1084 Mundpflege evidenzbasiert in der pädiatrischen Onkologie
K. Müller, C. Offermann, K. Schwab
Bern, CH
Einleitung: Entzündungen, Ulzerationen und Infektionen der Mundschleimhaut gehören zu den häufigsten Komplikationen systemischer Chemotherapie und radiologischer Bestrahlung im Kopf und Halsbe-
Schmerzlinderung

1087
Von den Guidelines zum klinischen Alltag in der Behandlung chronischer Schmerzen – Erfahrungen mit einem Qualitätsmanagementprojekt

S. Navarra, P.R. Müller, M. Andrey
Bern, CH


The gap between guidelines and clinical practice in the treatment of chronic pain – a quality management approach

S. Navarra, P.R. Müller, M. Andrey
Berne, CH

There is no lack of practice guidelines for the treatment of chronic pain. However, surveys in the US and Europe suggest that the actual treatment is far from satisfactory.

The Swiss Cancer League offers a quality management project with the aim of bridging the gap between expert knowledge and clinical practice. The project accompanies health care institutions in a 12-month process to achieve 10 quality criteria. During this process standard procedures for assessment, documentation and treatment of chronic pain are defined by an interdisciplinary group within the institution. This work includes the development, adaptation and implementation of appropriate tools as well as the education of the staff. The process is monitored and evaluated with regular audits.

The data gathered in these audits are of a non-experimental nature. The results of the 8 institutions that have achieved the 10 quality criteria show significant improvements on a structure level (e.g. a dossier on pain management for new staff members), a process level (e.g. systematic pain assessment) and a level of patient outcomes (e.g. pain-intensity).

The results and our experience indicate that a quality management approach is feasible and leads to satisfactory results.

1088
Schmerzelastung bei Tumorpatienten unter Radio- und Chemotherapie – eine multizentrische Studie

F. de la Fuente, K. Budeschesky, H. Bischoff, P. Drings
Heidelberg, Mainz, D


Die Literaturanalyse macht deutlich, dass der Bedarf an Unterstützung von Patienten mit Krebs im ambulanten Setting gemessen werden soll, weil so gezielt auf die unerfüllten Bedürfnisse des Patienten eingegangen werden kann.


Die Literaturanalyse macht deutlich, dass der Bedarf an Unterstützung von Patienten mit Krebs im ambulanten Setting gemessen werden soll, weil so gezielt auf die unerfüllten Bedürfnisse des Patienten eingegangen werden kann.
nem validierten Patiententagebuch über einen Zeitraum von 5 Tagen. Die Auswirkungen von Übelkeit und Erbrechen auf die Fähigkeiten des Patienten, seinen täglichen Aktivitäten nachzugehen, wurden anhand des ‘Functional Living Index Emesis’ (FLIE) – Fragebogens ermittelt. Gleichzeitig wurden die antiepileptischen Behandlungseitlinien der Insti-
tute beurteilt. Ergebnisse: Bei 70% der Patienten stellt Übelkeit kein Problem mehr dar, aber bis zu 30% der Patienten leiden an diesen onko-
logischen Zentren noch unter Übelkeit. Für 13% der Patienten stellt die akute Emesis, für 38% die verzögerte Emesis noch ein Problem dar.

**Schlussfolgerungen:** Die Studie konnte zeigen, dass chemotherapie-indu-
zierte Übelkeit und Erbrechen trotz antiemetischer Therapie in der Pra-
xis dieser onkologischen Zentren immer noch auffüllt. Auftretende Übel-
keit übt einen ungünstigen Einfluss auf die Lebensqualität der betroffe-
nen Patienten aus. Deshalb muss diesen Nebenwirkungen noch ausrei-
chende Aufmerksamkeit geschenkt werden.

### 1092 Lebensqualität in Familien mit einem onkologisch erkrankten Mitglied – pflegerische Handlungsoptionen

K. Lex Clausthal-Zellerfeld, D

In Deutschland nimmt die Dauer der Pflegebedürftigkeit onkologischer Patienten tendenziell zu; dadurch steigt auch die Belastung und das Kri-
enpotential der betroffenen Familien. Bei der Krankheitsbehandlung des Patienten scheint der familiäre Kon-
text (Familienstruktur, sozioökonomischer Status, Kommunikationsmüs-
ter etc.) von immenser Wichtigkeit für die Lebensqualität der gesamten Familie zu sein. Finanzielle Sorgen, Inkongruenz zwischen Hilfsangeboten und Bedürfnis-
en, und Schwierigkeiten, Hilfe anzunehmen sind Barrieren, die verhin-
dern, dass Familien ihr Bedürfnis nach 'Hilfe von außen' artikulieren (Lai$	ext{si}	ext{er$ et$ al.$} 1993$).

Diese Hindernisse haben eine erhebliche Auswirkung auf die Lebensqua-
lität der betroffenen Familie. Lebensqualität beinhaltet physische, psychi-
 sche, soziale, spirituelle und finanzielle Aspekte des Lebens. Interventionen zur Verbesserung der Lebensqualität reduzieren emotion-
alen Stress, mindern Tumorschmerzen, verbessern den Immunstatus, re-
duzieren Nebenwirkungen bei Chemotherapien und festigen die Bezie-
hungen innerhalb der Familie. Pflegepersonen sollten darum versuchen, eine lebendige Beziehung zwi-
schen den betroffenen Patienten und der Familie anzuspanen (Isaksen et al. 2002).

Auch bei Gesprächen zwischen Patienten spielen Pflegepersonen eine wichtige Rolle, da sie entscheiden, welcher Patient mit welchem anderen Patienten das Zimmer teilt. Informationsaustausch zwischen Patienten kann nämlich eine wichtige Hilfe beim 'coping' sein. Essentiell ist, dass Pflegepersonen Familien nach ihren Bedürfnissen und Wünschen fragen und entsprechend reagieren. Leider haben sehr viele Familien noch große Informationsdefizite, nicht nur was die Erkrankung selbst betrifft, sondern, v.a. Auswirkungen auf andere Lebensbereiche und Möglichkeiten wie z.B. finanzielle oder staat-
liche Unterstützung.

**Ausgaben:** Die Studie konnte zeigen, dass chemotherapie-indu-
zierte Übelkeit und Erbrechen trotz antiemetischer Therapie in der Pra-
xis dieser onkologischen Zentren immer noch auffüllt. Auftretende Übel-
keit übt einen ungünstigen Einfluss auf die Lebensqualität der betroffe-
nen Patienten aus. Deshalb muss diesen Nebenwirkungen noch ausrei-
chende Aufmerksamkeit geschenkt werden.

### 1093 Ausgebildete und begleitete Freiwillige als Teil des ambulanten pflegenden Netzwerks: Rolle und Aufgaben, Anforderungen und Ausbildung der Freiwilligen und Erfahr-
ungen aus der Zusammenarbeit mit professionellen Institutionen

P. Lack Basel, CH

**Beschreibung:** Die Begleiterinnen und Begleiter von GGG Begleiten gehen nach Hause zu behinderten, schwerkranken und sterbenden Men-
schen und ihren Angehörigen. Seit Start des Begleitdienstes im Jahr 1999 waren es vor allem KrebspatientInnen, die in der letzten Lebensphase be-
gleitet und ihre Angehörigen so entlastet wurden. Da es sich bei Krebs um eine oft schnell fortschreitende Krankheit handelt, werden oft die letzten Wochen vor dem Tod für Angehörige zu einer grossen Belastung. Oft warten Angehörige von schwerkranken Menschen zulange, bis sie Hilfe von aussen in Anspruch nehmen. Aufgrund einer fehlenden Sterbe-
kultur und der Tabuisierung von Krankheit und Tod sind Angehörige auch oft isoliert, was zu einem Erschöpfungszustand führen kann. Oft bleibt dann nur noch die notfallmässige Hospitalisierung des kranken An-
gehörigen. Diese kann vermieden werden, wenn die pflegenden Angehö-
rigen Unterstützung durch freiwillige BegleiterInnen erhalten.

Die Aufgabe von Freiwilligen besteht in der Unterstützung von Angehö-
rigen. Dies betrifft sowohl die Phasen der Krankheitsbewältigung des Pati-
enten, als auch die Phase des Todes. Die BegleiterInnen sollten sich in der Frage der Unterstützung und Betreuung von Patienten mit onkologischen Erkrankungen umfangreich und intensiv informieren und trainieren, um eine effektive Betreuung zu gewährleisten.

**Vorteile:** Die BegleiterInnen unterstützen die Angehörigen in der Pha-

**Nachteile:** Die BegleiterInnen müssen ihrerseits eine hohe Mobilität und Flexibilität aufbringen, um den Bedürfnissen der Familie gerecht zu werden. Die BegleiterInnen müssen auch lernfähig und flexibel sein, um sich auf die verschiedenen Situationen einzustellen. Sie müssen auch eine hervorragende Kommunikationsfähigkeit und eine ausdifferenzierte Kenntnis von rechtlichen Aspekten haben.

**Zusammenfassung:** Die BegleiterInnen von GGG Begleiten haben eine wichtige Rolle bei der Betreuung von Patienten und ihren Angehö-
rigen. Sie bieten emotionalen und praktischen Unterstützung und erbrin-
gt eine wichtige Funktion in der Betreuung von Patienten mit onkologischen Erkrankungen. Es ist wichtig, dass die BegleiterInnen sich umfangreich und intensiv informieren und trainieren, um eine effektive Betreuung zu gewährleisten.
die Patienten abzugeben v.a. in der Endphase Ihres Lebens. **Fazit**: neben dem Hochgefühl der freien WE nimmt das Team im wesentlichen positive Veränderungen wahr. Eine erhebliche Verbesserung der Befindlichkeit; die Kritikfähigkeit wird auf hohem Niveau gepflegt; die Führung der Pati
tenendokumentation hat sich verbessert; aus der Notwendigkeit des Rap
ers ergeben sich konstruktive Auseinandersetzungen; die Reflexion der eigenen Arbeit wird vertieft. Gegenseitiges Vertrauen und gutes Ein
durchwegs guten Erfahrungen während der Versuchsphase befürwortet
t das Team die Beibehaltung dieser Regelung.

**1095 Individuelle und im Beratungsgespräch erstellte Patientenver
fügungen als zuverlässiges Instrument der Willensäußerung und Teil einer Bewältigungsstrategie bei Menschen mit einer chronischen Krankheit: Beratungskonzept, Angebote und Erfahrungen**

**P. Lack**
Basel, CH

Beschreibung: Personen mit bestehender Krankheitsdiagnose wünschen sich auch im Falle von Nicht-Ausserungsfähigkeit eine selbstbestimmt
den und nach ihren Vorstellungen durchgeführte Behandlung. Besteht Ge
gar, dass die im Voraus festgehaltenen Wünsche respektiert und umge
etzt werden, kann eine Quelle möglicher Verunsicherung und Angst re
duziert werden. Vorformulierte PatientInnenverfügungen sind vor allem für
die Menschen mit einer bestehenden Diagnose zu allgemein gefasst und
erhalten konsequent weniger Beachtung. Bei Menschen mit einer bestehenden Diagnose gewinnen die medizinischen Verfügungen gegenüber der Wer
teklarung an Gewicht. Eine PatientInnenverfügung ist für Krebspatient
tInnen besonders geeignet, da der Verlauf der Krankheit abschätzbar ist. Im Voluntas Beratungsprozess kommen das je eigene Fachwissen der Volun
tas-Beraterin, des PatientIn / des Patienten und der / des medizinischen SpezialistIn /en zusammen. Die Voluntas-BeraterIn hat die Aufgabe der Moderation und der Prozessbegleitung.

Die Auseinandersetzung mit dem möglichen Verlauf der Krankheit und den persönlichen Vorstellungen und Einstellungen über Leben, Krank
halt, Sterben und Tod sind zum Teil der Bewältigungsstrategie. Diese
die Auseinandersetzung wird durch BeraterInnen unterstützt, die die Klien
tInnen durch Krankheits- und verfügungsrelevante Themen führen und bei der Abfassung der entsprechenden Dokumente behilflich sind. Voluntas-PatientInnenverfügungen werden bei der Medizinischen Not
erzentralliegen und sind so jederzeit abrufbar. Damit ist gewährleistet, dass in einer Notsituation der Wille des Patienten schnell ermittelt
den kann und respektiert wird.

**1097 Braucht es stationäre Hospize?**

*L. S. Thut*
Zufikon, CH

Am 07. Oktober 1994, d.h. genau vor 9 Jahren, wurde der Aargauer Hospiz
Zurich berufte die Schwerkranken als ambulantes Voluntas-Hospiz ge
ggründe. Ursprünglich stammt der Begriff “Hospiz” aus dem lateinischen (Hospes = Gast, Hospitium = Gastfreundschaft) und beschreibt einen Ort der Gastfreundschaft und der Begleitung. Ein Hospiz war eine Wegenstadt, ein Ort, wo man sich auf einer langen Reise ausruhen konnte.

Der Begriff ‘Hospiz’ beschreibt heute eine weltweite Bewegung und Phil
dosophie, welche den Kranken Menschen in seiner letzten Lebensphase
zusätzlich zu ambulanten Versorgungsangeboten und in den Kantonen Basel-Stadt und Aargau angeboten wird, möchten wir über die Möglichkeit, warum die SEOP kontaktiert wird. Diese lassen sich in 3 Bereiche unterteilen:

1. Organisation und Koordination beim Spitalaustritt und bei komplexen Pflegesituationen zu Hause.
2. Beratung und Unterstützung bei der Symptomkontrolle
3. Führen von Gesprächen bezüglich Krankheitsverlauf und Sterben.

Auseinandersetzung wird durch BeraterInnen unterstützt, die die KlientIn
en besonders geeignet, da der Verlauf der Krankheit absehbar ist. Im Voluntas Beratungsprozess kommen das je eigene Fachwissen der Volun	atas-Beraterin, des PatientIn / des Patienten und der / des medizinischen SpezialistIn /en zusammen. Die Voluntas-BeraterIn hat die Aufgabe der Moderation und der Prozessbegleitung.

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erzentralliegen und sind so jederzeit abrufbar. Damit ist gewährleistet, dass in einer Notsituation der Wille des Patienten schnell ermittelt
den kann und respektiert wird.

Um das finanzielle Risiko möglichst tief zu halten, scheint ein Anfang mit vier Zimmern optimal zu sein. Es wäre somit eine möglichst flexible, kostengünstige und trotzdem auf die Bedürfnisse der BewohnerInnen abgestimmte Lösung, die von einer gemeinsamen Trägerschaft und dem Engagement für die Hospiz-Idee geprägt ist. Auch das Sterben in einem Hospiz kostet Geld, aber die Kosten sind nicht vergleichbar mit denjenigen in einem Akutspital. Was ein Patient pro Tag kosten könnte, wird im Vortrag erläutert. Die Steigerung der Lebensqualität in ihrer letzten Lebensphase ist das dringendste Anliegen dieses Projektes. Der Bedarf, der anhand verschiedener Beispiele eruiert werden kann, die Verbreitung des palliativen Therapie-Ansatzes und nicht zuletzt die Anfragen der Betroffenen machen ein dringendes Bedürfnis für Hospiz deutlich.

**Palliation – Institutionen**

### Palliativpflege im Regionalspital

A. Lanz
Reinach, CH

Ist es möglich in einem Regionalspital gute Palliativmedizin zu betreiben?

Dank einer innovativen Geschäftsleitung, einer Onkologiepflegenden und zwei leitenden Ärztinnen (der medizinischen Klinik und der Schmerztherapie) konnte im Spital Menziken eine 10% Stelle für eine Palliativschwester realisiert werden. Seit einem Jahr werden die Pflegenden und die Assistenzärzte bei der Betreuung terminaler Patienten mit verschiedenen Diagnosen von einer Palliativschwester unterstützt.


Beider Arbeit mit sterbenden Krebspatienten, haben folgende Punkte Priorität:

1. Eine optimale Symptomkontrolle (Schmerzen, Atemnot, Übelkeit und Erbrechen)
2. Eine ganzheitliche Betreuung von Patient und Angehörigen mit dem Ziel einer Erhaltung einer guten Lebensqualität unter Ausschöpfung der vorhandenen Ressourcen