Abstracts

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Guest Editors

*Joachim Dreves, Heinz-Herbert Fiebig, Florian Otto,
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GPI-Linked CEACAM6 Molecule Expression in Prostate Cancer

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Background: Several members of the human carcinoembryonic antigen-related cell adhesion molecule (CEACAM) family are cellular surface molecules which belong to the immunoglobulin superfamily. They display a complex expression pattern in normal epithelia, but often show deregulated cell surface expression in malignant tissues. CEACAM6 has been found to be of prognostic value in colorectal and pancreatic cancer. In prostate cancer downregulation of CEACAM1 has been shown to correlate with loss of cell polarity, increased proliferation, and Gleason grade 3 to 4 transition. **Methods:** Expression of GPI-linked CEACAM6 was determined in comparison with transmembrane-linked CEACAM1 in primary PCa cells and established prostate cancer (PCa) cell lines under various *in vitro* and *in vivo* (xenograft) conditions. **Results:** Primary PCa cells were established from spheroids of total prostatectomy specimens of different Gleason (5-9) grades. Epithelial Primary Spheroid Outgrowing Cells (PSOCs) grew from individual spheroids during the first passages without adding any supplements, secreted significant amounts of PSA (prostate specific antigen) and displayed PSMA (prostate specific membrane antigen) in about 50% of PSOC cultures. PSOCs showed a varying but significant expression of CEACAM6 and/or CEACAM1 molecules in most patients. Androgen-dependent cell lines LNCaP and CWR22 or their sublines LNCaP-abl or C4-2 did not express any CEACAM-molecules. In contrast, two hormone-refractory, metastatic PCa cell lines, PC-3 and Du145, showed a weak CEACAM1 and a strong CEACAM6/CEACAM1 expression, respectively. CEACAM-expressing cell lines did, in contrast to other lines, grow *in vivo* also without adding Matrigel (a solubilized basement membrane preparation) after s.c. application. In contrast, orthotopically transplanted LNCaP cells also did grow *in vivo* without adding Matrigel as shown by luciferase imaging. 'Orthotopic PCa spheroid *in vitro* co-culture' induced in LNCaP cells significant expression of CEACAM6 or CEACAM1 after 4-21 days, not observed by *in vitro* treatment (96 h) with dihydroxy-testosterone (DHT) enhancing PSA secretion in hormone-dependent cells or subcutaneous transplantation in non-castrated mice. **Conclusions:** CEACAM molecules seem to be markers of epithelial Primary Spheroid Outgrowing Cells (PSOCs) from prostatectomy specimens and of individual hormone-refractory, metastatic PCa cell lines. The latter show a more aggressive growth *in vivo* than other cell lines. After orthotopic (in contrast to ectopic) transplantation also the less aggressive, androgen-dependent LNCaP cells did grow without adding Matrigel *in vivo*. An 'orthotopic *in vitro* culture' model showed that these cells do express CEACAM6 or CEACAM1 on their surface. Our results raise the question whether CEACAM6 and/or CEACAM1 may be markers of aggressiveness of PCa cells?

Generation of a Conditionally Transformed MEF Cell Line Using a Tetracycline-Regulatable IGF-R Allele

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Background: There is ample evidence for a role of the IGF1-receptor system in oncogenic transformation, as well as growth, survival, invasion, and metastasis of tumors. To develop specific inhibitors against the IGF1-R, a good cellular model, applicable to both *in vitro* and *in vivo* studies, is required. **Methods:** A 3T3 cell line that expresses a myc-tagged IGF1-R under the control of a tetracycline-regulatable promoter was generated via stable transfection. The resulting cell line was extensively investigated *in vitro* and *in vivo* for IGF1-R mediated growth characteristics. **Results:** In the absence of doxycycline, the resulting cell line expressed high levels of the IGF1-receptor, and was responsive to IGF1. Both these characteristics could be abolished by the addition of doxycycline to the medium. When implanted into nude mice, these cells formed tumors, but the onset of tumor growth was very slow. Further increases of the oncogenic properties of the cells were achieved by an additional selection in soft-agar assays. The resulting cell lines showed an accelerated proliferation rate and an increased sensitivity to IGF. They formed tumors in nude mice within 20 days that could be brought into transient remission by repression of IGF1-R expression indicating that development of these tumors strictly depend on IGF1-R expression. **Conclusion:** These cell lines can be used to test known and novel IGF1-R inhibitory compounds *in vitro* and *in vivo* in the mouse model.

Marking Cells with Luciferase for in vivo Metastases Detection

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Background: Orthotopic metastasizing mouse models are valuable tools for testing novel anticancer drugs. A major drawback of these models so far has been the difficulty to detect the metastases in live animals, which is important to establish the status of the animals at the onset of the treatment, and to follow the effect throughout the treatment period. Amongst others, bioluminescence has recently been described as a powerful novel imaging technique. **Methods:** Cell lines of human and mouse origin were transduced with a retroviral vector encoding a Luciferase-Neomycin fusion protein, selected using G418, and tested for Luciferase activity *in vitro*. These cells were then orthotopically implanted into mice. To visualize the primary tumor and potential metastases, the mice were injected with luciferin, and the emitted light was detected using a CCD camera system. After sacrificing the mice, the individual organs were homogenized, and assayed for luciferase activity. **Results:** Depending on the cell lines, the luciferase activity measured was sufficient to allow the detection of 1-100 cells. When these cells were implanted into mice, primary tumors could be detected before they were palpable, and in some of the mice, metastases were found. The results from the *in vivo* imaging were correlated with data obtained from necropsic analysis, as well as testing homogenized organs for luciferase activity. **Conclusion:** These cell lines and the luciferase system are very useful for efficient testing of novel anti-cancer compounds *in vitro* and *in vivo* in the mouse model.

Protein Tumor Lysate Bank from 150 Patient-Derived Tumor Xenografts for Target Expression Studies

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Oncotest has developed models for all major solid human tumor types by directly transplanting tumors from patients subcutaneously into immune-compromised nude mice. More than 400 solid tumors have been established in serial passage. We have generated a protein tumor lysate bank based on 150 of these tumor models, including carcinomas of the bladder, brain, breast, colon, head and neck, kidney, liver, lung, ovary, pancreas, prostate, stomach, testicle, and uterus as well as melanomas and sarcomas. Using Bead Suspension Assays the expression and phosphorylation status of a multitude of molecular markers involved in signal transduction, cell cycle, apoptosis, etc. is being determined. The complete molecular profiles are used to identify biomarkers predictive for drug response or resistance. Another application of Bead Suspension Assays is the monitoring of target expression and activity (e.g. phospho-EGFR) during therapy experiments in tumor-bearing nude mice. The detailed molecular characterization of Oncotest's tumor collection allows an optimal choice of tumor models for the preclinical profiling of new promising anticancer agents in vitro and in vivo and thereby accelerates and improves the preclinical evaluation of novel target directed compounds.

Cell-Based High Throughput Screen Using Target-Defined Tumor Cell Lines and Bioinformatics for Anticancer Drug Discovery

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Modern anticancer drug discovery uses primary screens in high throughput format in which thousands of compounds can be analyzed within a few days. Common approaches are isolated target screens and cytotoxicity assays based on permanent tumor cell lines. Oncotest has developed a large scale primary screen based on 36 cell lines most of which were established from patient-derived tumor xenografts. Therefore, for all cell lines corresponding tumor xenografts growing in nude mice are available. For each of 11 tumor types (bladder, CNS, colon, lung, mammary, melanomas, ovary, pancreas, prostate, renal and uterus) 2–5 representative cell lines are included. The read-out of the screen is propidium iodide based fluorescence as an indicator of viable cell number. Most of the cell lines are at low passage, are well differentiated and display relatively slow growth with doubling times between 30 and 48 hours, thus allowing 4-day incubation with test compounds. Since the NCI 60 cell line screen is based on fast growing, undifferentiated high passage cell lines and allows only 2-day incubation of test compounds, the Oncotest and the NCI screens are likely to identify non- or only partially overlapping sets of anticancer agents.

At Oncotest, the following strategy for compound identification and profiling in three stages was established. As a first step, compounds are tested at one high concentration (e.g. 3–30 µg/ml) in a panel of 6 cell lines with a throughput of over 2,000 compounds per week. Active compounds are subsequently tested at 5 different concentrations. Main compound selection criteria are potency (IC₅₀, IC₇₀ and IC₉₀-values) and tumor selectivity. During the third phase compounds are tested in all 36 cell lines, resulting in specific activity profiles. The IC₅₀, IC₇₀ or IC₉₀ profiles are compared with the profiles of 40 registered anticancer agents and 120 additional compounds with known mechanisms of action. This Compare Analysis gives clues as to the mechanism of action and allows the identification of compounds with a potentially novel mechanism of action. For all 36 cell lines, the expression patterns of 34,000 genes (Affymetrix chip HU 133) and of several key signal transduction proteins (Bead Suspension Assays) were determined. Correlation of the IC₇₀ values for a particular

compound with gene expression patterns allows the identification of sets of genes that are either over or under expressed in sensitive cells. The compounds identified as most promising in the 36 cell line screen are further investigated in a clonogenic assay in vitro using established patient derived tumor xenografts, and in vivo therapy studies in tumor xenograft bearing nude mice. The approach is currently being used for large scale screening of natural products.

Clinical Phase I Study with DOXO-EMCH, an Albumin-Binding Doxorubicin Prodrug

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Background: Doxorubicin is one of the most important anticancer drugs but its use is critical due to its unique toxic dose-related side effects as cumulative cardiotoxicity and extravasation with necrotic potential. Serum albumin accumulates in solid tumors, thus coupling of an anticancer pro-drug to albumin is a strategy for delivering the drug to its target site and preventing its diffusion into healthy tissue. An albumin-binding prodrug of doxorubicin with acid-sensitive properties, i.e. (6-maleimidocaproyl) hydrazone of doxorubicin (DOXO-EMCH), was developed to meet two features: (a) rapid and selective binding to the cysteine-34 position of endogenous albumin after intravenous administration, and (b) release of albumin-bound doxorubicin in the acidic environment of the tumor cell due to the incorporation of an acid-sensitive carboxylic hydrazone bond between the drug and the spacer molecule. Preclinically DOXO-EMCH has shown superior antitumor efficacy and an improved toxicity profile compared to doxorubicin. **Methods:** The objectives of this phase I trial is to evaluate the safety profile and pharmacokinetics of DOXO-EMCH in order to assess the maximum tolerated dose (MTD), evaluate dose-limiting toxicity (DLT), and to preliminarily assess antitumor activity. To date, cohorts of 3–6 patients (pts) with advanced cancer disease were treated with an intravenous infusion of DOXO-EMCH over 30 minutes once every 3 weeks at dose levels of 20, 40, 80, 135, 150, 180, and 200 mg/m² doxorubicin equivalent. Assessment of tumor size was performed before and after every 2nd cycle. **Results:** 27 patients (14 female, 14 male) were treated with DOXO-EMCH so far. No DLT was observed by a treatment with DOXO-EMCH up to 180 mg/m² doxorubicin equivalent. Somnolence (grade 3) was observed at 200 mg/m² in 2/6 patients but the causal relationship to the treatment remained unclear. No objective tumor response (partial or complete remission) but disease stabilization has been observed so far. PK analysis is ongoing, first results will be presented. **Conclusion:** Intravenous administration of DOXO-EMCH every three weeks was well tolerated up to 180 mg/m². Valid conclusions with regard to anticancer efficacy cannot be done due to the pretreatment conditions (the majority of patients were chemotherapy resistant) and the very heterogeneous tumor types included. Expansion at the dose of 200 mg/m² (or even higher) is ongoing in order to determine a safe dose recommendation for phase II studies as well as a dose level with antitumor activity.

Multiplexed Biomarker Determination

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Over the past years, research activities as well as the development of tests to routinely quantify soluble markers has put ProQinase in the position to offer a full service package, including the timely transfer of results together with an optional detailed data analysis. ProQinase is committed to improve its activities in the field of clinical biomarkers by evaluating new technologies. The multiplex analysis of soluble markers gains top priority because small amounts of patient samples can be characterized with respect to a whole panel of factors.

Today several new technologies appear on the market that allow the parallel determination of biomarkers in one assay. This report describes the validation of a 4-plex biomarker assay using the electrochemiluminescence reaction developed by MesoScaleDiscovery (MSD, USA). Measurements on the MSD platform are compared with values obtained using the well established Quantikine® Immunoassay kits from R&D (#DVE00 for VEGF and #HSFB75 for high sensitivity bFGF).

Custom coated plates made available from MSD enable the parallel quantification of VEGF, bFGF, sFLT-1 and PlGF. This is accomplished by spotting 4 specific capture antibodies per well in a defined spatial pattern. After binding of the proteins present in the sample, detection is performed using a mix of 4 respective detection antibodies. These antibodies are specifically tagged to emit light after electrochemical stimulation (Reader: MSD Sector 2400). Both ELISA kits from R&D and the MSD system translate the signal into protein concentration by comparing the signal intensity of unknown samples with the signal obtained of known amounts of recombinant standard protein. The comparison focussed on VEGF and bFGF and besides direct correlation of data we evaluated the recovery of defined amounts of recombinant proteins on the background of a regular plasma sample.

Calibration curves of both systems are linear with a very limited degree of variability. Direct comparison of VEGF and bFGF levels of patient samples determined with R&D ELISAs and the MSD system shows a highly significant level of correlation ($P < 0.0001$). The recovery of recombinant VEGF and bFGF in spiked samples is satisfactorily and demonstrates the expected additive behaviour.

In addition, besides the advantage of determining 4 parameters in parallel, the needed sample volume is much smaller. Another plus is the ease of handling of the MSD platform, which markedly reduces assay duration. By introducing this new technology ProQinase guarantees that our customers will benefit accordingly.

Bisphosphonates: Antiangiogenic and Antitumoral Activity in vitro and in the Murine Renal Cell Carcinoma Model (RENCA)

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Background: Bisphosphonates are successfully used to treat skeletal complications in patients with bone metastases from solid tumors. In addition to this application, bisphosphonates showed direct antitumoral activity in vitro, in vivo and when investigated in clinical trials. In this report, we particularly studied the antiangiogenic properties of bisphosphonates in vitro and in vivo. **Methods:** The effects of two different bisphosphonates (Clodronate, Zoledronic acid) on proliferation of endothelial cells, 6 different tumor cell lines and human osteoblasts were evaluated with the Cell Proliferation ELISA, BrdU (Roche Diagnostics, Germany). In vivo we examined the effects of these drugs on primary tumor and metastases in two independent murine renal cell carcinoma model experiments. The anti-angiogenic properties were investigated by immunohistochemical staining of the tumors for CD31 to determine vessel density (MVD) and for flk-1 (VEGF-R2). Furthermore, we determined mouse VEGF-serum levels and examined the effects of bisphosphonates on a subset of 12 protein kinases relevant in angiogenesis. **Results:** Bisphosphonates inhibited the proliferation of endothelial cells and human osteoblasts at lower concentrations when compared to different tumor cell lines. In this respect Zoledronic acid proved to be more potent than Clodronate. Significant effects on the primary tumor growth in the RENCA model were solely seen with Zoledronic Acid (200 µg/kg/d). Vessel density (MVD) was decreased in all treatment groups. Both drugs showed equal efficacy, with Zoledronic acid being more potent. Flk-1 staining showed no differences between control animals and the group treated with bisphosphonates. However, murine VEGF-serum levels in mice bearing a RENCA tumor were significantly increased. Treatment with bisphosphonates led to reduced VEGF-serum levels. In addition, Zoledronic acid showed inhibitory potential on almost all tested protein kinases, in contrast to Clodronate, which had no

effect. **Conclusion:** In summary, these results indicate that bisphosphonates, especially Zoledronic acid, exert antitumoral and antiangiogenic activity. Further investigations are required to clarify the exact antiangiogenic mechanism.

Costs and Outcomes of Chemotherapy in an Observational Setting in Patients with Advanced NSCLC (ACTION)

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Introduction: Chemotherapy in the palliative setting for NSCLC patients did make progress during the last decade. Although this benefit is widely accepted, health care providers and decision makers have been alerted by the possibility of higher treatment costs. Instead of drug costs, total treatment costs while using newer chemotherapy regimens will become of more importance in the future. Especially newer agents, like gemcitabine, showed significant survival benefit and improved quality of life. Available data concerning total treatment costs for these newer agents currently only exist from retrospective assessments of large randomized, controlled clinical trials. Therefore clinical data from routine clinical care settings associated with related total treatment costs are of high interest. **Methods:** Instead of elaborating data from a restricted patient collective, which is always the case when analyzing clinical trial data, ACTION has an open, prospective setting. Individual physician's treatment decision will be documented including patient characteristics chemotherapy, toxicities and quality of life for an observational period of 18 month. Treatment outcome and related total costs will be assessed to evaluate the benefit of newer chemotherapy agents in the treatment of NSCLC in a wider population, documenting every day's use. **Status:** ACTION was carried out in 5 European countries (Finland, Germany, Netherlands, Portugal and UK). During an 18 month period 971 patients in about were recruited. The prospective, open, observational setting with the intention to evaluate total treatment costs is especially of high interest in Germany which is undergoing a change of the health care system in the hospital domain to a diagnose related groups (DRG) system. In Germany 574 patients are enrolled. Distribution of chemotherapy regimens will allow for an analysis of patient cohorts receiving gemcitabine regimens and patients receiving any other mono- or combination chemotherapy.

Dementia, Gait Disturbance and Incontinence, Seven Years after Curative Chemoradiation of a Central Nervous System (CNS) Lymphoma. Toxicity or Relapse? – Case Report

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Background: After combination chemoradiotherapy primary CNS lymphoma is cured in about 30% of patients below the age of 60 years (y) and in less than 20% of patients above 60 y. Treatment-related leukoencephalopathy is the leading toxicity after successful treatment. Neurologic symptoms including gait disturbance, urinary incontinence and dementia may start within months after treatment but have also been observed later during the course of the disease. MRI scans usually show cerebral atrophy, ventricular dilatation and hyperintensity on T2 images. Patients may need nursing home care and usually die after prolonged suffering. Autopsies are rarely carried out. **Case Report:** In 9/93 the 66 y old male patient was admitted because of mental confusion. His past history was unremarkable. On CT multiple lesions were seen in the left temporal lobe and signs of right intraocular lymphoma were observed. A stereotactic biopsy revealed large B cell lymphoma. A primary CNS lymphoma was diagnosed and treated with 2 cycles of COPP BLAM (Procarbazine at that time was thought to be especially active in the CNS) and whole brain radiation

including the orbitae (40 Gy). He did well until 6 y later when he started to have memory problems and was suspected to develop dementia. Extensive neurologic tests were carried out: In a CT ventricular distension and cerebral atrophy was seen. This was confirmed on MRI. No masses were seen. CNS fluid showed an elevated protein but no abnormal cells were found. He was put on a choline esterase inhibitor but his mental situation deteriorated. 40 months after the first signs of mental change he died of pneumonia. **Autopsy** confirmed degenerative changes and enlarged ventricles. Surprisingly, diffuse infiltration of large B-cell lymphoma was found in both hemispheres, the mesencephalon and in the brainstem. The findings were consistent with a relapse of the primary CNS lymphoma diagnosed 10 years earlier. **Conclusions:** Cerebral leukoencephalopathy may be a frequent late complication of combination treatment of CNS lymphoma especially in the elderly. Although comprehensive non invasive restaging exams were done in this patient, no indications of active lymphoma were detected. We may speculate that a repeat stereotactic biopsy would have confirmed the diagnosis. This may have been of consequence since retreatment with high dose Methotrexate has been described to induce second long term remissions in such patients. This case again confirms the importance of an autopsy in seemingly routine cases.

Primary Stage, Grading and Receptor Status Are Prognostic Factors in Metastatic Breast Cancer

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Background: We aimed to ascertain the influence of primary TN-staging, grading and hormone receptors on the prognosis of survival in breast cancer patients at the time they develop metastases. **Methods:** We searched Basel University Hospitals medical oncology data base and identified 50 female patients who were diagnosed between 1/1994 and 12/2003 with primary breast cancer without metastases and who later went on to develop metastases. At the time of diagnosis we looked at five different prognostic factors (tumor size, lymphnodes, grading, hormone receptors and histologic type) and went back to look at their influence on survival after the patients had developed metastases. **Results:** We observed that from the above five prognostic factors, grading, T-stage, N-stage and hormone receptors play a significant role. These factors displayed prognostic significance also at the time metastases had been diagnosed. Histologic type had no influence on prognosis. **Conclusion:** Lower tumor staging, lower grading and positive hormone receptors initially at the time of breast cancer diagnosis signify a more favourable prognosis for breast cancer patients at the time they develop metastases. This finding indicates that the above prognostic factors define distinct biologic subgroups.

Anti-EGFR Immunoliposomes Mediate Specific and Efficient Drug Delivery to Target Cells Including Multi-Drug-Resistant Sublines

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We have developed immunoliposomes (ILs) that bind to EGFR or mutant EGFRvIII and internalize into target tumor cells, enabling intracellular delivery of potent anticancer agents.

ILs were constructed modularly with various MAb fragments, including C225 (cetuximab)-, EMD72000 - Fab' and novel human scFvs from phage antibody libraries, covalently linked to liposomes containing various drugs or probes.

Fluorescence-labeled anti-EGFR ILs efficiently bound to EGFR-overexpressing cells (A-431, MDA-MB-468, U-87) demonstrated extensive in-

ternalization in the cytoplasm of target cells consistent with receptor-mediated endocytosis. Non-targeted (no Mab) liposomes and irrelevant (anti-HER2) immunoliposomes did not bind to or accumulate in these EGFR-overexpressing cells. Quantitative studies of uptake and internalization showed binding to MDA-MB-468 cells within 5 minutes, followed by intracellular accumulation detectable at 15 min and increasing to a plateau after 240 min. Total uptake of ILs at 240 min was 1.70 fmol phospholipid/cell corresponding to approx. 13'000 ILs/cell.

Anti-EGFR ILs were used to deliver various drugs (doxorubicin, vinorelbine, methotrexate) to a variety of EGFR-overexpressing 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. Immunoliposomal cytotoxicity was comparable to that of the corresponding free drug which easily penetrates cell membranes. On the contrary, in a multi-drug-resistant and EGFR-overexpressing cell line (MDA-MB-231 Vb100) ILs loaded with doxorubicin or navelbine produced greatly more cytotoxicity than the corresponding free drug. Comparing the IC50's, anti-EGFR ILs were able to increase the cytotoxicity of doxorubicin approx. 400-fold in this multi-drug-resistant cell line (IC50 ILs-dox = 0.6 versus IC50 free dox = 240 µg dox/ml).

PK and biodistribution studies of liposomes and ILs 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 (e.g. doxorubicin, epirubicin and vinorelbine) 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 conclusion, ILs provide efficient and targeted drug delivery to EGFR or EGFRvIII-overexpressing tumor cells, and might be able to overcome drug resistance mechanisms. In principle, this targeting approach can be used for the delivery of various probes, drugs and genes.

Successful Treatment of a Patient with Myelodysplastic Syndrome (RAEB) with Darbepoetin Alfa in Combination with Pegfilgrastim

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Background: The myelodysplastic syndromes (MDS) are a heterogeneous family of hematologic disorders characterized by ineffective hematopoiesis. For the majority of patients with MDS, no curative option exists. Supportive care has a major role for the treatment of patients with MDS. Erythropoietin (rEPO), a growth factor, is perhaps the most commonly used supportive care after transfusion. However, the response rates of treatment with rEPO reported in the literature are only 0–40%. **Patients and Methods:** 12/99 a 67-year-old man presented to our hospital with a mild leukopenia and thrombocytopenia. Bone marrow biopsy and aspirate revealed an abnormal proliferation, differentiation, and maturation with an excess of blasts. A refractory anemia with excess of blasts (RAEB) according to the French-American-British classification system was diagnosed. Until 12/01 no treatment was required. 01/02 the MDS progressed and we started with ciclosporine-A. After an initial rise of blood cell counts the MDS further progressed. Thalidomide was also ineffective and 07/03 the patient became transfusion dependent. He received darbepoetin alfa 150 µg once weekly and the transfusion intervals could be prolonged. Severe septic complications occurred. **Results:** 07/04 we combined the treatment with pegfilgrastim 6 mg every 4 weeks (Leucocytes 1.2/nl; Hb 7.4 g/dl; Thromb 23/nl). The treatment was well tolerated and all three cell lines responded: Leuco 3.1/nl; Hb 11.8 g/dl; Thromb 75/nl. The patient is now transfusion independent and no infectious complication occurred. **Conclusion:** Treatment with darbepoetin alfa alone of a patient with MDS was not very effective. However, the combined treatment with pegfilgrastim was highly effective and improved even the thrombocyte counts. Further investigation within a clinical trial is warranted.

Chemoimmunotherapy with Capecitabine and Peginterferon- α 2a (PEG-IFN) in Advanced Renal Cell Carcinoma (RCC): Six Consecutive Cases

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Background: Untreated metastatic RCC have an overall median survival of no more than 12 months and a 5-year survival of less than 10%. Because conventional therapy regimens such as single-agent chemotherapy or hormonal therapy have shown unsatisfactory results, new strategies are needed. Promising results were reported with the use of high dose recombinant cytokines (interleucin-2 and interferon), however, those treatments were associated with considerable drug-related toxicities. We report on six consecutive cases with metastatic RCC treated with a chemoimmunotherapy regimen consisting of peginterferon- α 2a and capecitabine. **Patients and Methods:** Between 10/03 and 10/04 three women (age 56, 58, 73 y) and three men (age 55, 69, 71 y) with metastatic RCC presented to our hospital with progressive disease. All patients had been nephrectomised before and most of them were treated with tamoxifen. Only patients with progressive disease were included in this survey. The sites of metastases were: the lung (5), lymphnodes (2), bone (1), liver (1), and brain (1). Patients were treated with 3–9 cycles of peginterferon- α 2a (180 μ g s.c. weekly) and capecitabine (1000 mg/m² bid days 1–14 repeated every 4 weeks). **Results:** The treatment was well tolerated without grade 3 or 4 toxicities. Two patients showed a nearly complete response after 6 and 9 cycles respectively. One patient showed a partial response and three patients were stable. So far only one patient progressed after 5 months of treatment and died. **Conclusion:** The combination of capecitabine and peginterferon- α 2a seems to be active in the treatment of advanced renal cell carcinoma with a favourable toxicity profile. Due to these promising observations a clinical trial is planned. More detailed data and the follow-up of the patients will be presented at the meeting.

Image Fusion between PET¹⁸F-FDG/MRI/CT for Radiotherapy Planning in Post-Operative High Grade Brain Tumor: Investigation of Usefulness for Target Volume Delineation

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Background: Post-operative radiotherapy after surgery (macroscopically complete and partial resection) is the standard care of the treatment for high grade brain tumors. Currently, two questions are not yet solved: what is the target volume definition and what is the optimal clinical margin around the tumor bed or around the residual tumor. To investigate these two questions, we performed a feasibility study to evaluate the usefulness of PET¹⁸F-FDG/MRI/CT image fusion, for the target volume delineation. **Method:** MRI is performed before surgery (image of the tumor) and 48 hours after surgery (image of residual tumor if partial excision or tumor bed if complete excision). PET¹⁸F-FDG is performed 3 weeks after surgery (just before simulation for radiation treatment). The three types of images are automatically fused with a precise algorithm. The tumor volume (initial site or residual tumor) or tumor bed visualised on each image method are delineated. The clinical tumor volume (CTV) is the result of addition of the three images. An acute margin (10 mm for 40 Gy, than 5 mm from 40 to 60 Gy) is chosen. **Results:** The accuracy of the fusion process is very high on PHILIPS PINNACLE treatment planning system (SYNTEGRA SMARTSIM). Coregistered PET¹⁸F-FDG/RMI/CT shows a good fusion accuracy on the PHILIPS PINNACLE treatment planning system (via SYNTEGRA SMARTSIM). Bound to the PET¹⁸F-FDG and MRI images, therapy plans using information from the data set are compared in terms of volume of normal brain tissue irradiated, and topography of the target delineated. Our experience is based on 44 high grade tumors treated from January 2002 to December 2004, 28 PET¹⁸F-FDG were performed during this period; and 17 PET¹⁸F-FDG showed an uptake (60 % of the cases). In 35% of the cases, PET¹⁸F-FDG-CTV is not included in CT-CTV (average volume difference is 23%), in

12% of the cases, PET¹⁸F-FDG-CTV is larger than CT-CTV. The better definition of target volume allows a reduction of the margins in 12 of the 17 using PET¹⁸F-FDG fused image. In these cases, the result is an 1.62 average decrease of the CTV. **Conclusion:** We observed that the accurate setting of post-operative high grade brain tumor is a new feasibility, available for clinical practice regarding PET¹⁸F-FDG/MRI/CT image fusion, for better target delineation in radiotherapy planning. A new field is now probably open: escalation of the dose. **Process Developing Study:** On basis of these premises (and literature results) we are currently in a process of developing a feasibility study which could include dose escalation in the area of PET¹⁸F-FDG uptake by IMRT method, associated with concomitant chemotherapy by TEMOZOLOMIDE.

Fransnod Trial: French Randomized Prospective Study Comparing Periareolar (PA) and Peritumoral (PT) Injection of Radiotracer and Blue Dye for the Detection of Sentinel Lymph Node (SLN) in Patients Treated by Breast Sparing Procedures

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Background: One of the most avidly debated issues in lymphatic mapping is where the tracers are best deposited in patients with breast cancer. The aim of this study was to compare on a randomized basis periareolar (PA) and peritumoral (PT) injection of radiotracer and blue dye for the detection of sentinel lymph node (SLN) in patients treated by breast sparing procedures. **Methods:** From October 2001 to January 2003, 459 patients with biopsy-proven operable invasive breast cancer (139 T0, 320 T1, N0) underwent breast conserving procedures with SLN biopsy. Informed consent was obtained from all patients enrolled in 10 institutions, before randomization in two groups PA and PT. Radiotracer (11–37 MBq of 99mTc-labeled sulfur rhenium colloid) and blue dye (2 ml Patent Blue) injections were both delivered into the PT (n = 231) or PA location (n = 228). SLN was assessed by standard hematoxylin eosin (HE) stained sections, serial sections with HE and immunohistochemistry. **Results:** The two groups were similar according to age, previous biopsy type, tumor size, location and histology. The mean number of SLNs identified was 1.67 (range 1–5) in PT group and 1.88 (range 1–6) in PA group. The SLN identification rate was 99.1% for both groups. The preoperative lymphoscintigraphy success rate was 72.9% for PT group and 85.2% for PA group (p = 0.002). The gamma probe success rate was 96.0% for PT group and 98.2% for PA group. The blue dye success rate was 93.7% for PT group and 95.6% for PA group. The isotope-blue dye joint SLN detection was 90.6% for PT group and 94.7% for PA group. The SLN was invaded in 36.5% of cases. Complete level I and II axillary lymph node dissection was performed for 107 patients in PT group, and 92 patients in PA group. The non SLN involvement rate was 22.4% in PT group and 23.9% in PA group. No per-operative adverse effect nor any postoperative morbidity was observed. At a median follow-up of 23 months (range 16–32), no axillary recurrence was recorded. **Conclusions:** This randomized study demonstrates clearly that peritumoral and periareolar injection show the same high detection rate of SLN (99%). On grounds of other advantages in many cases (non palpable tumors or upper quadrant location), the periareolar injection site can be privileged with confidence.

A Phase II/III Randomized Study in Advanced Non-Small Cell Lung Cancer (NSCLC) with First Line Combination versus Sequential Gemcitabine and Docetaxel: Second Interim Study Results

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Background: Single-agent gemcitabine (G) and docetaxel (D) are not cross-resistant and are effective in chemotherapy-naïve and pretreated patients (pts). The study objective was to compare clinically relevant haematological toxicity among pts treated with G and D either in combination or sequentially. **Methods:** Pts were stratified and proceeded through a sequential 4-step Phase II study, continuing into Phase III, with 327 evaluable pts planned per treatment arm. Three-week cycles of G 1000 mg/m² and D 75 mg/m² were given in 2 study arms: A (G: Days 1,8 + D: Day 8 for 6 cycles) and B (G: Days 1,8 for 3 cycles followed by D: Day 1 for 3 cycles). Pts in Arm B switched to D earlier if tumour progression occurred during treatment with G. NCI-CTC grade 3–4 thrombocytopenia requiring transfusion, anaemia requiring transfusion or febrile neutropenia requiring i.v. antibiotics were considered to be clinically relevant toxicities (CRT). **Results:** After the 2nd step of the phase II study 201 pts are analyzed. The characteristics of 107/94 pts (arm A/B) are: Gender (m/f) 83/24 (arm A) and 73/21 (arm B); median age in years (range) 63.3 (39.8–78.7) (arm A) and 65.7 (39.7–81.2) (arm B); WHO PS (0 or 1/>1) 93/14 pts in A and 82/12 pts in B; histology: adeno/squamous/others 50/35/22 pts in A and 37/27/30 pts in B; stage IIIB/IV 13/94 pts in A and 13/81 pts in B. Number of cycles given (A/B): 385/413. CRT per cycle (primary endpoint from phase II study) for (A/B): Anemia/transfusion 8/5; thrombopenia/transfusion 1/1; febrile neutropenia/antibiotics i.v. 6/1 with $p=0.0463$ for comparison of single variable. Number of all received transfusions and antibiotics per cycle (A/B): Red blood cell transfusions (total units) 20 (40) / 14 (34); platelets transfusions (total units) 1 (1) / 1 (4); antibiotics i.v. (treatment days) 38 (286) / 25 (159) with $p=0.0457$ for comparison of single variable. CRT is not statistically significant different in both study arms ($p=0.1048$). Survival data do not differ significantly (A/B: median 7.1/8.6 months with confidence intervals [5.2; 9.5] / [5.9; 10.4]). **Conclusion:** These interim data suggest that sequential treatment is as effective as the combined one. Though total CRT is not statistically significant different in both study arms single important toxicity values are statistically significant in favor of sequential treatment.

Galectin 3, Oncofetal Fibronectin and Emmprin Expression in Thyroid Tumors

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Currently fine needle aspiration biopsy is the best diagnostic technique for thyroid nodules. This method has some limitations, such as, its lack of sensitivity for vesicular tumors and may not be informative in 5 to 20% of fine needle aspiration samples. This is usually due to an insufficient number of thyroid cells in the sampling. The use of molecular markers of malignancies could improve the diagnostic performance of these non-informative biopsies by typing the few cells sampled. The choice of these markers needs to be performed on tissue samples.

The aim of this study was to analyse surgical thyroid tissue samples, to determine the expression of 3 genes that may be effective for cancer diagnosis: galectin 3, fibronectin and emmprin, a metalloproteinase inducer. Eighty samples were analysed and divided into 5 types: 13 normal thyroid tissue, 16 adenoma, 37 papillary carcinoma, 8 vesicular carcinoma and 6 non differentiated carcinoma. For each sample, the gene expression was

assessed compared to an ubiquitary gene 'RPLPO' using the quantitative real time RT-PCR from total messenger-RNA. The ratio concentration of studied genes over RPLPO represented the level of expression of the studied gene in each sample. For the 3 studied genes, medians of this ratio were calculated for each type of tissue sample. These ratios were compared using an ANOVA and Scheffe tests (proc GLM Sas Institute). The emmprin levels of expression were comparable in all 5 types of tissue studied ($p=0.6$). Galectin 3 and fibronectin levels of expression increased significantly only in papillary carcinoma compared to normal tissue. The mean value of galectin was 1.19(IC 95%:0.94–1.44) vs 0.13(IC 95%: 0.08–0.18) and mean value of fibronectin was 4.48(IC 95%:2.5–6.06) vs 0.04 (IC 95%: 0.02–0.07).

The emmprin gene didn't seem effective in distinguishing different tissue types. The significantly increased levels of fibronectin and galectin found in papillary carcinoma compared to normal tissue may confirm the interest in these 2 genes as molecular markers of papillary cancers. An evaluation of these genes in cytology samples is now being conducted.

Long-Term Results after Consolidation Radiotherapy on Residual Disease < 1 cm in 106 Stage III Ovarian Cancer Patients

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Background: Attempts to increase survival of stage III ovarian cancer patients (pts), with macroscopic complete response at second-look laparotomy, have included prolonged chemotherapy, dose intensification, and consolidation radiotherapy. We present long term survival of 106 pts treated between 1983 and 1993 for stage III ovarian adenocarcinoma with first-look debulking, cisplatin-based chemotherapy, second-look surgery with a residual disease < 1 cm and consolidation radiotherapy. **Method:** Median age was 52 years (range 16–73). Residual disease after first look surgery was < 1 cm for 40.5% of pts. Residual disease < 1 cm was obtained in all pts after second look surgery, with 33% of pts without residual disease. Radiation was performed using a linear accelerator. The whole abdomen dose was 22 Gy with antero-posterior fields and a maximal kidney dose of 20 Gy. 71 pts received a 25 Gy pelvic boost. 33 patients received an additional 12 Gy lombo-aortic boost. **Results:** Median follow-up was 14 years. Radiation was stopped for acute toxicity in 11 pts. Long term toxicities included radiation enteritis in 21 pts with 9 pts requiring surgery for bowel obstruction, radiation cystitis in 4 pts, acute leukemia in 2 pts, dysmyelopoiesis in 1 pt, skin cancers in the radiation field in 1 pt, bilateral femoral head necrosis in 1 pt. 4 pts died because of radiation complications. Second cancer was diagnosed in 12 pts with 7 breast adenocarcinomas, 3 colon adenocarcinoma, 1 non-small cell lung cancer, and 1 pancreatic cancer. Overall survival (OS) at 5 and 10 years was respectively 53% and 36%. The main prognostic factor was residual disease after initial surgery, with a 10-year survival of 54% with no or microscopic residual disease, 48% with < 1 cm residual disease, 25% with 1–2 cm residual disease. **Conclusion:** Long term survival of our patients compares favorably to those of similar patients treated in phase II studies with consolidation high-dose chemotherapy (5 year survival < 40%), at the price of high toxicity incidence (3,7% of toxic deaths).

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