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Guest Editor

Christine Landmann, Basel, Switzerland

Investigations of Molecular and Immunological Markers in Patients with Lung Cancer

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Background: Soluble mediators which are secreted by malignant cells can significantly influence the capacity of peripheral lymphocytes to secrete immunomodulatory cytokines. Many tumors have been found to secrete immunosuppressive molecules such as transforming growth factor β 1 (TGF β 1), interleukin-10 (IL-10) and Fas ligand. In addition, genetic and epigenetic alterations of neoplastic cells can directly influence the biological behavior of the tumor.

Methods: We assess the capacity of peripheral lymphocytes to secrete cytokines upon stimulation with PHA. Molecular changes can be monitored in the periphery after purification of genomic DNA from serum or plasma of lung cancer patients. Upon chemical treatment with sodium bisulfite the DNA was subjected to a methylation-specific PCR.

Results: TGF β 1 is secreted by SCLC and we have demonstrated that suppression of IL-2 in SCLC-patients is correlated with reduced survival. Currently, the influence of cytokine secretion on the efficiency of a vaccination therapy is being investigated. In NSCLC, increased production of immunosuppressive IL-10 by the tumor and increased plasma IL-10 concentrations have recently been correlated to reduced survival. In NSCLC-patients we could indeed demonstrate an impairment of IL-2 secretion by these immunocompetent cells. More important, this reduction correlated with reduced survival. Thus different types of lung cancer may use distinct molecules to escape immune defense with clinically relevant success. Hypermethylation analyses of promoter regions encompass DNA repair enzymes (MGMT), apoptosis-related (DAP kinase) and tumor suppressor genes (p14, p16, RAR β , RASSF1A). Furthermore, point mutations in codon 12 of *k-ras* and the β -tubulin gene (codons 147, 245 and 246) were investigated.

Conclusions: The concomitant assessment of immunological and molecular data provides the basis to collect a whole set of different biological data from a single patient. The combination of such markers may be of much greater prognostic and predictive value than each of them alone. A thorough analysis of these parameters may lead to a better prognosis and may potentially provide the basis of an improved customized therapy for an individual patient. Finally this may lead to a novel approach which may allow a tumor treatment which is based on its biological properties rather than its histogenetic origin.

Standard (SCT) versus Dose-intensified Chemotherapy (ICT) with Sequential Re-infusion of Peripheral Ood Cells (PBC) in SCLC

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Objective: To determine whether a dose intensification of ICE chemotherapy may be associated with a survival prolongation in pts with good prognosis SCLC.

Methods: Eligibility criteria included: Manchester Score (MASC) 0–1; < 70 years, no prior tumor therapy, normal hematologic, renal, hepatic function, no brain metastasis. Chemotherapy consisted of Ifosfamide, 5 g/m² d1 (24h), Carboplatin 300 mg/m² d1, Etoposide 180 mg/m² d1+2. In pts on ICT, cycles were repeated q2w x6 and G-CSF (300–480 μ g) was given d4 to d13. Whole blood (750 ml) was collected on d15 prior to ICT (cycles 2–6) and reinfused on d3. In pts on SCT, cycles were repeated every q4w x6.

Results: From 10/96 to 9/01 a total of 70 pts have been entered, (ICT n=35, SCT n=35). Median age was 54 years, 36 pts had a MASC 0, 34 a MASC 1. Male/female ratio was 55/15; LD/ED ratio was 66/4. A total of 388 CT-courses have been delivered (ICT: 201, SCT: 187). The main hematologic toxicity was thrombocytopenia with a significant difference between treatment arms (d14 G 3/4 60,4% in ICT and 38,5 in SCT). No difference was seen in non-hematologic toxicity between ICT and SCT. 63 pts responded (CR/PR): 33 pts on ICT (16 CR/17 PR), and 30 pts on SCT (13 CR/17 PR). In pts on ICT, MS was 29.8 months, in pts on SCT 17,4 months 1-year-S and 2-year-S was 83% and 51% on ICT and 77% and 29% on SCT.

Conclusion: ICE with whole blood – peripheral blood stem cell support is feasible, effective and shows statistically significant superiority over SCT (p=0,023).

Targeted Radiotherapy for Small Cell Lung Cancer Using ⁹⁰Yttrium-DOTATOC, an Yttrium-labelled Somatostatin Analogue: Preliminary Results of a Pilot Trial

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Background: SCLC is of neuroendocrine origin and has been shown to express neuroendocrine markers, such somatostatin receptors. SCLC is therefore a candidate for targeted radiotherapy, using ⁹⁰Y-labelled octreotide, a somatostatin analogue, called DOTATOC.

Objectives: The objectives of this pilot trial were to assess response rate, progression free survival and toxicity in patients with SCLC, having failed ≥ 1 regimen of chemotherapy and presenting with a positive ¹¹¹In-octreotide scintigramm.

Methods: Patients were treated with one cycle of 60 mCi/m² DOTATOC iv. every 3 weeks, for a total of three cycles. Patients had to be ≤ 75 years old, have measurable tumor lesions, normal creatinine clearance, a PS of ≤ 2, adequate bone marrow function and had given written informed consent. Before therapy 500 ml Hartmann-Hepa solution was administered to avoid nephrotoxicity.

Results: So far 6 patients have been treated (3 men and 3 women). Three patients had a 2+ octreotide scintigramm (tumor as positive as liver), 3 patients a 3+ (tumor more strongly positive than liver). Median age was 58 (47–66). Two patients received DOTATOC as second line therapy, 3 in third line and one in 4th line. Two patients had previous mediastinal radiotherapy and one had brain irradiation. Three patients had elevated LDH levels at baseline and > 10% weight loss in 6 months before treatment. Median number of cycles was 2 (1–3). One patient received both iv. and intrathecal ⁹⁰Y-DOTATOC (20 mCi). All six patients had progressive disease, median PFS was 37.5 days (28–52) and median OS was 80 days (28–269). The only grade 3 toxicity observed was fatigue in 2 patients and dyspnea in 1 patient. None of the side effects could be attributed to DOTATOC treatment. Specifically, there were no severe renal and hematological toxicities observed.

Conclusions: In contrast to well differentiated neuro-endocrine tumors, ⁹⁰Y-DOTATOC seems to be inactive in SCLC. New, more specific targets for SCLC need to be identified.

Final Results from Two Randomised Phase II Studies with Single Agent Gemcitabine and Docetaxel Given Sequentially in Various Doses and Schedules Are Effective in Advanced NSCLC

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Gemcitabine (G) and docetaxel (D) have been shown to be effective in chemotherapy-naïve and pre-treated patients and are not cross-resistant. We have been testing G and D in various doses and schedules (Proc ASCO 2001, 337a(1346)) giving G or D initially up to 6 cycles and in case of tumor progression switching to the opposite drug (up to 6 cycles). 480 patients entered the studies (IIIB/IV 13%/87%; PS 0 or 1/>1: 78%/28%), and 380 have been analysed. Median survival (MS) differs significantly between IA and IB (Kaplan-Meier [KM]; log-rank p=0.023; ranksum p=0.012), but not between IIA and IIB. So far, IIA and IIB can be statistically considered as equally effective, however 1/2-year and 2-year survival for IIA and IIB show an inverse structure (all available data were used).

IA vs IIA and IA vs IIB show no difference in KM. IIB is significantly superior to arm IB (log-rank p=0.015 and rank sum p=0.002).

The evaluation of the quality of life forms as given in EORTC-30 query showed no difference between IA and IB. Final analysis data for the 480 patients included will be presented.

The use of G and D as carried out in IA, IIA, and IIB is effective and indicates that G and D given sequentially may be an alternative to conventional combinations in CT.

ARM	IA-Q4W	IB-Q4W	IIA-Q3W	IIB-Q3W
1 st line	G:d1,8,15	D:d1,8,15	G:d1,8	D:d1
mg/m ²	1000	35	1250	100
Patients (N)	95	48	120	117
MS (months)	8.0	5.0	6.4	9.2
Confidence Interval	5.5;10.5	3.0;6.5	5.2;7.5	7.1;10.4
1-year survival %	31	21	27	28
censored obs.	5	0	23	24
1/2-year survival (n/N)	55/95	19/48	70/139	82/132
1/2-year survival %	58	40	50	62
censored obs.	5	0	46	57
2-year survival (n/N)	11/95	2/48	6/45	3/47
2-year survival %	12	4	13	6
censored obs.	5	0	1	0

A Phase I Study of Two Doses of ZD 1839 in Combination with Docetaxel in Patients with Advanced Non-Small Cell Lung Cancer

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Background: ZD 1839 ('Iressa') is an orally active, selective EGFR-TKI (epidermal growth factor receptor tyrosine kinase inhibitor) that has been investigated for the treatment of locally advanced and metastatic NSCLC. ZD 1839 has shown antitumor activity and is generally well tolerated as monotherapy in pts. with advanced NSCLC. Docetaxel has been established as second line treatment for advanced NSCLC.

A phase I study was performed to assess the safety of ZD 1839 in combination with docetaxel in pts. with advanced NSCLC.

Methods: Pts. with locally advanced NSCLC or metastatic NSCLC, either untreated or pretreated. 13 Pts. have been enrolled: median range age, 59 (46–72) years; M/F, 9/4; performance status 0/1, 1/12; disease stage IIIB/IV, 2/11.

Chemotherapy Regimen: On day 1 pts. received the standard chemotherapy regimen with docetaxel (75 mg/m² iv). On day 2 the treatment of ZD 1839 was commencing and was given in the dosage of either 250 or 500 mg once daily. Additional standard cycles of docetaxel were administered every three weeks concurrently with ZD 1839 for up to 6 cycles in total.

Results: Adverse event (AE) data are available for 12 pts. (250 mg 6 pts.; 500 mg 6 pts.). At 250 mg/day no dose limiting toxicities (DLTs) were observed. ZD 1839 related toxicities included G1/2 skin rash (4 pts.), G1 diarrhea (1 pt.), and docetaxel related AEs included leukopenia (G1/2, 2 pts.; G3, 4 pts.), neutropenia (G1, 1 pt.; G3/4, 5 pts.), fatigue (G2, 4 pts.), mucositis/stomatitis (G1, 4 pts.), and nausea (G1/2 3 pts.). In the 500 mg/day dose group, 2 pts. had DLT; G3 diarrhea lasting over 4 days (1 pt.) and G3 skin rash (1 pt.). At this dose, the common ZD 1839-related AEs were diarrhea (G1/2, 3 pts.; G3, 3 pts.) and skin rash (G1/2, 3 pts.; G3, 1 pt.) and docetaxel-related AEs included leukopenia (G2, 2 pts.; G3/4, 4 pts.), neutropenia (G2, 1 pt.; G3/4, 5 pts. of whom 3 had febrile neutropenia with no proven sepsis), and mucositis/stomatitis (G1/2, 4 pts.; G3, 1 pt.). Recruitment is ongoing at the dose 500 mg dose. Pharmacokinetic data and antitumor activity will be presented.

Conclusion: The combination of ZD 1839 and docetaxel did not cause any unpredictable toxicity in pts. with advanced NSCLC.

'Iressa' is trademark of the AstraZeneca group companies.

Pemetrexed (ALIMTA): a New Effective Drug in Chemotherapy of Malignant Pleural Mesothelioma (MPM)

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No approved or effective chemotherapy has been identified for MPM. In a phase I trial of cisplatin and pemetrexed (ALIMTA), a novel antifolate targeting key enzymes in purine and pyrimidine synthesis, a promising antitumor activity was observed in MPM patients (pts.). In a randomized single-blind multi-center phase III trial with survival as primary endpoint Arm A consisted of pemetrexed 500 mg/m² i.v. followed by cisplatin 75 mg/m² on day 1 q3 weeks. Arm B consisted of cisplatin 75 mg/m² d1 q 3 weeks. After 118 pts had been enrolled, an unacceptable rate of severe toxicity including drug-related death occurred in arm A. To improve the safety profile and to maintain blinding, all subsequent pts in both arms received a low dose folic acid and vitamin B12 (FA/B12). In total, 456 pts were randomized, 228 Arm A and 228 Arm B from 5/99–3/01. Of these 170 pts in Arm A and 168 in Arm B were randomized after required FA/B12 supplementation. At the final analysis, median follow-up was 9.3 months with 304 of 448 possible death having occurred. Pts in Arm A had a longer median survival. Longer time to progressive disease, and higher response rate compared to Arm B. Pts in Arm A had more toxicity including Grade 3/4 neutropenia (ANC), and stomatitis than in Arm B. However FA/B12 clearly reduced the frequency of toxicity in Arm A without adversely affecting efficacy. Quality of life and pulmonary function tests

were evaluated in the study. The Lung Cancer Symptom Scale components of dyspnea and pain were significantly improved in Arm A compared to Arm B at cycle 6. Lung function also improved significantly for Arm A as measured by vital capacity and forced expiratory volume (FEV1) at cycle 6. In addition to survival advantage, the treatment with Pemetrexed and cisplatin is associated with greater symptomatic benefit for MPM pts. Pemetrexed and cisplatin supplemented with FA/B12 should now be considered standard treatment for pts with non-resectable MPM.

Adjuvant Combined Radiochemotherapy for Advanced Gastric Cancer – First Experiences with Acute Toxicity

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Background: To present our experiences with regard to acute toxicity of adjuvant Radiochemotherapy for gastric cancer

Methods: Since February 2001 18 patients with gastric cancer were treated with combined radiochemotherapy according to the protocol published by MacDonald et al. Median age was 61 years (39–69). Male/female-Ratio was 11:7. Karnofsky-performance-index was between 60 and 100%. Initially patients received 425mg 5FU/20mg FA for 5 days, followed then by radiochemotherapy with a total dose of 45 Gy with single daily fractions of 1,8 Gy, five days per week. During radiotherapy chemotherapy was given on day 1–4 and 23–25 at reduced dose of 400 mg 5FU/20mg FA. Two more 5-day cycles of full dose chemotherapy were given 4 and 8 weeks after radiochemotherapy.

Results: 12 of 18 patients finished therapy. 2 patients showed progress of disease during therapy, three patients declined therapy, and one patient died due to peritoneal spread before the last chemotherapy-cycle. Mean weight-loss amounted to 4.5 kg (0–10 kg). 13 patients suffered from gastrointestinal toxicity ≥ Grad 3 according to the CTC-Scale. Hematologic side effects ≥ Grad 3 were not observed.

Conclusion: Adjuvant combined Radiochemotherapy after resection of gastric cancer shows a high toxicity. Special supportive treatment is necessary.

Increasing Doses of Caelyx® in Combination with Infusional 5-Fluorouracil, Sodium Folinic Acid and Mitomycin C in Patients with Gastrointestinal Cancer: a Phase I Study

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Background: Infusional 5-fluorouracil (5-FU), folinic acid (FA) and mitomycin C (MMC) have demonstrated activity in patients (pts) with gastrointestinal cancer. Using 3-weekly bolus MMC 10mg/m² with weekly infusional 5-FU/FA (AIO-schedule) as first-line therapy in patients with metastatic gastric cancer we observed a response rate of 55% and overall survival of 10.6 months in gastric cancer patients (pts). In order to improve this treatment schedule, we added another drug (Caelyx®) to this regimen. We have launched a phase I study in pts with pretreated gastrointestinal cancer adding increasing doses of Caelyx® (liposomal, pegylated adriamycin) to infusional 5-FU/FA and MMC. Dose-escalation schedule: 5-FU 2,000 mg/m² and sodium FA (Oncofolinic®) 500 mg/m² mixed in one pump weekly × 6, MMC 7 mg/m² days 8 and 36, Caelyx 15–25–30–35 mg/m² (level I, II, III, IV, respectively) days 1 and 29. The cycle is repeated on day 57.

Results: 24 pts have been recruited so far. The pts suffered from pancreatic cancer: n=11; gastric cancer n=10; gallbladder cancer n=3. They had been pretreated with 5-FU/capecitabine n=7 and/or gemcitabine n=14. Two pts were withdrawn because of anaphylactoid reaction during the first Caelyx administration. No dose limiting toxicity (DLT) has been observed on level I (n=3). On level II, 3 out of 4 patients experienced DLT between treatment days 14 and 16 (leucopenia grade III–IV n=3, mucositis grade III n=3). Therefore, Caelyx 25 mg/m² was determined as maximum tolerated dose (MTD). Because of the large gap between the dose levels of 15 and 25 mg/m² we decided to add a 20 mg/m² level (Ia). 13 pts are evaluable on this dose level so far. 11 pts completed the first cycle without DLT, and in 2 pts DLT was observed (leucopenia grade IV n=1, diarrhea grade III n=2). Treatment in these pts was interrupted. Apart from these, treatment delay on dose level Ia (19 cycles) was only necessary in 2 pts and dose reduction in 1 pt. Antitumor activity and clinical benefit responses have been observed on all 3 dose levels.

Conclusions: The recommended dose of Caelyx in combination with weekly infusional 5-FU/FA and 4-weekly MMC is 20 mg/m². The study is ongoing on this dose level, now allowing for the recruitment of pts with advanced gastric cancer as first-line treatment.

Intraoperative Radiotherapy (IORT) and Post-operative Radiochemotherapy in the Palliative Treatment of Advanced Pancreatic Cancer

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Background: Most patients with non-resectable and relapsed carcinoma of the pancreas suffer from severe local pain and show a mean survival-time of only 2 months. Palliation of symptoms to maximize quality of life should be the primary aim of every therapy. We analyzed the effectiveness of the external beam radiotherapy (EBRT) combined with or without intraoperative radiotherapy (IORT), respectively chemotherapy.

Methods: From 06/91–12/01 48 patients with an advanced adenocarcinoma of the pancreas were treated by irradiation. 29 patients received a combined radiochemotherapy (5-FU or gemcitabine), 19 patients were treated by radiotherapy alone. 14 patients had intraoperative radiotherapy with electrons (IORT) during the trial laparotomy. The mean IORT-dose was 15.9 Gy (15–20), combined with additional postoperative radiotherapy of 30–43.2 Gy.

Results: Patients treated with a higher dose respectively with additional IORT showed an increase of mean survival-time from 2 to 6, respectively 8.5 months. The combination with 5-FU did not show any improvement of survival, but in combination with gemcitabine there was a survival increase of 7.5 months. 42 patients had the typical abdominal pain syndrome before therapy. Immediately at the end of the treatment 31 patients (74%) had pain reduction. In combination with IORT we saw a higher rate of complete pain relief (13/14 patients) and patients with IORT showed a faster pain reduction. No patient had severe gastrointestinal side effects.

Conclusion: An increase of survival and a good pain relief was achieved by the multimodal therapy concept. In respect to the palliative treatment intent IORT offers a possibility to a faster pain relief combined with a shorter treatment time.

STRAP, a Negative Regulator of TGF- β Signaling, Is a Prognostic and Predictive Marker in Colorectal Cancer Patients Treated with Adjuvant 5-FU-based Chemotherapy

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Purpose: Adjuvant 5-FU chemotherapy has been shown to increase survival in resected stage III colorectal cancer by 10–12%. Frequently patients with stage II disease receive ad-

juvant therapy too, although its benefit in that situation is uncertain. Molecular markers predicting the benefit of adjuvant treatment are urgently needed. Since genetic alterations of the TGF- β pathway play an important role in colorectal tumorigenesis, we aimed to analyze negative regulators of this pathway with regard to prognosis and prediction of adjuvant 5-FU chemotherapy benefit.

Experimental Design: By using quantitative real-time PCR, copy numbers of the TGF- β pathway genes *ski*, *SnoN* and *hSTRAP* were analyzed in a large series of paired normal and tumor tissue biopsies, which had been collected from a randomized multicenter trial of 5-FU/MMC adjuvant chemotherapy of the Swiss Group of clinical Cancer Research.

Results: We observed deletions of *ski*, *SnoN* and *hSTRAP* in 36.5, 50 and 41%, respectively, whereas amplification occurred in 8.7, 13 and 20% of the tumors, respectively. Patients bearing tumors with an amplification of *hSTRAP* had a significantly better prognosis than patients without a change in the *hSTRAP* locus (hazard ratio for overall survival: 0.262 ($p=0.0054$)). More importantly, while patients carrying a *hSTRAP* amplification were found to be harmed by 5-FU/MMC adjuvant treatment (hazard ratio for overall survival: 3.29 ($p=0.0241$)), those with normal *hSTRAP* gene copy numbers clearly benefited from adjuvant chemotherapy (hazard ratio for overall survival 0.33 ($p=0.001$)).

Conclusions: Our findings suggest that *hSTRAP* amplification is a positive prognostic and a negative predictive marker of chemotherapy benefit and thus might help to protect a substantial subgroup of colorectal cancer patients with a favorable prognosis from a deleterious treatment effect.

Experience with a Reduced Weekly Regimen of Irinotecan in Patients with 5-FU Refractory Colorectal Cancer

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Background: The feasibility of Irinotecan in an outpatient setting is challenged by a marked toxicity profile. Our observational study was intended to investigate the tolerability and efficacy of a reduced weekly regimen.

Methods: We report 30 patients which were treated with a weekly Irinotecan regimen on an outpatient basis. 9 of these patients were treated with a regular dose of 125 mg/m² (122 $\pm$ 2.6 mg/m² SD), the other 21 Patients received a reduced regimen with a weekly dose of 80 mg/m² (77.9 $\pm$ 4.1 mg/m² SD). Other patients characteristics are shown in table 1.

Results: For Patients who received a weekly Irinotecan regime with 125 mg/m² the median time to progression (TTP) was 3.5 months (0,1–11,4). For those patients with a reduced weekly scheme of 80 mg/m² the median TTP was 3.8 months (1,0–10,5) (see figure 1).

There was a remarkable difference in side effects between these two treatment groups. With an identical premedication consisting of atropine and dexamethasone we observed the following side effects (80 mg/m² vs. 125 mg/m²): Gastrointestinal cramps 9.5 vs. 33.3 %; nausea/vomiting: 14.3 vs. 33.3 %, diarrhea 23.8 vs. 55.6 % and asthenia 14.3 vs. 22.2 %. These results are also shown in figure 2.

Conclusion: The presented data of 30 Patients treated for advanced colorectal cancer underlines the efficacy of Irinotecan. The significant toxicity of weekly Irinotecan regimens was never the less a substantial argument and often an obstacle for the use of Irinotecan in this palliative setting. Our observation shows a marked improvement of tolerability when a reduced dosing scheme of 80 mg/m² vs. 125 mg/m² is used. In this small group the improvement side effects was not related to an impaired response rate. We think this data is a strong argument for the conduction of controlled phase III trials to verify this hypothesis.

Appendices and figures:

<i>Histology</i>	
Colon-Ca.	16
Rectal-Ca.	14
<i>Sex:</i>	
Female	14
Male	16
<i>Age:</i>	
Age (Min/Max)	59 (39–73)

Tab. 1. Patients characteristics

Fig. 1. Time to Progression (TTP) under weekly Irinotecan Regimens

Fig. 2. Toxicity with two different Irinotecan regimens

Randomized Phase III Study of Adjuvant Radiochemotherapy with Capecitabine versus 5-Fluorouracil (5-FU) in Rectal Carcinoma

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Background: Combined modality treatment (5-FU based chemotherapy plus concurrent pelvic irradiation of 50.4 Gy) is the recommended adjuvant therapy for patients with clinical pT3/4 or pN + resected rectal cancer. In the postoperative setting this treatment has been shown to decrease local recurrence rates and to improve overall survival. Although advances in surgical techniques (e.g. total mesorectal excision [TME]) have improved results, radiation therapy is still mandatory for local control. Capecitabine, an oral prodrug of 5-FU is at least as active as bolus 5-FU in the treatment of metastatic colorectal cancer. It has been shown that capecitabine can safely be combined with pelvic radiation therapy for rectal cancer (recommended dose capecitabine 1650 mg/m² daily). Capecitabine may also be beneficial for the adjuvant setting of radiochemotherapy. Thus, we have launched a randomized phase-III study within the German «Arbeitsgemeinschaft für Internistische Onkologie» comparing pelvic irradiation (50.4 Gy) in combination with either capecitabine (arm A) or 5-FU (arm B) after complete removal of the primary with TME.

Study design: The primary study endpoint is survival after 3 and 5 years. Secondary endpoints are recurrence rates and disease-free survival after 3 and 5 years. 520 patients will be randomized between both treatment arms in order to prove equivalence between oral (capecitabine) and intravenous (iv) 5-FU. Statistics are based on the assumption that capecitabine is equieffective (i.e. non inferior to iv 5-FU allowing for a maximum relative inferiority rate of 10% at the time point 5 years after the stop of recruitment). Patients randomized to arm A receive 2 cycles of capecitabine (2500 mg/m² days 1–14, repeated day 22) followed by radiochemotherapy (50.4 Gy and capecitabine 1650 mg/m² daily during the irradiation). 4 weeks after the completion of radiochemotherapy, another three cycles of capecitabine will be administered. Pts randomized to arm B will receive iv 5-FU-based radiochemotherapy according to the recommendations of the «Deutsche Krebsgesellschaft». The study has recently been started. Centers interested in participation are invited to participate.

Oncology Family Clinic in a University Hospital. A Pilot Demonstration Project

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Background: Forty percent of cancer patients have one or more near relative 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 university 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 Counseling [2].

Results: 46 individuals have been referred consecutively during the first 5 years: 36 female, 10 male, median age 40 years (range: 27–77); 29 (63%) had one or two neoplasias, 17 (37%) were unaffected and 2 (4%) had cancer with a negative family history. The family cancer patterns were: 14 (32%) breast-breast, 8 (18%) breast-others, 7 (16%) colorectal-colorectal, 4 (9%) gastric-gastrointestinal, 3 (7%) breast-ovary, 8 (18%) other combinations.

Gene testing was done in 10 (22%) families (5 BRCA1+2, 3 hLMH1+hMSH2 and 2 p53).

3 pathogenic mutations have been identified (2 hMSH2 and 1 hLMH1). Several families are studied by research consortia.

All probands and their families are counseled 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. Counseling and mutation testing is facilitated within a research oriented environment.

1 Calzone KA, Biesecker BB: Genetic testing for cancer predisposition. *Cancer Nursing* 25:15–27, 2002.

2 Pichert G, Stahel RA: Organizing cancer genetics programs: the Swiss model. *J. Clin. Oncol.* 18:65s–69s, 2000.

Pharmacokinetics of Amifostine, WR-1065 and the Disulfid Metabolites: Subcutaneous vs. Intravenous Administration

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Background: WR-1065, the active metabolite of amifostine (Ethyol®), serves as a protector against chemotherapy- and radiation-induced toxicities. Determination of amifostine,

WR-1065 and the resulting disulfid metabolites in plasma was done after subcutaneous (s.c.) or intravenous (i.v.) application of 500 mg amifostine in patients (pts.) with head and neck cancer receiving postoperative radiotherapy.

Methods: 448 blood samples of 5 pts. after s.c. and 378 blood samples of 4 pts. after i.v. drug administration were enrolled into this study. Amifostine was given 30 min prior to daily fractionated irradiation, five days a week for 6–7 weeks during radiotherapy. Plasma analysis was performed on day 1, 4, and 5 of week one, and for the first days of all the following weeks of treatment. For the quantification of amifostine, WR-1065 and the disulfides a RP-HPLC method was performed.

Results: For amifostine the mean values of C_{max} were 11418 (i.v.) and 3557 (s.c.) ng/ml, for T_{max} 6 (i.v.) and 35 (s.c.) min. The plasma clearance was 1.87 (i.v.) and 1.68 (s.c.) l/min. The mean terminal half life was 107 (i.v.) and 79 (s.c.) min. For WR-1065 the mean values of C_{max} were 2229 (i.v.) and 1362 (s.c.) ng/ml, for T_{max} 6 (i.v.) and 28 (s.c.) min. The plasma clearance was 13.7 (i.v.) and 8.0 (s.c.) l/min. The mean terminal half life was 111 (i.v.) and 31 (s.c.) min. The s.c. administration led to comparable AUCs of amifostine and WR-1065 as was obtained after i.v. dosage. After repeated s.c. administrations there is evidence for an accumulation of the disulfides in plasma, leading to an increase of C_{max} as well as AUC. The disulfides were cleared much slower from the plasma than amifostine and WR-1065.

Conclusion: These data provide that s.c. administration of amifostine is suitable during full course of RT. The subcutaneous route of administration seems to be an attractive choice and offers a more practical route of administration for cancer patients during radiotherapy.

Thermoablative Treatment of Liver Metastases

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Background: Standard treatment for solitary liver metastases is ablative surgery. Some patients cannot tolerate this procedure due to advanced age or comorbidity etc. There are other local interventions like ethanol-injections or thermoablation. In our institution there is now increasing experience with radiofrequency ablation (RFA), a special form of thermoablation.

Methods: More than 20 patients have been treated so far at our institution with RFA for hepatic metastases. The number of metastases treated in the individual patients ranged from 1–3. In some patients the intervention was performed on several occasions.

Results: Based on this heterogeneous group of patients, we will discuss technical aspects of the procedure, patients selection and outcome in correlation to pretreatment variables. In

some patients the shooting-up of satellite metastases in close neighborhood of the RFA lesion shortly after the intervention has been observed. In this context the possibility of ‘growth stimulation’ due to hypoxia induced VEGF release leading to proliferation of endothelial and tumor cells has to be considered (YB Kim, et al., Histopathology 38, 160–166, 2001). Therefore the possible indication of adjuvant treatment after RFA has to be discussed.

Conclusion: RFA is a potential effective palliative treatment in patients with solitary liver metastases. A prospective trial will be necessary to assess the putative benefit of an angiogenesis inhibitory treatment concomitant to RFA intervention.

Results of Therapeutic Splenic Irradiation

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Background: To assess subjective and objective improvements after splenic irradiation.

Methods: Patients with hematological disease and symptomatic splenomegaly were treated with low dose radiotherapy. The spleen size was measured clinically, sonographically and radiologically before/during and after radiation. Symptoms were recorded carefully. Regularly hematological controls were mandatory always before the next X-ray application.

Results: 31 patients received 65 radiation series, e.g. more than 2 series/patient (1 to max. 8). Gender: 19 men, 12 women. Median age: 68 years (8 to 86 years). Average radiation dose was 210 cGy (120–360 cGy), applied 2 to 3 times/week. Patients complained signs of upper left abdomen symptoms in 63/65 series at start of RT, after RT only in 13/65. Reliefs: abdominal pressure 98%; nausea 97%; pain 93%. Improved situation after RT in 80% of all series. Size of spleens: clinical before RT: 18.4 cm (5–40 cm), after RT: 10.1 cm (3–28 cm); sonographic before RT: 23.6 cm (10–35 cm), after RT: 15.0 cm (7–21 cm). Kissing phenomena: before RT 26%, after RT: 0%. Hematological parameters: severe anemia (Hb < 100 g/l): before RT Hb 79 g/l, after RT Hb 87.6 g/l, leucocytes: only moderate decrease (0.4–1.1 G/l), thrombocytes remained stable. Leucocytosis (hematological disease) improved distinctly: before RT 63.3 G/l, after RT 20.6 G/l. Time to next radiation course: average 10 mts (1–81 mts). 13 patients received chemotherapy before RT, 12 patients were treated with cytostatics after RT. In 16 out of 65 series blood transfusion was performed during radiotherapy, after RT decrease of blood substitutions was in some cases reported. Survival after 1st radiation course: average 21 mts (1–92 mts), median 17 mts. 13 patients died due to their hematological disease.

Conclusions: Splenomegaly due to hematological disease can be treated easily with low dose radiotherapy with improve-

ment of almost 80%, hematologic parameters tend to improve as well. Splenic irradiation is a true alternative specifically in those cases where medical treatments or splenectomy cannot be offered.

Radioimmunotherapy with Lutetium-177-DOTA-Rituximab: a Phase I/II Study in Patients with Follicular and Mantle Cell Lymphoma. An Interim Analysis

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Background: Radioimmunotherapy with ^{131}I and ^{90}Y labeled anti-CD20 antibodies showed convincing results mainly in the treatment of indolent lymphomas. We analyzed the pharmacokinetics and dosimetry of ^{177}Lu labeled, chimeric (human-mouse) anti-CD20-antibody Rituximab. The antibody was modified with up to 4 DOTA-chelators with retained immunoreactivity. ^{177}Lu was chosen because of its low β -energy (0.497 MeV) and its imageable γ -energies. These are favorable physical characteristics for the treatment of small tumors or tumors that diffusely infiltrate visceral structures.

Methods: 7 patients (2 f, 5 m) with relapsed lymphoma were included so far. After pretherapeutic staging (^{18}F -FDG-PET, CT, bone marrow biopsy) patients received 250 mg/m² Rituximab on day 1. On day 8 patients received another course of Rituximab followed by the injection of 20 mCi/m² ^{177}Lu -DOTA-Rituximab (32–40 mCi). Scintigraphic images were obtained up to 15 d p.i. to determine biodistribution and total dose. Blood samples were collected up to 15 d p.i.. Urine was collected by male patients up to 72 h p.i.. Calculations were done with dose-factors published on 'RADAR' by M. Stabin et al.[1].

Results: Early γ -camera images showed mainly blood pool activity. Over time, in 6 of the 7 patients a clear specific uptake at tumor sites was seen. The effective half-life of the whole-body activity ranged from 95 to 136 h (mean 117 h $\pm$ 15 h), leading to diffuse whole-body-doses of 1007 mGy to 1459 mGy (mean 1150 $\pm$ 123 mGy). The dose to the red marrow ranged from 393 to 539 mGy (mean 447 mGy $\pm$ 55 mGy). Excretion of activity was found to be low in urine with a maximum of 10% of the injected activity excreted within 72 h by the kidneys. The application of ^{177}Lu -DOTA-Rituximab was well tolerated. Clinical results and toxicity will be presented at the meeting.

Conclusion: ^{177}Lu -DOTA-Rituximab seems to be an interesting, well tolerated compound with clinical activity already in a low dose range. Dose escalation is in progress.

[1] www.doseinfo-radar.com

Idiotypic Vaccination for the Treatment of Non-Hodgkin's Lymphomas

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Normal and neoplastic B cells carry membrane-bound immunoglobulins as antigen receptors. The peptide sequences of these receptors are diversified by the rearrangement process of immunoglobulin gene segments and somatic hypermutation. As a result, unique sequences exist for each lymphoid clone, which are referred to as the 'idiotype' of each clone and represent clone-specific antigens. For over a decade, attempts have been made to generate specific immune responses against lymphoma-specific idiotypes.

In lymphomas with monoclonal gammopathies (multiple myeloma and lymphoplasmocytic immunocytoma), an idiotype vaccine can be produced simply by purification of the paraprotein from the sera of affected patients. In non-secreting lymphomas, soluble idiotype protein can be obtained by somatic cell hybridization of viable lymphoma cells with a permanent hybridoma cell line and purification of immunoglobulin from the cell culture supernatant.

To generate a sufficiently immunogenic vaccine, the idiotype is commonly conjugated to the immunogenic carrier protein KLH and frequently injected with concomitant administration of GM-CSF. This type of idiotype vaccine induces protective, specific anti-lymphoma in a variety of animal models. In phase II clinical immunization trials conducted in patients with follicular lymphomas during clinical remission, induction of measurable anti-idiotype immune responses was associated with improved disease-free and overall survival. In addition, idiotype vaccination has been reported to result in the disappearance of minimal residual disease as assessed by sensitive PCR assays. Idiotype-presenting dendritic cells may represent an even more immunogenic anti-lymphoma vaccine. However, the superiority of this approach to first-generation idiotype vaccines has yet to be demonstrated.

Unfortunately, the considerable effort to manufacture individual idiotype antigen has hitherto prevented large-scale clinical testing. Therefore, our group has developed a rapid, PCR-based protocol for rapid expression cloning of lymphoma-derived immunoglobulin in *E. coli*. A clinical Phase I immunization protocol with this recombinant idiotype vaccine is currently being conducted at our institution in patients with advanced B-cell lymphomas. In 11 patients vaccinated as of October 2002, the vaccine was well tolerated. Preliminary results seem to indicate the potential to induce idiotype-specific immune responses despite an overall immunosuppression due to previous antineoplastic therapies. While no clinical remissions could be documented, the majority of patient had stable disease during and after the scheduled vaccination course. Based on these phase I experiences, phase II trials for patients with low tumor burden and less suppressed immune status are being designed and may be initiated in the near future.

Cellular Immune Therapy – from Vaccine to Adoptive Transfer

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The concept of cellular immune therapy is based on the observations that tumors are immunogenic and that T cells can recognize and eliminate tumor cells. According to the *immune-surveillance hypothesis*, tumors express neo-antigens resulting from genetic instability of tumor cells and generally induce an immune response against those foreign, or non-self, tumor associated antigens (TAA). The development of tumors in the face of circulating lymphocytes was thought to be the result of various immune escape mechanisms that tumor cells employ to avoid elimination by the immune system. The observation that tumor specific CTL in melanoma patients are directed against normal melanocyte differentiation antigens led to a paradigm shift linking tumor immunology to autoimmunity in that most tumor antigens are self antigens and that mechanisms of tumor tolerance induction resemble those to avoid autoimmunity.

Stimulation with antigen loaded dendritic cells (DC) can prime naïve T cells and break T cell tolerance in vitro and in vivo. TAA loaded DC applied s.c. or i.v. can elicit tumor specific responses in tumor patients. In prostate cancer, several tissue specific TAA have been characterized. Other groups reported encouraging clinical results after vaccination with peptide pulsed DC in patients with metastatic prostate cancer. We will report preliminary results of an ongoing clinical phase I/II study on the vaccination with peptide loaded, autologous DC for patients with refractory, metastatic prostate cancer.

Alternatively, T cells can be primed with autologous DC in vitro and be re-transfused after expansion. The most powerful form of adoptive T cell transfer known today, however, is the transfusion of allogeneic T cells, or donor lymphocyte infusions 'DLI', which has been particularly effective for the treatment of relapsed CML, but less so for patients with acute leukemia or lymphoma. The transfer of TAA specific donor T cells instead of polyclonal DLI, i.e. a potentially highly effective, well tolerable and specific immunotherapy would thus be a desirable alternative. Current laboratory research is focused on the generation of tumor antigen specific donor T cells in patients with AML or NHL.

Effect of 5-AZA-2'-Deoxycytidine (DAC) on Cell Growth and Differentiation-enhancing Using CD34+

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DNA methylation is a major epigenetic mechanism controlling tissue- and development-specific gene regulation. During normal hematopoietic cell maturation, methylation of myeloid-specific genes (e.g. myeloperoxidase [MPO], lysozyme [LZM], CSF receptors) is decreased concomitantly with differentiation (Blood 87:447–455, 1996; Leukemia 13:530–534, 1999). Conversely, the S-phase-specific demethylating agents 5-azacytidine and 5-aza-2'-deoxycytidine (DAC) have shown a moderate differentiation-inducing activity on myeloid cell lines. To establish an in vitro model of normal hematopoietic differentiation modulated by demethylation, we used CD34+ cells from apheresis specimens (AP) and analyzed proliferation, viability, CD15, LZM and MPO expression (FACS) with and without DAC treatment. Since DAC is ineffective on cells in G0, CD34+ cells from AP (being mostly in G0/G1) were cultured with serum containing medium (IMDM plus 10% FCS) (SCM, n=4) or serum-free medium (SFM, n=10) in the presence of Flt3, SCF, and IL-3 for 72 hours. Treatment with DAC was performed on day (d) 3 of culture, as three 24 hr pulses with two low-cytotoxic concentrations (10, 50 nM). Cells were harvested on d 7 of culture. Without DAC treatment, cell expansion under these conditions was 11.9 fold (range 0.36–22.8). With DAC pretreatment, growth inhibition was observed relatively to the expansion culture (median 13.9%, range -4.4–27.6% [10 nM DAC]; median 17.3%, range -13.9–47.7% [50 nM DAC]), as well as a slightly decreased cell viability (median 8.8%, range -5.8–46% [10 nM DAC]; median 17.7%, range -23.3–73.1% [50 nM DAC]). Interestingly, DAC induced an increase in LZM+ cells (median 14.1%, range 4.3–36.8% [10 nM DAC]; median 19.8%, range 8–56.5% [50 nM DAC], n=7), and a lesser increase in MPO+ cells (median 0.4%, range -10.2–17.5% [10 nM DAC]; median 5.3%, range -9–19.5% [50 nM DAC]) and CD15+ cells (median 7.6%, range -10.8–22.4% [10 nM DAC]; median 4.6%, range -24.7–33.8% [50 nM DAC]). In order to ask whether DAC pretreatment increases responsiveness of the cells to G-CSF, DAC treatment was followed by G-CSF (10 µg/ml) for 48 hours. Expectedly, G-CSF alone led to a substantial increase of LZM+ cells (median 36.7%, range 33–86.8%, n=5), MPO+ cells (median 21.1%, range 3.8–22.6%) and CD15+ cells (median 26.2%, range 14.8–44.1%). With DAC pretreatment, we detected an additive increase in LZM+ cells upon G-CSF-induced differentiation (median 49.1%, range 13–127.4% [10 nM DAC]; median 50.8%, range 39.1–63.2% [50 nM DAC]), whereas no additional effect on expression of MPO (median 19.4%, range 4.6–50.5% [10 nM DAC]; median 20.2%, range 6.8–29.8% [50 nM DAC]) or CD15 (median 23.7%, range -21.6–44% [10 nM

DAC]; median 33.2%, range 6.5–62.5% [50 nM DAC]) was noted. In conclusion, at low-cytotoxic concentrations DAC induces a dose-dependent inhibition of cell growth, and moderately increases differentiation markers in cytokine-expanded normal hematopoietic precursor cells. This effect was particularly evident for lysozyme expression. This model should be suitable for global analyses of multiple differentially methylated genes, to determine possible patterns of methylation and gene expression being established during lineage-specific maturation.

Characterization of Differential Gene Expression of Granulocytic versus Monocytic Differentiation in Estrogen-dependent E2A/PBX1 Myeloid Cell Lines by Gene Expression Profiling

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The molecular pathways of normal myeloid differentiation, as well as the mechanism by which oncogenes disrupt this process, remain poorly understood. A major limitation in approaching this problem has been the lack of suitable cell lines that exhibit normal, terminal and synchronous differentiation in the absence of endogenous oncoproteins and in response to physiological cytokines, and whose differentiation can be arrested by ectopically expressed human oncoproteins. In this report we use a clonal, GM-CSF-dependent myeloid cell lines that exhibit these properties. The cell lines were established by conditional immortalization of primary murine marrow progenitors with an estrogen-regulated E2A/PBX1-estrogen receptor fusion protein. Clones were identified that proliferated as immortalized blasts in the presence of estrogen, and that exhibited granulocytic or monocytic differentiation upon estrogen withdrawal. Differentiation was normal and terminal as evidenced by morphology, cell surface markers, gene expression and functional assays. The cell line showing lineage specific granulocytic differentiation (ECoM-G) was compared with the one showing monocytic differentiation (ECoM-M) by gene expression profiling to identify tentative lineage specific genes.

Specific Peptide Ligands Binding to Acute Myeloid Leukemia Cells

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Background: Cytotoxic drugs used for acute myeloid leukemia (AML) are often ineffective or have side effects preventing their application with sufficient antileukemic effect. Ligands binding specifically to leukemic cells may be used for targeting drugs or gene therapy vectors in AML patients, resulting in a more effective treatment with less toxic side effects. Little is known about receptors specifically expressed on AML cells or ligands thereof. We intended to identify such ligands based on a peptide selection system.

Methods: We used random phage display libraries to select for peptides binding to AML blasts but not to non-neoplastic blood or bone marrow derived cells (NCs). We performed sequential negative selection eliminating phage binding to NCs, followed by positive selection on AML blasts. Cell bound phage were separated from unbound phage by differential centrifugation and subsequently recovered and amplified by bacterial infection. Additional selection rounds with increasing stringency were performed to enrich AML-specifically binding phage. Binding of enriched peptide displaying phage on AML cells was compared with binding to NCs. The peptide inserts of randomly picked clones recovered from AML cells after several rounds of selection were determined and analyzed indirectly by DNA sequencing. Single clone binding assays were performed to evaluate specificity of the enriched peptides, using bone marrow samples from AML patients while non-neoplastic bone marrow cells were used as a controls.

Results: More than five selection procedures applying the above system on AML blasts were performed. One procedure revealed an up to 100-fold enrichment of peptide displaying phage from the 1st to the 3rd round, defined by the output/input ratio of applied clones. Enriched phage bound considerably stronger to AML cells than to NCs. DNA sequencing revealed the enrichment of two phage clones displaying unrelated peptides. One of these clones showed a 60-fold stronger binding to AML cells than to NCs.

Conclusions: We established a method to select clones from a random phage peptide library that specifically bind AML cells rather than non-neoplastic leukocytes. Using this system, we selected one AML-binding peptide clone that may be used for the development of AML-targeted therapies.

Mobilization and Transplantation of Philadelphia Chromosome-negative Peripheral Blood Progenitor Cells in Patients with CML: a Mature Follow-up Report

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A phase II trial investigating mobilization and transplantation of peripheral blood progenitor cells (PBPCs) in patients with chronic phase chronic myelogenous leukemia (CML) was conducted between March 1995 and October 1996. 17 patients were mobilized using combination chemotherapy with idarubicin, cytosine arabinoside, and etoposide (ICE) followed by simultaneous administration of rhG-CSF and rhIL-3 or rhG-CSF alone. Autologous transplantation of mostly Ph⁺ apheresis products was performed in 16/17 patients after myeloablative chemotherapy. Post-transplantation the majority of patients received consolidation therapy with IFN- α . 12 of 16 transplanted patients are alive at a median follow-up after initial diagnosis CML of 86+ months (range 28–106+ months) and after autologous transplantation of 71+ months (range 19–85+ months), respectively. 10 patients achieved a complete or major cytogenetic remission (CCR/MCR), of whom in 2 patients the bcr/abl-transcript could not be detected by PCR. 6 patients did not respond cytogenetically. At last follow-up 9 of 12 surviving patients remained in complete hematological remission (CHR) under IFN- α of whom 5 were in CCR and 2 in molecular CR. 2 patients who relapsed in 2nd chronic phase CML reached CHR and MCR after initiation of therapy with STI571. Our data show that this very efficient treatment modality is able to induce major and sustained cytogenetic responses in the majority of patients and possible to prolong overall survival. Autologous transplantation offers an additional treatment option for patients with CML lacking an allogeneic donor.

Imatinib (Glivec®) and Pegylated Interferon alpha 2a (Pegasys®) Phase I/II Combination Study in CML Patients in Early Chronic Phase

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Imatinib and interferon alpha (IFN) are active in the treatment of CML; synergistic effects were demonstrated in vitro. To define the tolerability and efficacy of combination therapy with imatinib and pegylated IFN α 2a (PEG-IFN, Pegasys), a phase I/II study was performed in 32 Ph⁺ chronic phase CML patients (pts, 22 m, 10 f; median age 41, range 21–70 years), recruited within 6–355 days (median 82) from diagnosis. Primary objective was to determine safety and tolerability of PEG-IFN (sc) once a week, given in combination with imatinib (po) once daily. Secondary objectives were to explore hematologic, cytogenetic, and molecular response rates. Following a 2 week period of imatinib therapy, PEG-IFN was added on day 15 and then given once weekly, during 8 weeks. Cohorts comprised (1) 400 mg imatinib + 90 μ g PEG-IFN (n=7); (2) + 180 μ g PEG-IFN (n=6); (3) 300 mg imatinib + 90 μ g PEG-IFN (n=4); (4) +180 μ g PEG-IFN (n=8). A 5th cohort received 400 mg imatinib monotherapy for six weeks, 300 mg from day 43 combined with 180 μ g PEG-IFN for 8 weeks (n=7). **Efficacy:** Within the study period 30/32 pts (94%) reached a complete hematologic remission, 19/28 (68%) a major cytogenetic response (Ph⁺ <35%), 8 (29%) being complete. One patient progressed into myeloid blast crisis 7 weeks after start of therapy. **Toxicity:** Grade 3 non-hematologic adverse events were reported in 5 pts and comprised flu-like symptoms, bone pain, elevation of transaminases, pharyngitis, and unspecific colitis. Grade 3/4 cytopenias was observed in 13/32 pts (41%; 4/7 in cohort 1, 3/6 in cohort 2, 1/4 in cohort 3, 4/8 in cohort 4, and 1/7 in cohort 5). Overall, pts received 71% of the planned PEG-IFN and 95% of imatinib doses. The median dosages administered within cohorts were (1) 63% and 100%, (2) 56 and 95%, (3) 88 and 100%, (4) 69 and 100%, (5) 100% PEG-IFN and 100% imatinib, respectively.

Conclusion: The combination treatment of imatinib and PEG-IFN is feasible and results in a high hematologic and cytogenetic response rates. Predominant dose limiting toxicities are cytopenias, which are less frequent if PEG-IFN therapy is commenced after 6 weeks imatinib monotherapy in order to allow restoration of Ph⁺ neg. hematopoiesis. The combination of imatinib, 400 mg/day and PEG-IFN, 90 μ g/week starting after 6 weeks with individual escalation to 180 μ g/week is suggested for further studies.