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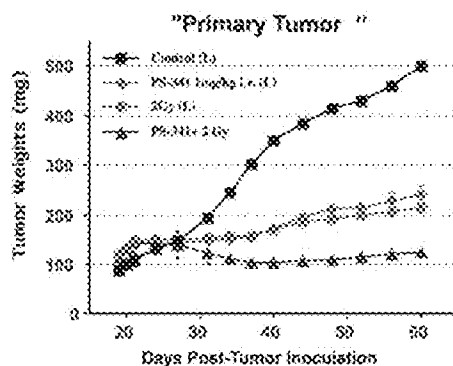
Pre-clinical studies of concomitant PS-341 and ionizing radiation therapy: local and systemic anti-tumor effects

S. Formenti¹, S. Demaria², L. Liebes³, B. Ng³, M. Devitt¹, J. Babbs⁴, F. Muggia³. ¹New York University, Radiation Oncology, New York, USA; ²New York University, Pathology, New York, USA; ³New York University, Medicine, New York, USA; ⁴New York University, Radiology, New York, USA

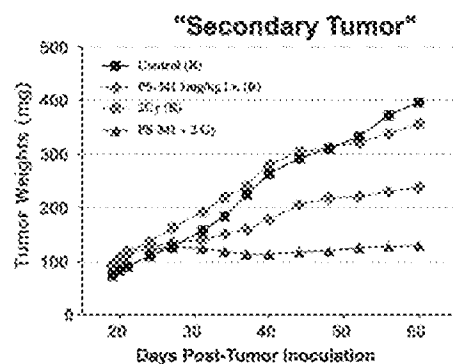
Background: We have used a pre-clinical breast cancer model to test whether the combination of PS-341 and local radiation can induce protective anti-tumor immunity in tumor-bearing hosts. The hypothesis tested is that local treatment by ionizing radiation (RT) or concomitant chemotherapy and RT, may have systemic anti-tumor effects mediated by the immune system.

Methods: BALB/C mice were injected on day 0 with the syngeneic mammary carcinoma cells 67NR subcutaneously in two sites: 1×10^5 cells were injected in the left flank ("primary" tumor) and 5×10^4 cells were injected in the right flank ("secondary" tumor). When primary tumors reached approximately 100-150 mg, treatment was started. Four treatment groups, each comprising 3 mice, were established: 1) no treatment; 2) PS-341; 3) RT; 4) PS-341 + RT. The proteasome inhibitor PS-341 was administered i.v. at 0.3 mg/kg body weight on day 19, RT was delivered at a single dose of 2 Gy on day 20 exclusively to the "primary" tumor. Mice were then followed up to day 52 and tumor measurements obtained every 3 days. Data were analyzed using repeated measure analysis of variance.

Results: Measurements of tumor size from day 21 to day 60 were determined. RT and PS-341 used as single modality treatment were both able to significantly delay growth of the primary tumor ($p < 0.001$ starting at day 37)



In contrast, growth of the secondary tumor was delayed by PS-341 but not RT.



The combination of PS-341 and RT was more powerful than each treatment alone in reducing the size of the primary tumor. Remarkably, in the presence of PS-341 RT to the primary tumor had an effect also on the secondary tumor, leading to tumor growth delay that was significantly better than PS-341 alone ($p < 0.005$ from day 40).

Conclusions: 1) The combination of RT and PS-341 showed results superior to single modality-treatment in reducing the weight of the irradiated tumor. Administration of PS-341 may allow the use of lower doses of radiation to achieve greater local tumor control. 2) The combination of RT and PS-341 can elicit anti-tumor mechanisms capable of controlling tumor growth at a remote site. This finding is extremely important as it suggests that it may

be possible to use this combination regimen to treat cancer patients and at the same time vaccinate them against their tumor.

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Transcriptional profile of response during senescence or apoptosis of colon carcinoma cells after SN-38 treatment

M. Bhonde¹, D. Moorthy¹, M. Hanski¹, R. Magrini¹, M. Notter², H. Scheruebl¹, M. Zeitz¹, C. Hanski¹. ¹Gastroenterology, ²Hematology, Berlin, University Clinic Benjamin Franklin, Germany

Introduction: CPT-11 in combination with 5-fluorouracil, has been recently approved as the first line chemotherapy for colorectal cancer. Both CPT-11 and its active metabolites SN-38 are topoisomerase I inhibitors and interfere with DNA replication, transcription, recombination and repair. The genetic determinants of the response, are not known; the effect of SN-38 appears to be mediated via p53-dependent as well as p53-independent pathways. The understanding of the role of p53 is essential since more than 50% of colorectal carcinomas are mutated in this gene. We investigated in several colorectal carcinoma cell lines with known p53 status the response to SN-38 in order to characterise the p53-dependent and p53-independent reactions.

Materials and

Methods: Isogenic and established colon carcinoma cell lines differing in their p53 status were used as model systems. The cells were treated with 10 nM SN-38 for 48 h after which the drug-containing medium was removed, the cells were washed with PBS and cultured further in fresh drug-free medium. The cell cycle distribution, apoptosis and gene expression on RNA and protein level were determined. The transcriptional response of cells to SN-38 was analysed using Affymetrix Genechip oligonucleotide microarrays.

Results: SN-38 induces a p53-independent G2/M arrest. If the p53 gene is intact, the G2/M arrest is prolonged, which eventually leads to cellular senescence. On the other hand, cells harbouring a p53mut undergo massive apoptosis following their release from the arrest. Both processes lead to a similar lack of colony-forming potential in clonogenic cell survival assay *in vitro*. The analysis of gene expression revealed that either process, senescence or apoptosis, is accompanied by a different, non-overlapping set of alterations. The alterations detected by means of DNA microarrays have been confirmed by RT-PCR. Several of the affected genes could be related to either process, the significance of other alterations is under study. **Conclusions:** 1. Colorectal cancer cell lines with p53mut react to SN-38 treatment with apoptosis and those with p53wt with senescence. 2. These diametrically different processes result in a similar final outcome, if sensitivity in clonogenic assay is used as the readout. 3. Either process is associated with transcriptional activation of a different set of genes. 4. The knowledge of the pathway activated by treatment may allow to select suitable ways to enhance either process.

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PLENARY SESSION 3

Cytokines and angiogenesis in cancer biology and treatment

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IL-7 as a therapeutic: immunorestorative agent and vaccine adjuvant

T. Fry, G. Melchionda, C.L. Mackall. National Cancer Institute, Pediatric Oncology Branch, Bethesda, USA

Endogenous antitumor immune responses are not sufficient to prevent growth of clinically evident cancer, but it is now clear that tumors commonly express antigens which can be recognized by the adaptive immune system and emerging evidence suggests that weak priming to tumor antigens occurs concomitant with primary tumor growth. Thus, it remains possible that naturally acquired, T cell mediated immunity directed toward tumor antigens may play a role in controlling minimal residual disease. As cytotoxic agents which are used for cancer therapy often induce significant T cell depletion,

our work has focused on developing approaches to enhance T cell regeneration as a means for targeting minimal residual neoplastic disease. IL7 is a potent immunorestorative which enhances both thymic-dependent and thymic-independent pathways of T cell regeneration in mice. Administration of IL7 to normal primates resulted in dramatic increases in total body T cell number as evidenced by 3-5 fold increases in circulating CD4+ and CD8+ T cell numbers and reversible lymphadenopathy. Further study of these effects revealed that both naive and memory cells increased in IL7 treated monkeys, that there was increased peripheral T cell cycling as evidenced by increased Ki-67 expression and increased BrdU incorporation upon ex vivo culture of cells from IL7 treated primates. Furthermore, TREC levels declined following IL7 therapy. Thus, IL7 therapy induced widespread T cell cycling, a biologic effect which is predicted to result in expansion of antigen specific T cell clones. In order to test whether IL7s effects on T cell cycling could enhance antigen specific responses, we administered IL7 in concert with a dendritic cell based vaccine in a murine model. We observed substantial increases in the numbers of both antigen specific CD8+ cells which responded to both immunodominant and subdominant Class I epitopes and CD4+ cells responding to immunodominant Class II epitopes. IL7 was a more potent vaccine adjuvant than IL2 or IL15. Furthermore, when tumor bearing animals were treated with IL7, we observed enhanced reactivity toward expressed tumor antigens. These results suggest that IL7 may be beneficial in the context of cancer therapy due to its capacity to enhance immune reconstitution, enhance immune reactivity toward weak tumor antigens and potentially to enhance the effectiveness of tumor vaccines.

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TNF revisited: New perspectives for a successful antivasculature cancer therapy

A.M.M. Eggermont, T.L.M. ten Hagen. *Erasmus Medical Center Rotterdam Daniel den Hoed Cancer Center, Department of Surgical Oncology, Rotterdam, The Netherlands*

Clinical Program: On the basis of the spectacular results of a multicenter trial in Europe TNF was approved by the EMEA for application of TNF in the Isolated Limb Perfusion (ILP) setting in combination with melphalan for irresectable soft tissue sarcomas of the extremities. In 246 patients limb salvage was achieved in the 71% of patients corresponding to a 76% objective response rate (CR+PR). The procedure is performed in over 30 cancer centers in Europe. It appeared from the clinical studies that a very rapid selective destruction of the tumor-associated vasculature (TAV) was the essential mechanism by which TNF mediates its antitumor effects in combination with melphalan.

Preclinical Models elucidating antitumor mechanisms: A number of crucial observations have been made in new tumor models in Rotterdam: the vasculo-toxic effects of the combination of TNF melphalan lead to haemorrhagic necrosis of the tumors but more importantly we have demonstrated that the addition of high dose TNF to the perfusate results in a 4-6 fold increased uptake by the tumor of the cytostatic drugs. This is true with different tumors in different tumor models and organ settings:

Isolated Liver Perfusion Setting: The same synergy between TNF and melphalan is observed. A very high antitumor response rate of almost 80 percent was observed in studies in patients.

Genetherapy: We have shown that Isolated limb perfusion is an interesting method for new treatment modalities such as adenoviral-vector mediated genetherapy. Other new developments in the field of TNF-based genetherapy have brought very interesting results recently. After intratumoral therapy with the adenoviral vector-TNF-gene construct "TNFerade" (Genvec) in combination with radiotherapy impressive antitumor effects have been observed in various large tumours in a broad phase I program.

Liposomes and New Opportunities for Systemic Application of TNF: We have demonstrated that low doses (clinically applicable) of TNF in combination with doxorubicin containing liposomes enhanced the uptake of the liposomes in the tumor and the intratumoral drug concentration significantly, resulting in a highly significant antitumor effect. This should be the basis to explore the utility of TNF at low doses in combination with liposomes in phase I-II studies.

Conclusions: TNF-based antivasculature therapy of cancer is here to stay and its potential needs to be studied further.

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IL-15 mediates good and bad

M.A. Caligiuri, USA

Abstract not received.

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First results of a phase I and pharmacokinetic study of SU011248, a novel oral anti-angiogenic agent, in patients with advanced solid tumours

E. Raymond¹, S. Faivre¹, K. Vera¹, C. Delbaldo¹, C. Robert¹, N. Brega², A. Achour¹, G. Massimini³, P. Schigalla³, J.P. Armand¹. ¹I. Gustave Roussy, Medecine, Villejuif, France; ²Pharmacia, Nerviano, Italy; ³Sugen, Peapack, USA

SU011248 is a novel orally bioavailable indolinone that inhibits VEGFR (Flk-1/KDR), PDGFR alpha and beta, Flt3, and c-kit tyrosine kinase activity at nanomolar concentrations. Dose-dependent antitumor activity was measured in a variety of human tumor xenografts in nude mice, and was well tolerated in these studies. In this clinical study, escalating doses of SU011248 were given orally for 28 consecutive days followed by a 2-week rest period to patients (pts) with advanced malignancies not amenable to conventional therapy. Based on preclinical toxicology data, the starting dose was 30mg/m² every other day (4 pts), then further escalated to daily 30mg/m² (6 pts), 42mg/m² (4 pts), and 59mg/m² (3 pts). Dose escalations were 100% and 40% in the presence of grade 0-1 and grade 2 toxicity, respectively. Seventeen pts (M/F: 9/8, median age: 53, range 33-73; median PS: 100, range 60-100) were entered including 3 renal cell carcinomas, 2 non-small cell lung cancer, 2 neuro-endocrine tumors, 2 uterine cancer, 2 angiosarcoma, 2 mesothelioma, 1 pancreatic carcinoma, 1 breast cancer, 1 colorectal cancer, 1 undifferentiated nasopharyngeal carcinoma. At the dose of 30mg/m² daily, grade 3 edema was observed in 1/6 pts allowing to resume dose escalation. SU011248 was well tolerated up to the dose of 42mg/m². At the dose of 42mg/m², drug-related toxicities were grade 2 asthenia (4 pts), grade 2 thrombocytopenia (1 pt), grade 2 neutropenia (1 pt), and grade 2 diarrhea (1 pt). Grade 2 sore tongue and mouth was observed at the highest doses. Consistent with a high volume of distribution of SU011248, a sustained and dose-dependent tanned gold coloration of the skin was observed in several pts. Progressive hair discoloration was observed in pts with the highest plasma levels, suggesting an effect on tyrosine kinase receptor driven tyrosinase transcription in the hair follicles. Pharmacokinetic data indicate good oral bioavailability with modest intra/inter-patient variability of SU011248 and its metabolite. Target plasma concentrations determined preclinically for activity were achieved in this study. Objective responses were observed in one pt at the first dose level and in 3 pts at the dose of 42mg/m² daily, with a prolonged 6-month tumor stabilization in a pt treated at 30mg/m² daily. The dose escalation is ongoing at the dose of 59mg/m² to define the maximum tolerated dose. The fair toxicity profile and preliminary evidence of activity encourage further exploration of SU011248.

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Poster Sessions

Anthracyclines

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Combination of novel sulfonamide anticancer drug, E7070, with CPT-11 "antitumor effect and synergistic mechanism"

Y. Ozawa, J. Kai, K. Kusano, T. Owa, A. Yokoi, T. Nagasu, M. Asada, K. Yoshimatsu. *Tsukuba Research Laboratories, Eisai Co. Ltd., Department of Cancer Research, Ibraki, Japan*

The novel sulfonamide anticancer drug E7070, N-(3-chloro-7-indolyl)-1, 4-benzenedisulfonamide, has demonstrated promising antitumor efficacy in pre-clinical models on the basis of its unique mode of action and antitumor spectrum. The phase II studies of E7070 monotherapy have been conducted in Europe and the US. As we reported previously, the combination of E7070 with CPT-11 exhibited synergistic antitumor efficacy in human tumor xenograft models in mice. There was no difference between monotherapy and combination in terms of pharmacokinetic profile of E7070, CPT-11 and SN38 (active metabolite of CPT-11). The synergistic effect of E7070-CPT-11 combination was observed in cultured cells, by the Combination Index method. It has been reported that SN38 treatment causes a transient up-regulation of topoisomerase II alpha mRNA. In our GeneChip analysis, E7070 was shown to decrease topoisomerase II alpha mRNA. Therefore, it was considered that synergism in E7070-CPT-11 combination occurred through the modulation of topoisomerase II alpha amount. To address this