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

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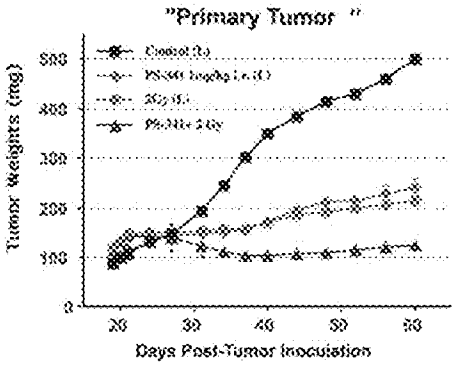
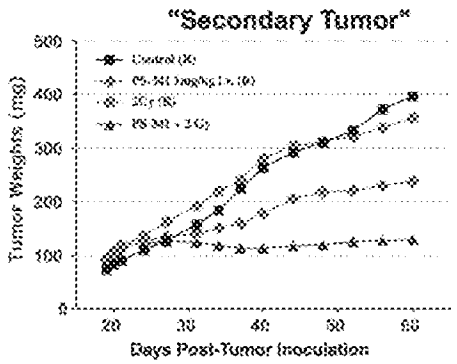
Background: We have used a pre-clinical breast cancer model to test whether the combination of PS-341 and local radiation can induce protective anti-tumor immunity in tumor-bearing hosts. The hypothesis tested is that local treatment by ionizing radiation (RT) or concomitant chemotherapy and RT, may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

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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 metabolits 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 beeen 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 diametrally 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.

Wednesday 20 November

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

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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,