cine vectors are able to do. Therefore, a strategy that facilitates the spread of antigen to other APCs may significantly enhance the potency of naked DNA vaccines delivered intradermally. We have recently enhanced the potency of DNA vaccines using herpes simplex virus (HSV-1) VP22, an HSV-1 tegument protein that has demonstrated the remarkable property of intercellular transport and is capable of distributing protein to many surrounding cells. We showed that HSV-1 VP22 (HVP22) was capable of enhancing intercellular spreading of linked protein, such as E7. Furthermore, we demonstrated that mice vaccinated intradermally with HSV-1 VP22/E7 DNA generated a significantly greater number of E7 specific CD8+ T cell precursors and stronger antitumor effect than mice vaccinated with wild-type E7 DNA. The impressive pre-clinical data based on these strategies have led to several nucleic acid vaccine trials tentatively scheduled to begin in 2003.

## Wednesday 20 November

WORKSHOP

# Novel targets and radiation response

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### COX-inhibitors and radiation

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Cyclooxygenase-2 (COX-2), the enzyme that converts arachidonic acid to prostaglandins, overexpressed in a variety of different tumors, including colon, pancreatic, prostate, lung and head and neck cancers. COX-2 is also observed within human tumor neovasculature, suggesting that COX-2 derived prostaglandins contribute to tumor growth by inducing formation of new blood vessels

Angiogenesis is the process by which new capillaries are formed from preexisting vessel networks. Angiogenesis into a newly growing tumor provides a pathway for escape and systemic dissemination via the blood or lymph system and represents an important therapeutic target. Tumors that demonstrate intense immunostaining for COX-2 also demonstrate co-localization for cytokines involved in angiogenesis.

Angiogenesis has been considered as a potential target for the treatment of cancer, either by the inhibition of endothelial cell proliferation and migration or by inhibition of the production of angiogenic factors by tumors. In contrast to tumor cells, endothelial cells, derived from the host, are genetically stable and have a low mutation rate. Kerbel has suggested that antiangiogenic therapy may be a strategy to bypass drug resistance. Celecoxib and rofecoxib, have been shown to posses potent inhibitor of angiogenesis and tumor growth.

A growing tumor requires a blood supply; and, thus, it secretes numerous angiogenic compounds that induce host endothelial cells to proliferate, migrate, and differentiate into patent vessels. It is proposed that by inhibiting the angiogenic processes of endothelial cells, tumor growth and metastasis will be inhibited as well. It is unclear at the point whether the target for selective inhibitors of COX-2 is tumor, tumor associated neovasculature or both. Clinical trials will help elucidate the role of this interesting class of agents in combination with cytotoxic therapy for the treatment of cancer. The use of COX-2 inhibitors in cancer therapy may complement current strategies while potentially minimizing the impact on quality of life.

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### Combination therapy with anti-angiogenic agents and radiotherapy

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Radiation therapy has been used for over 100 years in the treatment of cancer. The conventional explanation for the mechanism of the -radiation effect- against tumor cells is that DNA damage caused by ionizing radiation results in reproductive cell death. However, what if, damage to the DNA of tumor cells is not the primary target? What if the primary target is actually the supporting endothelial cell?

The oxygen effect on the radio-resistance of hypoxic tumor cells has been well demonstrated. It may be counterintuitive to some that the use of anti-angiogenic therapy may augment local control with radiotherapy. Classic dogma teaches that if the tumor bed is rendered more hypoxic with anti-angiogenic therapy, then the tumor cells must be less radiosensitive. Generating hypoxia with anti-angiogenic therapies may also select cancer cells that have acquired hypoxia-resistance and have a higher metastatic and invasive potential. Fortunately, three pre-clinical studies have shown that the treatment of tumors with anti-angiogenic drugs actually increases the tumor pO2. In 1992, Teicher published the first paper using a combination of anti-angiogenic therapy and radiotherapy against a primary tumor. She demonstrated, in a tumor growth delay study, that the combination of minocycline (a weak metalloproteinase inhibitor), TNP-470 and radiotherapy was synergistic against Lewis lung carcinoma cells in mice. These results triggered a paradigm shift in the rationale for combining anti-angiogenic therapy with radiotherapy.

Since this landmark study, radiotherapy has been used in combination with numerous anti-angiogenic agents including angiostatin, endostatin, anti-VEGF therapy, thalidomide, as well as numerous other agents, in pre-clinical models. Numerous clinical trials are also ongoing with many newer combinations in the planning phase.

It is the objective of this session to review the data concerning anti-angiogenic therapy and its effects on tumor vasculature and to describe the potential use of anti-angiogenic therapy from the point of view of the radiation oncologist. We will then explore the promising evidence and rationale for combining anti-angiogenic drugs and radiotherapy to enhance local control.

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### Protein expression and tumor hypoxia

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There is strong evidence that poor oxygenation (hypoxia) influences important physiological and pathological conditions. This includes development, ischemia, stroke and cancer. In cancer, hypoxia is a negative prognostic factor and is implicated in carcinogenesis, metastasis, angiogenesis and therapy resistance. The influence of hypoxia is due in large part to changes in gene expression. We are investigating the both the mechanisms of gene regulation during hypoxic stress and attempting to identify hypoxia responsive genes. Most of the known hypoxia-induced genes are regulated at the level of transcription through HIF-1. We have found that a second important mode of gene regulation during hypoxic stress occurs through inhibition of mRNA translation. The eukaryotic initiation factor 2-alpha (eIF2-alpha) becomes phosphorylated at Ser51 within 1 hr of hypoxia, resulting in a rapid decrease in protein synthesis. At higher oxygen concentrations (0.05% - 1%), the phosphorylation occurs later, and is less pronounced. This effect is specific to hypoxia and is independent of HIF-1alpha. Prolonged hypoxia causes further inhibition of translation through inhibition of the mRNA cap binding protein eIF4E. The eIF4E binding proteins (4E-BPs) become dephosphorylated, resulting in increased binding to eIF4E. Concomitantly there is loss of association of eIF4E with the scaffolding protein eIF4G, which brings eIF4E, the mRNA and the ribosomal subunit together. Finally, the eIF4E transporter (4E-T) also becomes dephosphorylated and both proteins relocalise to the cell nucleus. These changes demonstrate that cells respond to hypoxia by a rapid, co-ordinated and persistent down regulation of protein synthesis that has important implications for understanding protein expression in hypoxic tumors.

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### P53 differential radiosensitizing mechanism of a PKC-inhibitor (PKC412)

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The cellular response to ionising radiation (IR) is complex and includes a great number of intra- and extracellular targets. Increasing the tumor specific cell kill of IR with pharmacological sensitizers is an attractive goal. But so far only few genes, growth receptors and signaling proteins are known to have a key function for tumor selective radiosensitization. Based on recent data it seems unlikely that single target radiosensitizers will have a major impact for radiocurability in advanced solid human tumors. However, targeting entire survival signaling pathways with known molecular aberrations in tumor cells is an attractive concept. P53 mutations are common in locally advanced solid human tumors and might confer a radioresistant phenotype in some tumors. Growth stimulatory protein kinase C (PKC) antagonizes IR-induced cell death. Likewise activation of the phosphatidylinositol 3-kinase/Akt survival pathway suppresses pro-apoptotic signals. Compounds

that antagonizes PKC and downregulate the PI3K/Akt survival-pathway could be of potential interest as novel anticancer agents or radiosensitizers. Here we discuss the radiosensitizing effect of a PKC-inhibitors (PKC412, Novartis Inc.) with a differential p53 dependent mechanisms if combined with IR. Further data indicate that a crucial intracellular signalling target is the PI3/AKT-survival pathway.
**Material and Methods:** A novel potential radiosensitizer, PKC412 or N-benzoyl staurosprine (a broad PKC inhibitor and staurosporine derivative with a broader therapeutic index), was used for *in vitro* and *in vivo* experiments. The results were assessed in tumor cell cultures with defined p53 status and in human tumor xenografts using a transplantable mouse model system. Efficacy of radiosensitization was assessed by cell survival *in vitro* and tumor growth delay *in vivo*. To better understand the mechanism of PKC412 apoptosis assays, cell cycle analysis, mRNA and protein regulation of the PI3/AKT survival pathway with IR alone +/- the PKC inhibitor were used.
**Results:** The novel compound PKC412 has a clear radiosensitizing effect *in vivo* if combined with low dose, fractionated irradiation (4x 3 Gy) in a human tumor xenograft model system in absence of a functional p53 gene. The compound alone comprises low toxicity and therefore a broad therapeutic index. PKC412 induces massive apoptotic cell death in p53 wild type tumor cells and increases the G2 arrest in p53 dysfunctional tumor cells when combined with IR. *In vivo* PKC412 exerts a substantial growth delay effect in two different p53 dysfunctional murine and human tumor xenograft models. Furthermore the inhibition the PI-3K/Akt survival pathway by PKC412 is relevant for its apoptosis-inducing, radiosensitizing effect. A PKC412 dose dependent decrease of Akt phosphorylation was noted, as well as reduced phosphorylation of the Akt-substrate GSK3-alpha. Expression of a dominant-active form of Akt (myristoylated) abrogates the PKC412-mediated cytotoxic effect.
**Conclusions:** A tumor-associated, activated PI3K/AKT-survival pathway might contribute to a high treatment threshold for radiotherapy but represents an attractive target for radiosensitization (e.g. by PKC412) in p53-wildtype and p53- deficient tumors.

## Wednesday 20 November

WORKSHOP

# Preclinial toxicology

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### Value of human tumor xenograft models for predicting pharmacodynamic and toxicological endpoints in preclinical development of molecular drugs

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Human tumor xenografts derived from both, human tumor cell lines and patient explants, have been proven to be valuable predictors of clinical response for most of the currently registered antitumor agents (Br. J. Cancer 84: 1424-31, 2001). Their use has therefore been recommended by the regulatory agencies such as the EMEA in the "note for guidance on the pre-clinical evaluation of anticancer medicinal products".
The shift of paradigms in cancer drug development during the past decade away from exploiting the therapeutic window of cytotoxic agents to molecularly designed therapeutics, however, has led to a new thinking of preclinical in vivo testing. The molecular target and its modulation is now the major focus. Thus, in vivo efficacy in a particular xenograft model should be clearly related to target effects in the tumor tissue or a surrogate and the commonly used parameter "maximal tolerated dose" (MTD) is being replaced by "target effect dose" (TED). In order to assess preclinical pharmacodynamics and toxicology, contemporary in vivo tumor models require a convincing demonstration of target levels.
The Freiburg human tumor xenograft panel, which is derived from patient explants, comprises a collection of over 450 established tumor models of 25 different histologies. More than 80 xenografts have been characterized for in vivo response to 14 standard agents and 33 validated molecular targets. Moreover, 47 tumor models were profiled on 12K Affymetrix chips enabling a rapid identification of mRNA expression levels for approximately 1/3rd of the most prominent genes of the human genome.
We have utilized this knowledge for example to a) revisit the mode of action of established clinical agents such as mitomycin C, b) evaluate molecular targeted drugs in target-depending xenografts such as the VEGF antibody HuMV833, c) examine the pharmacodynamics of telomerase inhibition under chronic treatment and serial transplantation, or d) to assess the influence of paraneoplastic syndromes like cachexia on the tolerability of anticancer drugs.
Taken together, our experience with human tumor xenografts in the preclinical evaluation of molecular agents has proven their continuously increasing value in new cancer drug development.

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### Examples on the role of drug administration on toxicity in mice and rats

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Preclinical evaluation of drug toxictiy and efficacy are essential parts of drug development. Toxicity studies are commonly performed in mice and primates with the aim of defining maximum tolerable doses and the dose-limiting organ toxicities, and from the information gained, decide on the starting dose to be used in phase-I clinical studies. Preclinical toxicity data for an immunotoxin studied in mice and monkeys will be reported.
Although it is well known that the toxicity may be different in tumor-bearing and non tumor-bearing animals, this aspect has received very little attention. One possibility would be to include toxicity studies in animals carrying human tumor xenografts in clinically relevant tissues, either by growing them in orthotopic sites and/or by using experimental metastasis models.
We have established a number of such models in nude mice and rats, making it possible (by injecting the tumor cells by the iv, intracardial, intratibial, intrathecal routes or into the internal carotid artery) to establish organ-preferenced metastasis in various organs mimicking the situation in patients. The toxicity and efficacy of a number of established and experimental agents have been evaluated in these models, and results also from studies in which some compounds have been given by different routes will be presented.

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### The use of in vitro bone marrow toxicity to predict clinical MTD in humans

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Despite animal studies, investigational oncology drugs still enter Phase I clinical trials with uncertainty about dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) in patients. In vitro tests of drug effects on clinically relevant functions of normal human cells which are affected in the dose limiting organ, or inter-species comparisons of effects on these normal target cells, should be able to predict clinical toxicity. The prototype in vitro safety test uses the difference in drug-induced inhibition of myeloid cell production by human and mouse CFU-GM progenitors to predict the dose difference that will cause Grade 3+ neutropenia (CTC v2.0 classification). This simplest of three published prediction models [Ann Oncol 1998] has been evaluated by our Phase 1 unit at the Karmanos Cancer Institute, by the NCI and formally by ECVAM (prevalidation) and found to predict human MTD within 4-fold of the actual value for 18 of 24 compounds (4 of 4 by Karmanos, 9 of 14 by the NCI, and 5 of 6 by ECVAM). A second model uses drug sensitivity of peripheral blood CFU-GM isolated from individual Phase 1 patients to develop an exposure-risk curve for severe neutropenia. Although only a small number of drugs have been studied, model performance predicting marrow MTD after accrual of initial patient cohorts is promising. All of these models predict marrow MTD, which will be the actual MTD, if neutropenia is dose limiting. If not, and other organs are more susceptible to drug toxicity, in vitro hematotoxicity testing provides an upper limit on tolerable human exposure levels, rather than the MTD. In other words, the CFU-GM assay predicts the risk of marrow toxicity as a function of drug exposure, but will not be useful to classify a drug as myelotoxic unless conducted in parallel with other predictive tests for non-hematologic toxicities. These studies show the utility of the CFU-GM assay for predicting the simple toxicity of neutropenia and its clinical outcome, as it is quantitative (linear), target cell-based, and uses clinically relevant endpoints. These results point to expanded use of the prediction principles to other, similar dose-limiting toxicities, such as mucositis and stomatitis. They also indicate the value of Phase 1 clinical trial data for developing and validating in vitro safety tests and prediction models for all therapeutic classes. Supported in part by NIH grants UO1-CA62487 and R21-CA93266 and contract NO1-CM-87028.