xenographs. As an alternative approach we have applied the power of mouse genetics to produce animal models that recapitulate the genetics and pathobiology of human malignancies to study treatment responses of tumors treated at their natural site. One model we have focused on is the Em-myc transgenic mouse. These mice overexpress the c-myc oncogene in B-cells, and develop malignancies that resemble human Non-Hodgkin's lymphomas. Using methods for rapidly producing Eμ-myc lymphomas with compound genetic lesions and experimental strategies that parallel clinical trials, we have characterized biologic and genetic determinants of drug resistance in vivo. These studies have identified potential mechanisms of drug sensitivity and resistance, and highlight the relationship between tumor cell genotype and its response to cancer chemotherapy. They also suggest rational strategies to reverse drug resistance in some tumor types.

13 INVITED

Patient and cell line derived human tumor xenograft models – preclinical/clinical correlations

H.H. Fiebig. *Oncotest GmbH, Institute for Experimental Oncology, Freiburg, Germany*

For all major solid human tumor types experimental models have been developed by engrafting patient tumors or permanent human cell lines into immunodeficient mice. Transferring the NCI 60-cell line panel – the largest cell line panel used for drug discovery – *in vivo* into nude mice resulted in sc growth in 47/58 cases (Fiebig et al 1989), or 49/60 cases (Plowmann et al. 1997), respectively. My group implanted more than 1600 solid tumors directly from patients, leading to more than 400 permanent xenograft models. The take rate was highest (38–51%) for melanomas, lung and colon cancers. 60 xenografts were characterized in detail by comparing histology, and 10 surface markers over 10 *in vivo* passages with the original patient tumor. More than 90% showed very similar histology and marker profiles. From 100 xenografts the histology, sensitivity to standard cytotoxic agents, 35 molecular drug targets and expression profiles of 34.000 genes (Affymetrix chips HU133) were determined and compared with the occurrence in the *in vivo* growing NCI-60-cell line panel. 85% of the NCI cell lines showed an undifferentiated or very poorly differentiated histology *in vivo* without the typical tumor architecture seen in e.g. adenocarcinomas of the colon or lung in contrast to the patient's and the patient derived xenograft models. Many cell line-derived xenografts grow faster than patient-derived ones. For colon xenografts, comparison of gene expression profiles showed some differences in cell line-derived models to patient derived xenografts in typical colon associated genes. Activity of standard cytotoxic agents (regression) was observed in cell line-derived xenografts only with alkylating agents, but not with Vinca-alkaloids, Adriamycin, VP16, 5-FU and Methotrexat. In the Freiburg xenograft panel we obtained regressions for 12 clinical active standard agents except 5-FU from which mice tolerate only 25% of the human dose. The response of the same tumors treated in the nude mouse and in the patient was investigated in 42 combinations and 38 single agent therapies. The xenografts showed a very similar response as the same tumor in the clinic, in 90% (19/21) for remission and in 97% (57/59) for resistance. The high correct predictivity validates the xenograft system for drug development. More recently, also target-directed compounds effected remissions and T/C values <10%, e.g. EGF-R-, VEGF- and HSP-90-inhibitors EMD72000, Erbitux, Avastin and 17-AAG. Modulation of the respective targets *in vivo* has been demonstrated. Patient derived xenograft models established in nude mice reflect very well the clinical situation except for metastasis and they are excellent models for tumor biology studies and for the discovery of target directed novel antitumor agents.

Wednesday 29 September 08:00–09:45

WORKSHOP 3

Mechanistic combinations

14 INVITED

Combined targeted agents with cytotoxic chemotherapy

L. Gianni, G. Bianchi, G. Mariani, S. Cresta. *Istituto Nazionale Tumori, Division of Medical Oncology A, Milan, Italy*

The availability of targeted drugs interfering with signal transduction and/or intracellular signaling has led to the investigation of the potential for using them in combination with conventional chemotherapy that still is the leading therapeutic option for medical treatment of cancer patients. The possibility for combination stands on several considerations. The cellular targets of chemotherapeutic and of targeted drugs are different, supporting the concept that combinations would not lead to cross-resistance, while expected toxicity would not be overlapping. Indeed, perturbation of signals involved in regulation of growth, survival, invasion and metastasis could be associated with enhanced sensitivity to chemotherapeutic drugs and eventually lead to synergistic antitumor effects. The possibility of combining chemotherapy and targeted drugs has been extensively explored in several cellular and animal models. In most cases, the combination of cytotoxic agents and drugs targeting erbB receptors, farnesyl-transferase, m-TOR, PTEN, proteasome, VEGF and VEGF-receptors, PDGFR and many other signaling pathways including those involved in apoptosis has indicated at least additive and often synergistic results. Such experimental evidence has served as a basis for designing clinical studies for many such combinations. Most molecular targeted agents could be combined with most cytotoxic agents at full or nearly full doses. In most instances, continuous exposure to the targeted drugs was associated with concomitant delivery of monochemotherapy or classical combination chemotherapies. Less frequently, the targeted drugs were given sequentially after the delivery of the planned chemotherapy. Outcome of these trials have sometimes posed problems of enhanced or unexpected toxicity, although tolerability of the combinations has rarely been a limiting factor. The clinical antitumor activity and efficacy has offered mixed results. In some case, most notably that of combinations of anti-erbB2 drugs and chemotherapy, significantly improved efficacy and possibly syergism was documented. In other examples, the preclinical evidence supporting supra-additive effects was not confirmed in the clinic. The mixed success of the approach is possibly related to the emerging awareness that in most cases the presence of the target is not the only variable dictating the sensitivity to the targeted agent(s). The future success of combinations of cytotoxic agents and targeted drugs will likely depend on the clarification of what makes the target competent of the tumor survival, and in which cases. That clarification will restrict the applicability of targeted drugs, but, at the same time, expand the possibility of exploiting and measuring expected synergisms from the application of these new combinations in selected subgroups of patients.

15 INVITED

Radiation with targeted agents

M. Brown. *Stanford University, Department of Radiation Oncology CCSR-South, Room 1255, CA Stanford, USA*

Two fundamental principles should guide the extrapolation of preclinical data with radiation and anticancer agents to the clinical situation as follows:

1. The mechanisms by which each agent produces its antitumor effect must be understood.
2. The extent to which the preclinical model mimics the human situation in those aspects relevant to the mechanisms of the agents must be known.

This presentation will review examples relevant to the combination of radiation with targeted agents with these two principles in mind. Specifically, we will review the extent to which the response of tumors to radiation is governed by the sensitivity of the tumor cells versus the sensitivity of the endothelial cells comprising the vasculature. The answer to this question will determine the efficacy and way in which radiation should be combined with antiangiogenic agents. Also of relevance to the combination of radiation with antiangiogenic drugs is the extent to which transplanted tumors reflect human spontaneous tumors in their reliance on neovasculature. We will show that preclinical models with transplanted tumors are likely to "overpredict" the efficacy of antiangiogenic therapy in the clinic because rapidly growing transplanted mouse tumors have a total reliance on the neovasculature whereas this is not the case with most human tumors. Second, we will review the extent to which preclinical models mimic the clinical situation when radiation is combined with hypoxic cell radiosensitizers or hypoxic cytotoxins. In this context many studies, particularly with hypoxic cell radiosensitizers, were conducted at doses of radiation and drugs that did reflect the clinical situation, and which let to inappropriate expectations in the clinic. Third, we will examine the extent to which short-term assays using apoptosis or tumor shrinkage are relevant to the response of human tumors to combinations of radiation with therapies designed to enhance tumor cell apoptosis.

16 INVITED

Targeted agent with targeted agents

P. Houghton. *St Jude Children's Research Hospital, Molecular Pharmacology, Memphis, USA*

Dysregulation of signaling cascades involved in cellular proliferation and survival is a hallmark of many human cancers, and thus presents potentially selective targets for therapeutic intervention. Agents that target signaling pathways dysregulated during transformation and tumor progression are entering clinical trials. However, with the exception of imatinib, other

kinase inhibitors have shown relatively modest activity as single agents. It is anticipated that clinical development of these agents will involve first combining them with cytotoxic agents and subsequently with other signaling inhibitors. However, the number of potential combinations of signaling inhibitors is daunting, and there is a lack of knowledge about human cancers that may be used to guide the use of individual targeted agents or for the rational choice of combinations. Further, inhibiting one pathway can lead to upregulation of other pathways as part of the cellular stress response. Understanding which pathways are critical for cancer cell proliferation and survival under physiological and stress conditions will be critical in understanding how best to combine agents.
Dysregulation of the phosphatidylinositol 3 kinase (PI3K)-Akt-mTOR pathway occurs frequently in human cancer. Oncogenes, overexpressed receptor tyrosine kinases and constitutively activated mutant receptors, amplification of the p110 catalytic subunit of PI3K, loss of PTEN phosphatase function, amplification of Akt2, inactivating mutations of the tuberous sclerosis proteins hamartin and tuberin (TSC1/2), and overexpression the small G-protein Rheb (in transformed cells) activate mTOR (mammalian target of rapamycin) a serine/threonine kinase that through control of translation initiation, regulates cell size, proliferation, survival and responses to cellular stress (nutritional deprivation, cellular energy charge and hypoxia). Overexpression or amplification of eIF4E (the RNA cap-binding protein) downstream of mTOR in many human cancers also indicates the role of this pathway in maintenance of the transformed phenotype. Here we will explore the role of preclinical models in identifying combinations of targeted agents building on mTOR inhibition. Examples to be considered are the combination of inhibitors in different signaling pathways that impinge on a common product, and combinations of inhibitors of different steps within the same signaling cascade that may circumvent stress-induced pathways.

Wednesday 29 September 10:15–12:00

WORKSHOP 4

Practical issues in tissue research

18 INVITED

US NCI perspective on tissue handling and banking

S.E. Taube. *National Cancer Institute, NIH, Cancer Diagnosis Program, Division of Cancer Treatment and Diagnosis, Rockville, MD, USA*

The National Cancer Institute (NCI) of the US has long supported the collection and distribution of human specimens to facilitate research. Different models have been developed to meet varying research needs. These have included procurement, traditional tissue banks and virtual tissue banks. The procurement model enables researchers to identify the types of specimens they require and the format they prefer; the resource staff coordinate with participating hospitals to acquire the necessary surgical specimens. Traditional banks are centralized repositories where specimens are submitted and held until requested. The virtual bank model the NCI has used involves centralizing the data associated with specimens, but leaving the specimens at the sites where they are archived until they are requested. The data associated with the specimens varies with the purpose of the collections and the needs of the research. Procedures have been developed for both pathology review and data quality assurance. Informed consent and other regulatory issues have also been addressed and modified as new regulations have been put in place. Rules for accessing specimens differ depending on how limited the availability of specimens is and the nature of the associated data. The various models will be presented with discussion of how well they are meeting research needs and what gaps remain. Challenges will be described as well as innovative plans to address anticipated needs.

19 INVITED

US Cooperative Group perspective on tissue handling and banking

R. Schilsky. *University of Chicago, Pritzker School of Medicine, MC 1000 Biological Sciences Division, Chicago, USA*

Conducting correlative science studies requires a coordinated system that includes centralized and standardized collection of patient specimens; storage under controlled conditions with appropriate safeguards; pathology quality control; a comprehensive inventory; a process to distribute specimens to approved investigators consistent with patient consent and to receive the research results from investigator laboratories; and policies to safeguard patient confidentiality. Ultimately, the results of the laboratory studies must be linked to and correlated with the clinical outcomes of patients treated on clinical trials. The US cooperative group program includes 9 NCI-funded groups that conduct multi-center studies of cancer prevention, treatment, biology and health outcomes. Each group operates one or more repositories that collect a variety of human specimens including frozen tumor tissue, paraffin-embedded tumor and normal tissue, germline DNA and serum. These specimens are collected from patients enrolled on clinical trials and are accompanied by detailed information on patient characteristics, treatment and outcomes. CALGB protocols contain instructions for specimen acquisition, handling and shipping and CALGB uses customized specimen tracking software to monitor sample shipping, receipt and distribution. Standardized procedures for specimen storage and distribution are in place at all CALGB repositories each of which is designed according to the guardian repository model. Investigators are asked to provide local IRB approval of research studies and to sign an investigator agreement before receiving specimens from a CALGB bank. Despite these well-established procedures, many barriers remain to correlative science research in the cooperative group program. These include variability in local IRB review/approval for such studies; variability in specimen handling across multiple sites and at multiple repositories with varying expertise/resources; lack of uniform policies and procedures for access to and use of human specimens; lack of harmonization of guidelines across the multiple federal and state agencies that oversee such research and lack of standard approaches to specimen/data ownership and intellectual property. The cooperative groups have begun to address these issues collaboratively with NCI through formation of the Group Banking Committee that will establish uniform minimum standards for specimen handling/banking across the cooperative group program.

20 INVITED

Current challenges of national and pan European human tumour banking

M.M. Morente. *Spanish National Cancer Center – CNIO, Spanish National Tumour Bank Network, Madrid, Spain*

Current oncological research has three main characteristics: The capacity for large-scale studies in genomics and proteomics, the high sensitivity of the current tools, and the transfer from basic to clinical research. All these three characteristics are dependent of especially procured tissues: Large-scale molecular studies need large numbers of cases to identify new parameters of clinical value, highly sensitive techniques require appropriately handled samples, and the translational research needs homogeneous tissue-sampling protocols avoiding the bias of multicentre studies.
Some of the more urgent challenges in Tumour Banking include:
A new hospital strategy
Tumour Banking requires collection, freezing and storage of neoplastic and normal tissues and these activities must be considered a routine in the Departments of Pathology although they must be considered from the hospital point of view including new type of Biorepositories in parallel such as: Serum banks, minimally passaged tumour cell lines for drug checking and, mainly, clinical data.
Networking
As previously mentioned, current oncological research needs of a large number of cases homogeneously treated, followed up and with tissue samples in the context of multicentre and multinational projects. For this reason, networking appears the best environment where TB must grow. Spanish, UK or EORTC networks can be taken like a model in this issue
Integration in clinical trials and projects of excellence
The best role to be play for TB in Translacional research is its close integration of Tumour Banks in clinical trials of excellence including molecular profiling by using frozen samples with protocolised clinical information.
Ethics and laws
Although there are broad principles regarding the use of human tissue material in Europe and the US, the various laws and customs in the different countries or States show that national laws and custom still dominate. Occasionally, there are conflicts in the laws and policies between these nations and states. To solve this diverse legislations is, perhaps, the most important challenge for Tumour Bank Networking in the very close future. Europe needs a common legislation which explicitly would cover the use for research, not only for clinical practice or genetic susceptibility studies, of surplus diseased and normal tissue, linked histopathological data and relevant clinical information, with a linked-anonimased design. This common legislation o directives, independently to what has presently been legislated by others, could start forming a common legislative body for European countries, so that it enables and enhances the development of international multicentre studies.