Materials and Methods: B16 F10 cells were implanted subcutaneously on the back of C57BL/6 mice. Animals were randomly divided into four groups and treated with 50,000 IU IL-2 daily by subcutaneous injection, 5 mg/kg MS-275 daily by oral gavage (5 days/week for two weeks), or a combination thereof. Treatment was started 3 days after tumor cell injection.
Results: Weekly measurement of tumor size and tumor weight after 3 weeks of treatment showed significant tumor inhibition (>60%) in the combination therapy-group compared to the IL-2 (no significant inhibition) or MS-275 (no significant inhibition) groups. Kaplan-Meier analyses showed a statistically significant increase in the survival rate of the combination group, compared with control and single agent-groups. The percentage of CD4+ and Fox-p3+ T cells decreased in the lymph nodes of tumor-bearing animals treated with the combination of MS-275 and IL-2, whereas in the control and single agent-group the percentage had increased. Similarly, the percentage of CD8+ and CD69+ (activated CD8) cells had increased in the lymph nodes of the tumor-bearing animals treated with the combination of MS-275 and IL-2 in comparison with the control and MS-275-treated groups. These results suggest that a combination of IL-2 and MS-275 has a synergistic antitumor effect in vivo in an immunocompetent murine model of malignant melanoma. The antitumor effect was associated with a decreased number of regulatory T cells and increased activated CD8 cells.
Conclusion: These data provide the rationale for clinical testing of the combination of IL-2 and HDAC inhibitors in the treatment of malignant melanoma patients.

67 POSTER

A relevant panel of human uveal melanoma xenografts directly established from primary and/or metastatic patient's tumor for pharmacological preclinical assays

F. Némati[1], X. Sastre[2], J. Couturier[3], P. Mariani[4], S. Piperno-Neumann[4], L. Desjardins[5], O. Lantz[2], A. Dahiani[1], S. Arrufat[3], M.F. Poupon[1], D. Decaudin[1]. *[1]Institut Curie, Translational Research, Paris, France; [2]Institut Curie, Tumor Biology, Paris, France; [3]Institut Curie, Genetics, Paris, France; [4]Institut Curie, Medical Oncology, Paris, France; [5]Institut Curie, Ophthalmological Oncology, Paris, France*

Background: Uveal melanomas, which are the most common intraocular malignancy, have a pejorative outcome with about 50% of the patients who die of metastases. No efficient chemotherapy was still available and new therapeutic approaches should be evaluated to improve the prognosis. Human cancer xenografts transplanted into immunodeficient mice, that mimic the patients' tumor genomic heterogeneity, constitute a useful preclinical tool for testing new agents and protocols and for further exploration of the biological basis of drug responses. The aim of this study was then to develop, establish, and characterize an in vivo panel of xenografts directly obtained from uveal melanoma patients.
Materials and Methods: Samples obtained from primary tumors after enucleation or from liver metastases, after patients' consent, were subcutaneously xenografted into immunocompromised mice. A characterization of the xenografts growing into mice was then performed and compared to originated tumors, including histopathological, genetic (karyotype or FISH, and CGH-array), and molecular assays, as well as in vivo response to conventional chemotherapy.
Results: Thirty-seven xenografts have been obtained among 95 patient's tumor sample transplantations in which 10 that have still grown after at least three transplantations in mice and that have been characterized. Pathological analyses of these ten xenografts confirmed the diagnosis of uveal melanoma and showed, for the five models derived from primary tumors, similar chromosome 3 status, namely 2 monosomies and 3 disomies in which one probable isodisomy. Bcl-2 protein was overexpressed in all but 2 xenografts. NA17 and Melan-A antigen expressions were positive in all tested samples, tyrosinase antigen expression was positive in all but 2 xenografts, and MAGE-(1/2/3/4/6/10), LAGE-1, and MAGE-C2 antigens expression were negative in all studied cases. Finally, In vivo therapeutic assessments are currently performed and will be further presented.
Conclusion: Our in vivo human uveal melanoma xenografts present the same histopathological and genomic characteristics of the patient's originated tumors. This observation supports the use of our panel for pharmacological preclinical evaluations that could serve as a bridge linking pre-clinical and clinical research, and drug development.

68 POSTER

Sensitivity of a disseminated in vivo model of L363 plasma cell leukaemia against antitumoral compounds sorafenib, bortezomib, and dexamethasone

J. Schueler[1], D. Wider[2], M. Wagner[1], H.H. Fiebig[1], M. Engelhardt[2]. *[1]Oncotest Institute – Freiburg, in vivo, Freiburg, Germany; [2]University Freiburg Medical Center – Freiburg, Hematology and Oncology, Freiburg, Germany*

For the better understanding of multiple myeloma (MM), the establishment of reproducible in vivo models is pursued worldwide. We have established a cell line-based, disseminated MM model in NOD/SCID-IL2-receptor-gamma-chain$^{-/-}$ (IL2$^{-/-}$) mice. In the current study, this model was validated in various treatment groups, using 1. bortezomib (B: 0.7 mg/kg/day (d); d: 0, 4, 11), 2. sorafenib (S: 200 mg/kg/d; d: 0−11), 3. dexamethasone (D: 3 mg/kg/d, d: 0−4 + 7−11), in comparison with 4. a control group. L363 cells were injected intratibialy (it) into IL2$^{-/-}$ mice and respective therapies were started 7 days after L363-implantation (d0). Tumor growth was monitored with (a) flow-cytometry (FACS; detection of human HLA-A, B, C + CD138), (b) daily monitoring of MM symptoms, (c) fluorescence-based in vivo imaging (FI) and (d) serum osteocalcin analyses. Tumor inhibition was calculated as the median percentage of MM cells at respective compartments of the test- vs. control-group multiplied by 100 (optimal test/control (T/C) in %). L363 engrafted reliably (take rate=100%) at the injection site and in distant organs, such as bone marrow (BM; 100%), spleen (38%) and rarely liver (8%). Control mice developed MM symptoms, such as hind limb pareses, weight loss and osteolyses. L363 cells were detected by FACS and FI, not only at injection sites, but also in the BM, hollow bones and spleen. Primary tumor development was markedly reduced by S (optimal T/C of 23% on d14), as well as with D or B, albeit to a much lesser extend (optimal T/C: 81% + 62% on d14, respectively). BM metastases were also significantly reduced by S with an optimal T/C value of 67% on d28. D and B, possibly due to subclinical doses (determined after titration and toxicity experiments), had no relevant influence on BM metastases (97% + 100% optimal T/C on d28, respectively). Thus, L363 engraftment into IL2$^{-/-}$ is a valuable in vivo model for MM which exhibits high reproducibility, take- and metastases-rates and closely mimics the clinical situation. Collection of whole-body FI data proved to be a time- and animal-saving analysis that allows to closely monitor MM growth. Further investigations will validate the very promising antitumor activity of S and evaluate the potentially synergistic effect of B and S. The evaluation of new therapeutic approaches in comparison to standard agents was thus successfully conducted, suggesting that this model serves as a valuable tool in the development of new anticancer strategies.

69 POSTER

Evaluating the duel kinase inhibitor lapatinib: Bioanalytical method development and pharmacokinetic analysis in mouse, rat, and human and determination of in vivo efficacy in a panel of EGFR wildtype and mutant human tumor xenograft models

J. Cooper[1], F.E. Nieves[1], J. Kuhn[2], C.H. Takimoto[1], A.W. Tolcher[3], M.J. Wick[1]. *[1]START (South Texas Accelerated Research Therapeutics), Pharmacology, San Antonio, TX, USA; [2]University of Texas/UTHSCSA, College of Pharmacy, San Antonio, TX, USA; [3]START (South Texas Accelerated Research Therapeutics), Clinical Research, San Antonio, TX, USA*

The EGFR/Erb-2 (Her2/neu) duel tyrosine kinase inhibitor lapatinib is currently approved as part of a combination therapy in some types of metastatic breast cancer. Because of its mechanism of action and positive preclinical results, clinical trials are underway examining the possibility of expanding the use of this agent in other indications both alone and in combination with approved chemotherapeutics or novel agents in later stages of development. Several early-stage compounds have shown promising single agent activity in preclinical studies and may be useful when combined with lapatinib in treatment of certain cancer types.
Our goal was to benchmark lapatinib in various preclinical systems for subsequent studies evaluating effects of early-stage anticancer agents on factors including pharmacokinetic (PK) parameters, tumor and tissue deposition, and antitumor activity as well as a rodent to human PK comparison. To accomplish this, we developed a bioanalytical method for quantification of lapatinib in mouse, rat, and human plasma and rodent tissue; experimental determinations included half-life with single and repeated dosing, oral versus intravenous bioavailability, and determination of plasma: tumor and tissue ratios. In addition, we screened a panel of human tumor xenograft models including non-small cell lung based on EGFR mutation status, practical tissue types, and published results. Activity of lapatinib was also compared to standard agents in several of these models.

In the lung xenograft models, lapatinib was active towards NCI-H292 ($EGFR^{wt}$) as well as HCC-827 (delE746-A750) and demonstrated increased activity compared with cetuximab in this model as well as the non small cell lung K-Ras mutant line NCI-H441; however, lapatinib was only marginally active towards NCI-H1650 (delE746-A750) and inactive in NCI-H1975 (L858R/T790M). In addition, lapatinib was active towards the SK-OV-3 ovarian tumor model and demonstrated comparable activity to the multi TKI compounds sunitinib and sorafenib towards the EGFR and VEGF expressing A431 model. In these studies, no significant differences were noted in plasma: tumor ratios with comparable drug concentrations in both sensitive and insensitive tumors. In addition, some species differences were reported in half life and bioavailability studies.
Results from these studies benchmark lapatinib pharmacokinetic characteristics in rodents and humans and antitumor activity in various preclinical model systems which can be used to aid in the preclinical development of early stage anticancer agents.

70 POSTER

In vitro activity of the multi targeted receptor tyrosine kinase inhibitor sunitinib against multiple myeloma cell lines is not predictive of in vivo xenograft response

M. Batey[1], J.L.R. Brito[1], H. Maitland[1], H. Leung[1], A. Hall[1], G. Jackson[1], D.R. Newell[1], J.A.E. Irving[1]. *[1]Newcastle University, Northern Institute for Cancer Research, Newcastle upon Tyne, United Kingdom*

The t(4;14) gene translocation in 15% of Multiple Myeloma (MM) patients leads to the ectopic expression of FGFR3 and is associated with a poor prognosis, suggesting that FGFR3 is a therapeutic target in this disease. In in vitro kinase assays, sunitinib has been identified as a potent FGFR3 inhibitor with an IC50 of between 120–300 nM. An interleukin 3 (IL-3) independent Baf/3 cell line engineered to express the ZNF198-FGFR1 fusion protein, and t(4;14) positive and negative MM cell lines, were used to screen for growth inhibition using the MTS assay. Sunitinib differentially inhibited the growth of Baf/3 cell transfectants compared to wild type cells, with a GI50 value of 730 nM versus 2.7 μM. Similarly, sunitinib induced differential growth inhibition of t(4:14) positive cell lines, with a GI50 of 1.2 μM compared to 4−5 μM for t(4:14) negative cell lines. No other FGFR isoforms were expressed in the MM cell lines. Sunitinib inhibited the activation of FGFR3 in t(4;14)-positive MM cells, and induced growth inhibition and apoptosis in a concentration and time-dependent manner in FGFR3-expressing MM cell lines. Consistent with growth inhibition, sunitinib was also shown to inhibit the activation of the MAPK pathway in MM cell lines through monitoring pERK1/2 levels by Western blot analysis. The in vivo efficacy of sunitinib was assessed using JIM1 (t(4:14) positive) and RPMI8226 (t(4:14) negative) subcutaneous xenografts grown in mice treated with vehicle control or 40 mg/kg sunitinib daily by oral administration for 21 days. In contrast to the in vitro data, treatment with sunitinib gave only a minor 2 day growth delay in the t(4:14) positive JIM1 xenograft model, whereas marked antitumour activity was observed in the t(4:14) negative RPMI8226 model with at least a 15 day growth delay. These results suggest that the in vivo efficacy of sunitinib is not solely due to cellular determinants, but is also dependent on other factors such as, potentially, host stroma interactions. Furthermore, these studies caution against using the t(4:14) translocation as a predictive biomarker for sunitinb sensitivity in MM.

71 POSTER

Features of chemo and radiotherapy response of a new model of breast cancer xenograft derived from a BRCA2 germ-line mutation carrier patient's tumour

E. Marangoni[1], L. De Plater[1], B. Froget[1], L. Durand[1], S. Piperno-Neumann[2], B. Sigal-Zafrani[2], D. Stoppa-Lyonnet[2], A. Laugé[2], D. Decaudin[1], M.F. Poupon[1]. *[1]Institut Curie, Translational Department, Paris, France; [2]Institut Curie, Medical Oncoloy, Paris, France*

Background: BRCA germline mutations predispose to breast and ovarian cancers that have high propensity to relapse rapidly after response to the initial treatment. Several clinical agents exert their cytotoxic effect through the formation of double-strand DNA breaks and the effect of BRCA2 on DNA repair might be exploited clinically. The purpose of this work is to characterize a new xenografts with BRCA2 gene mutation that can be useful for the study of the biology of BRCA2-deficient breast cancers and to test new drugs.
Patient's tumor, material and methods: A BRCA2-deficient breast cancer xenograft was established directly from a patient's tumor, of a 37-year old woman. The patient had a BRCA2 germ-line mutation and was treated by mastectomy, taxane-based chemotherapy and parietal radiotherapy. The clinical control of the disease was achieved with a cisplatinum-ifosfamide combination. Characterization of the HBCx-17 xenograft was performed and compared to that of patient's tumor, including histological analysis, BRCA2 gene sequencing, p53 status determination and genetic analysis by comparative genomic hybridization (CGHarray). Detection of gamma-H2AX repair foci was done by immunofluorescence microscopy. Sensitivity to different standard chemotherapies and radiosensitivity of the HBCx-17 xenograft were evaluated. A cell line was also obtained from the xenograft.
Results: The xenograft presented the same features than the primary tumor. HBC-17 was identified as a triple negative breast cancer (ER−, PR−, ERBB2−) with a high EGFR expression and a mutated p53. Ki67 staining revealed a high proliferation status in both patient and xenograft cancer cells and CGHarray showed a high number of gene alterations. Therapeutic assessment of the xenograft showed sensitivity to anthracyclin-based chemotherapy, to radiotherapy and cisplatin-based treatments and lack of sensitivity to taxane. Deficiency in DNA DSB repair was found by staining the phosphorylated γ-H2AX foci after γ-irradiation. Loss of heterozygosity at the BRCA2 locus was detected by poly-CA analysis.
Conclusions: This study describes the characterization of a new human breast cancer xenograft model obtained directly from a BRCA2 gene mutated patient. This tumor xenograft represents a unique *in vivo* model of triple negative BRCA2 mutated breast cancer conserving the same biological caracteristics of the patient tumor, providing a useful tool for testing new agents and protocols and for further exploration of the biological basis of drug responses.

72 POSTER

Preclinical evaluation of the tyrosine kinase inhibitor erlotinib: Bioanalytical method development and pharmacokinetic analysis and in vivo evaluation and comparison in a panel of human EGFR wildtype and mutant tumor xenograft models

F.E. Nieves[1], J. Cooper[1], G.L. Mangold[2], J. Kuhn[3], C.H. Takimoto[1], A.W. Tolcher[2], M.J. Wick[1]. *[1]START (South Texas Accelerated Research Therapeutics), Pharmacology, San Antonio, TX, USA; [2]START (South Texas Accelerated Research Therapeutics), Clinical Research, San Antonio, TX, USA; [3]University of Texaser/UTHSCSA, College of Pharmacy, San Antonio, TX, USA*

The epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor erlotinib is currently used for treatment of non-small cell lung cancer and as part of a combination therapy in some pancreatic cancers. Because of its mechanism of action and positive preclinical results, clinical trials are underway examining the possibility of expanding the use of this agent in other indications both alone and in combination with approved chemotherapeutics or novel agents in later stages of development. Several early-stage compounds have shown promising single agent activity in preclinical studies and may be useful when combined with erlotinib in treatment of certain cancer types.
Our goal was to benchmark erlotinib in various preclinical systems for subsequent studies evaluating effects of early-stage anticancer agents on erlotinib pharmacokinetics, tumor and tissue deposition, and antitumor activity. To accomplish this, we developed a bioanalytical method for quantification of erlotinib in mouse and rat plasma and tissue; experimental determinations included half-life with single and repeated dosing, oral versus intravenous bioavailability, and determination of plasma: tumor and tissue ratios. In addition, we screened a panel of human tumor xenograft models including non-small cell lung based on EGFR mutation status, practical tissue types, and published results. Activity of erlotinib was also compared to the EGFR-targeting antibody cetuximab in several of these models.
In the lung xenograft models, erlotinib was active towards NCI-H292 ($EGFR^{wt}$) as well as HCC-827 and NCI-H1650 (delE746-A750) and demonstrated increased activity compared with cetuximab in both 746–750 deletion mutants as well as the non small cell lung line NCI-H441 ($K\text{-}Ras^{mut}$); however, erlotinib was inactive towards the NCI-H1975 (L858R/T790M) model. No significant differences were noted in plasma: tumor ratios with comparable drug concentrations in both sensitive and insensitive tumors. In addition, some species differences were reported in half life and bioavailability studies.
Results from these studies benchmark erlotinib pharmacokinetic characteristics in rodents and antitumor activity in various model systems which can be used to aid in the preclinical development of early stage anticancer agents.