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.