273 POSTER

Use of bioluminescence imaging and quantitative RT-PCR to monitor tumor progression and treatment response in orthotopic AML mouse models: Application to the targeted cytotoxic agent F14512

L. Creancier[1], A. Pillon[1], J. Verdier[1], N. Chansard[1], S. Pourtau[1], I. Vandenberghe[1], C. Recher[2], N. Guilbaud[1], C. Bailly[1]. [1]*Institut De Recherche Pierre Fabre, Centre de Recherche en Oncologie Expérimentale, Toulouse, France;* [2]*CHU Toulouse, Service dhématologie Hôpital Purpan, Toulouse, France*

After xenotransplantation of human cells into mice, it is necessary to detect those cells as early as possible and at a low level to monitor their engraftment, dissemination and growth in immunodeficient mice. To this purpose, we developed two methods based on bioluminescence imaging and quantitative RT-PCR. HL-60 and KG-1 AML cell lines were constitutively modified to express the firefly luciferase (HL60-Luc and KG1-Luc) allowing thus in vivo detection by non-invasive bioluminescence imaging. We also developed a PCR method for the specific detection of human and mouse glyceraldehyde-3-phosphate-desydrogenase (GAPDH) mRNA. HL60-Luc and KG1-Luc cells were implanted intravenously or in the tibia bone marrow of irradiated NOD-SCID mice. Leukemic cell proliferation, monitored by optical imaging, was correlated to the quantitation of human leukemic cells in mouse blood and bone marrow by quantitative RT-PCR and FACS. In an effort to mimic the human disease, we injected approximately 10^6 AML cells (from patient LAM09–012) in irradiated NOD-SCID mice and allowed them to establish as xenografts. Circulating leukemic cells were detected in peripheral blood of these living mice by quantitative RT-PCR after 8 weeks. This technology was also used to assess the antileukemic activity of F14512, a potent spermine–drug conjugate exploiting the polyamine transport system for tumor cell delivery, against these models of primary AML. Multiple i.v. administrations of F14512 at 0.32 mg/kg, induced an extensive reduction of the number of leukemic cells in mouse blood and bone marrow (97−99%), assessed by quantitative RT-PCR and confirmed by FACS analysis and histology. In order to determine if key properties of leukemic stem cells such as self-renewal are targeted by F14512, a secondary transplantation of LAM09–012 was performed following in vivo treatment and was monitored by quantitative RT-PCR. While the LAM09–012 AML cells harvested from the drug-vehicle treated mice have maintained homing and repopulation to the bone marrow of secondary recipient mice, the repopulation capacity of LAM09–012 AML cells harvested from F14512-treated mice was abolished. No re-growth was observed after 25 weeks post secondary transplantation. In conclusion, the quantitative RT-PCR and bioluminescence imaging methods presented here provide a sensitive and reliable detection and quantitation of low numbers of human cells in immunodeficient mice. In addition, these methods permit a non-invasive monitoring of drug effects in vivo, reducing thus the extensive use of mice for conventional pharmacological studies. Furthermore, these results also demonstrated that F14512 exhibits a marked preclinical antileukemic activity in patient-derived AML models and support its on going phase I clinical trials.

274 POSTER

Bioluminescence imaging for monitoring tumor growth of GI-tract tumors

P. Schulz[1], C. Dierkes[1], A. Rexin[1], B. Wiedenmann[1], C. Grötzinger[1]. [1]*Charite Universitätsmedizin Berlin, Department of Hepatology and Gastroenterology, Berlin, Germany*

Introduction: Bioluminescence imaging (BLI) is an attractive non-invasive technique for monitoring and characterizing tumor growth. For this purpose different stable cell cancer cell lines (CRC, pancreatic, NET) expressing the luciferase gene (Renilla or firefly) were established and characterized *in vitro* and *in vivo*. By administrating the substrates luciferin or colelenterazine, growth of different tumor models (ectopic and orthotopic) were monitored.
Material and Methods: Several human gastrointestinal tumor cell lines (colorectal, pancreatic, neuroendocrine), stably expressing luciferase or renilla constructs were validated in vitro and in vivo. For the in vitro characterization proliferation assays and kinetic studies were performed. Furthermore different cell clones were tested for their in vivo growth characteristics. For this purpose the cells were inoculated either subcutaneously (5×10^6 cells) into the flanks of nude mice or orthotopically (1×10^6) into the head of the pancreas (PaCa) or into the wall of the colon ascendens (CRC model). Shortly before in vivo BLI, the substrates were either injected i.p. (Luciferin) or iv (Coelenterazine).
Results: Several gastrointestinal cell lines were successfully stably transfected with either luciferase (luc) or renilla (Rluc) constructs. In vitro analysis of the established cell lines showed high bioluminescence signals in comparison to purchased luc-expressing cell lines. In vitro, Rluc expressing cell lines showed a 10 fold higher bioluminescence signal (10.000–80.000 phtotons/sec) compared to luc expressing cells (1000–6000 photons/sec). In addition, kinetic studies in Rluc-cells showed a fast decrease of the signal within the first 10 min after adding the substrate, whereas Luc-cells showed a stable expression over 60 min. In vivo tumor growth of s.c. growing tumors was measured by BLI and calliper measurements which showed similar growth characteristics. Contrary to the in vitro results, in vivo, Luc-expressing cells showed higher BL signal compared to Rluc-cell lines. For the first time we could also measure the tumor growth of our orthotopically growing tumors by BLI which showed an increase of signal over time.
Conclusion: Our findings show hat BLI improves monitoring of tumor growth of subcutaneous growing xenografts and especially offer non invasive monitoring tumor growth of orthotopically growing tumors of the gastrointestinal tract by using fewer animals. BLI is a powerful tool for longitudinal monitoring of tumor load in orthotopic models with almost the same simplicity as ectopic tumors. The establishment of these gastrointestinal bioluminescent xenograft models are powerful tools for ongoing studies concerning the detection of metastases and for the application in pre-clinical therapy interventions.

275 POSTER

A collection of patient-derived pancreatic adenocarcinoma xenografts: pharmacological and molecular characterization

T. Beckers[1], A. Maier[1], J. Schueler[1], T. Giesemann[1], A. Ackermann[1], U. Hopt[2], T. Keck[2], I. Kohler[3], H.H. Fiebig[1], S. Kuesters[2]. [1]*Oncotest GmbH, Institute for experimental Oncology, Freiburg, Germany;* [2]*University of Freiburg Medical Center, Department of General and Visceral Surgery, Freiburg, Germany;* [3]*University of Freiburg Medical Center, Institute of Pathology, Freiburg, Germany*

There is a high medical need to identify new treatments for patients with pancreatic cancer, since in many cases only palliative treatment is possible. The standard 1st line chemotherapy in inoperable, locally advanced (stage II and III) and metastatic (stage IV) adenocarcinoma of the pancreas is Gemcitabine as a single agent with a median survival of about 6 months. In addition, Gemcitabine is indicated as adjuvant chemotherapy after surgery. Although the anti-metabolite 5-Flourouracil (5-FU) and the EGF-R inhibitor Tarceva (Erlotinib) have been approved for 2nd line treatment, new and more efficient drugs are urgently needed. In the present study, more than 60 samples of pancreatic carcinomas were transplanted subcutaneously (s.c.) into NMRI nude mice directly after tumor resection. In most cases, tumor material from chemonaive patients with defined histology and staging was used for implantation. Up to now, 20 tumor models were passaged in nude mice and characterized comprehensively. Except for less stroma, histology of the established xenograft was comparable to that of the primary tumor. Chemosensitivity *in vivo* was evaluated by treatment of tumor bearing nude mice with 5-FU (100 or 75 mg/kg, q7dx3, i.p.), Gemcitabine (240 mg/kg, q7dx3, i.v.) and Erlotinib (25 and 50 mg/kg, qdx21, p.o.). In general, tumor growth was not inhibited with best T/C values >50% highlighting the general chemoresistance of pancreatic cancer. Only in three models (PAXF 1872, PAXF 1998, PAXF 2011), a high sensitivity towards Gemcitabine was evident with best T/C values of 8%, 3.8% and 0%, respectively. Concerning Erlotinib, best T/C values ranged from 89.6% (PAXF 1876) to 38.5% (PAXF 1982) with no correlation to EGFR expression status. A more broad chemosensitivity profile was established with the *ex-vivo* clonogenic assay. Interestingly, several tumors responded strongly to treatment with mTOR Inhibitors ($IC_{50} \leqslant 10$ nM). Based on this data, in vivo treatment experiments with RAD001 (Everolimus) were performed with best T/C values around 50% for most tumors as tested and transient regressions in a few experiments. In summary, a unique collection of patient-derived pancreatic xenograft models of high clinical relevance has been established. These models are available for translational research studies including in-vivo efficacy testing of new investigational drugs. According to our data, mTOR Inhibitors might be useful in selected patients.

276 POSTER

Establishment and characterization of human luminal breast cancer xenografts

F. Assayag[1], P. Cottu[2], C. Elbaz[1], E. Marangoni[1], C. Guyader[1], J.J. Fontaine[3], A. Vincent-Salomon[4], F. Reyal[5], P. De Cremoux[6], D. Decaudin[1]. [1]*Institut Curie, Translational Department, Paris, France;* [2]*Institut Curie, Medical Oncology, Paris, France;* [3]*Ecole Nationale Vétérinaire, Pathology, Maisons Alfort, France;* [4]*Institut Curie, Pathology, Paris, France;* [5]*Institut Curie, Surgical Oncology, Paris, France;* [6]*Institut Curie, Tumor Biology, Paris, France*

Background: Luminal breast cancers (BC) are characterized by a persisting long term risk of relapse. Preclinical models of estrogen-dependent human BC are requested for a better understanding of estrogen

receptor (ER) and hormone resistance biology. Because of a very low tumor take in immunodeficient mice, most *in vivo* models of estrogen-dependent human breast tumors are derived from human cancer cell lines. We report here the establishment and the characterization of new primary human luminal breast cancer xenografts directly obtained from fresh human tumor samples.

Methods: As of December 2009, 453 fresh human BC samples have been engrafted in the interscapular fatpad of *nude* mice, of which 405 were retained for further studies (32 were non infiltrating or non-breast carcinoma, and 16 were axillary metastatic lymph node from a simultaneously engrafted primay tumor). ER was expressed in 313 tumors (77.3%), progesterone receptor in 175/291 informative tumors (60.1%), Her2 in 39/315 tumors (12.4%), and overall 60 tumors were triple negative. Validation of the xenografts was obtained by a large phenotypic and genotypic profiling including: pathological and immunohistochemical (IHC) examination, dedicated gene expression (RT-qPCR), genomic (BAC CGH arrays) and transcriptomic (Affymetrix u133 RNA chips) analyses, and therapeutic assessment (estrogen deprivation, ovariectomy, LHRH agonists, letrozole, tamoxifen, fulvestrant).

Results: Among the 405 human xenografted tumors, 8 luminal models have been established (2%), 7 from ER+/PR+ tumors and 1 from an axillary relapse of an ER−/HER2+ tumor. In all tumor/xenograft pairs, histopathological analyses showed an impressive morphological concordance. One had a strong mucinous component, and all of them were grade II/III tumors. Out of the 7 ER+/PR+ models, 3 were also HER2 positive. RNA expression by RT-qPCR confirmed ER, PR and HER2 status for the 7 ER+/ER+ tumors, and confirmed the ER+ status of the ER-/HER2+ derived tumorgraft. CGH arrays analyses demonstrated striking similarities of the genomic profile between the original tumors and their corresponding xenografts. Array CGH analyses were also performed at several passages, showing stable profile of the tumors during sequential *in vivo* passages. Transcriptomic profiling is ongoing. Therapeutic characterization of the xenografts showed that tamoxifen had a delayed but significant anti-tumor effect, whereas fulvestrant was the most efficient hormone therapy with durable complete responses observed in 3/3 evaluable models. Updated and extended results will be presented during the meeting.

Conclusions: We have durably established and characterized 8 primary human luminal BC xenografts. In order to identify new therapeutic approaches of hormone resistant BC, we have now planed to decipher in these well-defined preclinical models the molecular variations associated with emergence of resistance to hormone therapies.

277 POSTER

Preclinical antitumor assessment of bendamustine in human primary uveal melanoma xenografts

F. Némati[1], S. Piperno-Neumann[2], L. Desjardins[3], N. Karboul[1], C. Elbaz[1], A. Dahmani[1], X. Sastra-Garau[4], A. Berniard[1], P. Mariani[5], D. Decaudin[1].
[1]Institut Curie, Translational Research, Paris Cedex 05, France; [2]Institut Curie, Medical Oncology, Paris Cedex 05, France; [3]Institut Curie, Ophthalmological Oncology, Paris Cedex 05, France; [4]Institut Curie, Tumor Biology, Paris Cedex 05, France; [5]Institut Curie, Surgical Oncology, Paris Cedex 05, France

Background: Uveal melanoma (UM) is the most common primary cancer of the eye, with a pejorative outcome due to metastatic death in up to half of the patients. Apart complete resection of metastasis, few alkylating agents such as temozolomide and fotemustine were used in metastatic UM patients with a slight efficacy. Bendamustine hydrochloride, which is both an alkylating and an anti-metabolite cytotoxic drug, has been shown to possess clinical activity in cancer patients refractory to alkylating-based chemotherapy. The purpose of this study was therefore to determine the efficacy of bendamustine in primary human UM xenografts.

Materials and methods: Four well characterized models of human UM, obtained from patients after enucleation (primary tumors)(MP41, MP46, and MP80) or liver surgery (metastatic tumors)(MM26), were used for the *in vivo* experiments (Némati et al, CCR 2010). Bendamustine was administered intraperitoneally (IP) at a dosage of 11 mg/kg day 1 to 5 every 28 days; temozolomide was administered IP at a dose of 40 mg/kg day 1 to 5 every 28 days and fotemustine was administered IP at a dosage of 30 mg/kg every three weeks. Tumor growth inhibition (TGI) was calculated to measure the efficacy of various tested compounds.

Results: Bendamustine induced a TGI between 44% and 49% in the four human UM xenografts, as shown in the Table 1. Moreover, when bendamustine was compared to temozolomide and fotemustine, it appears less efficient than fotemustine in all tested tumors and more efficient than temozolomide in 2/4 xenografts.

Conclusions: Using 4 human UM models, bendamustine was less efficient than standard chemotherapies administered in metastatic patients. These data are correlated to the results of the only one clinical study evaluating bendamustine efficacy in relapsed or refractory metastatic UM patients and showing 11/11 progressive diseases (Schmidt-Hieber et al, Melanoma Res 2004). These data also suggest that human primary UM xenografts constitute relevant preclinical models for pharmacological assessment of new therapeutic compounds and new combination of treatments.

UM models	Bendamustine	Temozolomide	Fotemustine
MP41	49	38	64
MP46	46	93	94
MP80	49	14	75
MM26	48	95	96

278 POSTER

Myxoid liposarcoma tumors with different chimera subtypes xenografted in nude mice are characterized by different response to trabectedin and gene expression profile

S. Marchini[1], R. Frapolli[1], I.F. Nerini[1], S. Pilotti[2], P. Casali[3], F. Grosso[3], R. Sanfilippo[3], A. Gronchi[4], J.C. Tercero[5], M. D'incalci[1]. *[1]Mario Negri, Oncology, Milano, Italy; [2]Fondazione IRCCS Istituto Nazionale Tumori, Pathology, Milano, Italy; [3]Fondazione IRCCS Istituto Nazionale Tumori, Cancer Medicine, Milano, Italy; [4]Fondazione IRCCS Istituto Nazionale Tumori, Surgery, Milano, Italy; [5]PharmaMar, Scientific Development, Madrid, Spain*

Background: Trabectedin is a marine alkaloid isolated from Ecteinascidia turbinata, that is approved in Europe for the 2nd line of therapy in soft tissue sarcomas (STS). Among different STS, myxoid liposarcomas (MLS) are particularly sensitive to trabectedin, a clinical finding possibly related to the drug ability to block the trans-activating activity of the FUS-CHOP chimera gene, that represents the MLS pathogenic lesion. Different chimera subtypes seem to share different response to trabectedin in clinical setting. To define if this can be related to a different pattern of sensitivity to trabectedin tumor myxoid liposarcomas type II and type III were xenografted in nude mice, treated with trabectedin and analyzed for their gene expression profile.

Material and Methods: Fragments of type II and type III MLS were transplanted s.c. in female athymic NCr-nu/nu mice. Xenografts were established and characterized by morphology and molecular biology. Trabectedin 0.15 mg/kg was injected i.v. weekly for three times. The growing tumor masses were measured with a Vernier caliper. Drug efficacy was calculated as T/C %, where T and C are the mean tumor weights of treated and control groups, respectively. Whole gene expression experiments were performed with dual color labeling protocols and hybridized onto 44K oligos-array platforms commercially available. Analysis was performed with "R" package software. Pathway analysis was performed using Metacore software. qRT-PCR was used for data validation. Statistical analysis was performed using *Graphpad software.*

Results: The responsiveness to trabectedin in type II MLS xenografts was very high (T/C = 8%) whereas type III MLS xenografts appeared much less sensitive (T/C = 42%) to trabectedin. Gene expression analysis of both type II and type III subtypes identified a large subset of genes which expression is modulated by trabectedin in a dose dependent manner. Pathway analysis revealed that trabectedin treatment modulated different molecular pathways in the two FUS-CHOP subtypes models.

Conclusions: The overall data suggest that nude mice xenografted with different FUS-CHOP subtypes are associated with different sensitivity to trabectedin, mirroring clinical evidence. The differences appear to be related to a different modulation of gene expression by trabectedin.

279 POSTER

NC-001 induces tumor growth discontinuation and necrosis in a xenograft renal cancer rat model

H. Hedman[1], L. Buvall[1], U. Nilsson[1], J. Nystrom[1], B. Haraldsson[1].
[1]University of Gothenburg, Molecular and clinical medicine, Gothenburg, Sweden

Of all cancer malignancies in the world, renal cancer constitutes about 3%, which corresponds to 100,000 cases annually worldwide. The majority of renal cancer (75%) starts in the proximal tubular epithelial cells in the kidney, and is referred to as clear cell renal cell carcinoma (CCRCC). At diagnosis, about one third of the patients are presented with metastases. Subsequently, half of the patients who seemed to have a localized disease initially, will develop metastases even if the original tumor is successfully removed. Several new therapies are emerging in clinical trials, with mainly anti-angiogenic properties. These are combined and compared with conventional therapies, explicitly interferon-alfa and interleukin-2. However, none of these show true curative potential, although significant retardation of the disease have been reported for specific patient categories.