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« Du Laboratoire au Patient et du Patient au Laboratoire »

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SESSIONS PLÉNIÈRES

Jeudi 19 mars 2009

Vendredi 20 mars 2009

1er Colloque Inter-Régional Grand-Est de Recherche Translationnelle en Oncologie ONCOTRANS 2009

« Du Laboratoire au Patient et du Patient au Laboratoire »

19 et 20 mars 2009, Nancy

Le Centre Alexis Vautrin organise, sous l'égide du Cancéropôle Grand-Est, les 19 et 20 mars 2009, **le 1er Colloque Inter-Régional Grand-Est de Recherche translationnelle en oncologie, Oncotrans 2009**.

Ce colloque réunira au palais des Congrès de Nancy, chercheurs, biologistes et cliniciens issus des **4 Centres de Lutte Contre le Cancer** (Centre Alexis Vautrin, Nancy ; Centre Paul Strauss, Strasbourg ; Institut Jean Godinot, Reims ; Centre Georges François Leclerc, Dijon), des **cinq CHU** (Nancy, Strasbourg, Reims, Dijon, Besançon) et de l'ensemble des **universités des cinq régions** qui constituent l'inter-région Grand-Est (Lorraine, Alsace, Champagne-Ardennes, Bourgogne, Franche-comté). Nous étendrons également le domaine de ce colloque en contactant les acteurs de proximité frontalière allemands, luxembourgeois, suisses et belges.

Plan Cancer, Cancéropôles et Recherche Translationnelle

La recherche translationnelle, également appelée médecine expérimentale, est au cœur du Programme Recherche du Plan Cancer, lancé en 2003, qui doit assurer le continuum soins-recherche dans le domaine de la lutte contre le cancer. Dans cette logique, les "Cancéropôles" établis à l'échelle d'une région ou d'un groupe de régions ont vocation à développer la coordination opérationnelle de projets mobilisant des équipes de recherche académiques et/ou industrielles, des services de soins orientés vers l'innovation et des plates-formes technologiques mutualisées. Les Cancéropôles ont un rôle stratégique à jouer dans l'émergence d'une recherche translationnelle innovante et de haut niveau scientifique. La Cancéropôle Grand-Est coordonne les cinq régions du Grand-Est : Alsace, Bourgogne, Champagne-Ardennes, Franche-Comté et Lorraine et leurs structures de recherche académique, hospitalière et industrielle.

La recherche translationnelle est impliquée à tous les niveaux de l'innovation thérapeutique. Elle doit assurer un continuum entre la recherche biologique cognitive et la recherche clinique et ainsi permettre la mise en œuvre optimale des connaissances les plus récentes dans la pratique médicale. La recherche translationnelle nécessite par conséquent un lien fort entre les structures académiques de recherche, les établissements hospitaliers et les industriels impliqués dans la recherche clinique, notamment des essais précoces de Phase I et II.

Du laboratoire au patient et du patient au laboratoire

La recherche translationnelle se développe à proximité du patient afin de permettre un flux bidirectionnel des connaissances de la recherche cognitive vers son application au patient et des observations faites chez le malade vers la recherche cognitive, ou encore du laboratoire au patient et du patient au laboratoire.

Ainsi, la recherche translationnelle concerne à la fois la biologie, l'imagerie et la thérapeutique, elle nécessite des approches méthodologiques rigoureuses. Elle porte sur le transfert de connaissance ou de technologie, nécessairement innovant, et toujours en connexion avec un aspect de la biologie, vers les soins innovants. C'est en ce sens qu'elle s'inscrit dans un continuum entre recherche et soins et s'attache à :

- transférer et interpréter le plus vite possible les connaissances nouvelles et les nouvelles technologies vers des applications diagnostiques et thérapeutiques, au bénéfice des patients ;
- créer les conditions d'une réelle approche multidisciplinaire impliquant une concertation entre chercheurs académiques et hospitaliers, industriels ou structures impliquées dans le développement thérapeutique, médecins et patients ;
- contribuer à la mise à disposition la plus rapide possible des innovations validées en terme de rapport bénéfice/risque de d'amélioration du service médical rendu.

ONCOTRANS 2009

C'est avec beaucoup de plaisir que nous avons conçu et organisé, sous l'égide du Cancéropôle Grand-Est et de l'INCa, ce 1er Colloque Inter-Régional Grand-Est de Recherche Translationnelle en Oncologie "ONCOTRANS 2009".

Notre but est de rendre visible la richesse de nos équipes impliquées dans ce domaine passionnant de recherche au service des patients et de favoriser les interactions entre les différents acteurs de l'Inter-Région Grand-Est et d'ouvrir notre réseau de recherche vers l'industrie.

Nous souhaitons que ce colloque favorise l'émergence de projets structurés, et mette en avant les potentialités scientifiques et médicales regroupées au sein du Cancéropôle Grand-Est.

Pour le Comité d'organisation,
Pr Jean-Louis Merlin

Oncologie (2009) 11: S23-S32

Jeudi 19 mars 2009

Session 1 : Modèles expérimentaux et leur pertinence clinique

Modérateur : Pr Philippe Birembaut, Reims

Preclinical models applied to translational research

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Scientific background: Preclinical models of human cancer are needed to prioritize and select potential drug candidates for investigations in man. Human tumor xenografts established directly from patient biopsy material represent such valuable tools for *in vivo* drug testing. Indeed, we and others have shown that xenografted human tumors maintain their fundamental histological and genotypic features and that they also recapitulate the genetic heterogeneity of the original tumor. Using colon cancer xenografts and treatment with irinotecan as an example, I will illustrate how such models can be used to define the gene expression elicited by this drug and how such information can lead to the rational design of a novel and effective therapy combining irinotecan and mTOR inhibitors to target the mTOR/HIF-1 alpha axis in colon cancer.

Material and Methods: Irinotecan was first tested on 7 patient-derived subcutaneous colon tumor xenografts and its gene expression signature was determined using Affymetrix micro arrays. Data were analyzed using dedicated oligonucleotide probe masks that allow to efficiently measure human-specific transcriptional profiles in chimeric human-mouse samples. Based on these results, xenografts were treated with low doses of irinotecan alone, rapamycin alone or combination of both drugs.

Results: Gene expression analysis in xenografted tumors showed that two-third of the down-regulated genes after treatment with irinotecan alone are HIF-1 alpha target genes. This signature correlated with a complete inhibition of HIF-1 alpha protein accumulation *in vivo* and led to an important inhibition of tumor angiogenesis. Since this effect appeared independent of mTOR pathway inhibition, we rationally hypothesized that rapamycin, a potent mTOR inhibitor, could synergize at low doses with irinotecan.

Xenografted tumors treated with the combined treatment showed a dramatic reduction in tumor volume which was accompanied by a synergistic inhibition of the mTOR/HIF-1 alpha axis. *In vitro* experiments using siRNA targeting specifically HIF-1 alpha confirmed that cytotoxic effect was mediated through HIF-1 alpha inhibition. We also observed that constitutive activation of PI3K/Akt and Ras/MAPK pathways through oncogenic mutations render cells less sensitive to the combined treatment. However, sensitivity could be restored by combining irinotecan with selective agents targeting activated Akt (LY294002) or K-Ras (salirasib).

Conclusions: These results identify HIF-1 alpha as a promising target and provide a rationale for the clinical trials combining irinotecan and mTOR inhibitors in colon cancer that we are currently setting up within the Cancéropôle Grand-Est. This study provides a comprehensive example whereby xenografts of patient-derived tumors proved to be a valuable source of material which can be exploited in greater details to foster innovative translational research.

Are *in vivo* preclinical models adapted to the expectations of clinicians ?

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In vivo screening of anticancer drugs, started at the NCI in 1955 in murine tumor models (including L1210 leukemia), then in human tumor models, showed that approximately 30% of the drugs that were identified as potent in at least one human cell line was not detected in the murine cell lines and that no correlation was observed between preclinical and clinical activity when only one tumor line from one histotype was evaluated. Several studies aiming at precising the mechanisms of action of new molecules then demonstrated the interest of taking into account the pharmacokinetic and pharmacodynamic parameters. Among the 7 studies comparing responses achieved in animals and in humans, the predictive value of preclinical evaluation has been reported in 3/5 breast, 3/4 ovarian, 3/3 NSCLC and 0/1 colon cancer studies. In a study comparing the responses to 39 anticancer drugs in mice xenografts and in phase II trials, response prediction of the preclinical data was only observed in NSCLC, leading to the conclusion that preclinical studies were able to identify potent drugs but not to predict their activity in a particular tumor type. Drugs activity has also being compared *in vitro,* in humans and *in vivo* in tumor xenografts. Out

of 80 comparisons, a correct prediction for tumor resistance was observed in 97% and for tumor sensitivity in 90%. Independently of the known behavioral differences between clinical human tumors and xenografts, many methodological problems can explain the discrepancies between the tumor responses achieved in preclinical *in vivo* models: xenografting of cell lines vs tumor fragment, lack of standardization of the assays, assays in one vs several lines of the same histotype, heterotopic vs orthotopic grafting, difference in the chemotherapy and radiotherapy dose regimen and schedule, lack of PK/PD analyses…

Conclusion: The methodological defects observed in many preclinical models often led to separate *in vivo* preclinical evaluation from clinical research in humans and to discredit the predictive value of preclinical screening of anticancer agents. Such assays remain however mandatory before envisaging the clinical development of new therapeutic approaches in man and has the advantage of considering, beside toxicity, the primordial role of the tumor environment.

Session 2 : Gènes cibles, ciblage thérapeutique et thérapies ciblées

Modérateur : Dr Laurent Arnould, Dijon

Targeted therapies in gastrointestinal oncology: principles and future directions

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Biological therapies provided a critical improvement in the management of metastatic colorectal cancer (mCRC). The anti-angiogenic monoclonal antibody bevacizumab (Avastin®) improves survival in bevacizumab-naïve patients when added to first-line or second-line chemotherapy, but seems inactive in refractory-disease. Biologics targeting the epidermal growth factor receptor (EGFR) are effective in disease refractory to 5-FU, irinotecan, and oxaliplatin. Moreover, the molecular status of specific oncogenes and the development of pharmacogenomic biomarkers allow treatment individualization. However, despite recent advances in the treatment of CRC, which include the introduction of biotherapies and new cytotoxic molecules, oxaliplatin and irinotecan, the 5-year overall survival rates for these patients remain unacceptable, except for patients who are candidates for surgical resection of their metastases. Therefore, the development of alternative therapies is required.

The development of novel targeted therapies requires the identification of molecular pathways pivotal for both cell-autonomous cancer renewal and micro-environment dependent cancer progression. Indeed, the formation of carcinomas is often accompanied by a well-orchestrated stromal reaction, which involves the recruitment of host cells with both pro-tumorigenic and pro-angiogenesis activities. Certain models of cancer progression propose that recruitment of mesenchymal stem cells (MSC) within the tumor endows cancer cells with invasive and metastatic properties.

The endpoints of cancer therapies should include i) the removal of cancer cells with innate resistance mechanisms, ii) the deprivation of cancer cells from the promotion signaling provided by the micro-environment (angiogenesis and MSC), iii) the activation of the immune system which recognizes and eradicates the remaining tumor cells.

We have recently identified the STAT3 and neuropilin 2 proteins as fulfilling the criteria of such an integrative target for anti-cancer therapy.

Experimental studies to demonstrate the ability of STAT3 neutralization to restore cancer immunosurveillance and pre-clinical models conducted to define the interest of neuropilin 2 targeting will be presented.

New therapeutic targets, new hopes

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Modulating cell signalling pathways by small molecules to cure cancer, is a major challenge in oncology. A number of such target-based anticancer therapies are now successfully used in routine clinical practice. For example, in chronic myelogenous leukemia (CML), the Abelson tyrosine kinase inhibitor Imatinib (Gleevec) targets the activity of BCR-ABL oncoprotein while in acute promyelocytic leukemia (APL), all-trans-retinoic acid (ATRA) or arsenic trioxide (As2O3) target PML-RARa fusion. Their introduction in the treatment of APL or CML patients has significantly improved the management of these diseases, yet their use remain restricted. Novel therapeutic options, targeting a wider variety of cancer and exhibiting tumour selectivity are needed. One such approach includes the use of TRAIL (TNF related apoptosis-inducing ligand) a member of the TNF family.

TRAIL is a type II membrane bound ligand that engages with its membrane bound signalling receptors DR4 and DR5 to activate the extrinsic death signalling pathway. Proof-of-principle for the tumour-

selectivity of TRAIL, as well as its significant potential for the treatment of a broad range of solid malignancies, either as a single agent or in combination with chemotherapy, based on preliminary clinical results, fostered its clinical development. Therapeutic exploitation of the TRAIL pathway mainly relies on the use of recombinant TRAIL (Genentech/Immunogen, phase II clinical trial) or DR4 and DR5 activating antibodies (HGS, phase II clinical trial).

We have started to establish an entirely novel procedure to activate the TRAIL system using an approach based on the 3D structure of trimeric ligand mimetics for the CD40, another member of the TNF receptor family. This chemical approach will be presented and discussed in the light of the other targeted therapies based on the TRAIL system that offer great promise for the treatment of tumour malignancies.

DDB2: a potential and promising candidate in diagnosis of breast cancer

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Background: Despite advances in cancer research, breast cancer is by far the most common female cancer, and mechanisms involved in the tumor progression are not well defined. Our study reports the identification of the Damaged DNA binding 2 protein (DDB2) as a new potential marker for breast tumor growth and its relation with the manganese superoxide dismutase (MnSOD), an antioxidant enzyme which is already known to play a role in cancer. Previously, DDB2 was known to play a role in DNA repair and chromatin remodelling in normal cells. However, its role in cancer was not described yet. In this study, we report the characterization of a new biological activities of DDB2 in breast cancer cells.

Methods: We developed in vitro models of breast cancer cells in which DDB2 and MnSOD expressions were modulated by RNA interference, antisense RNA or expression vector strategies to study consequences on cell growth and invasiveness. Different approaches of molecular biology were used to define precisely the functional activity of DDB2 as transcriptional regulator.

Results: We showed that DDB2 is only overexpressed in estrogen receptor (ER)-positive breast cancer cells and tumors. With in vitro models of breast tumor cells in which DDB2 is down-regulated or overexpressed, we demonstrated that this protein is involved in the cell growth by activating the G1/S phase transition of the cell cycle. In addition, we purified and identified DDB2 in a systematical proteomic analysis, which was done to trap new potential transcription factors involved in the regulation of the MnSOD gene. The DDB2 binding DNA sequence was characterized precisely into the proximal promoter of MnSOD gene. Functional analysis of this proximal promoter allowed us to demonstrate that DDB2 regulates negatively the constitutive expression of this gene. In addition, we showed that DDB2 exerts, at least in part, a control of breast cancer cell growth through its negative regulation of constitutive expression of the MnSOD gene. Indeed, we observed that expression of this latter is inversely correlated with that of DDB2 in several breast cancer cell lines studied and with the cell growth. In parallel, we demonstrated that experimental down-regulation of MnSOD expression in metastatic and ER-negative breast cancer cells decreases concomitantly the MMP-9 activity and invasive ability of tumor cells, and that this action is mediated by MnSOD-dependent H_2O_2 production.

Conclusions: Taken together, these data give for the first time a supporting evidence that DDB2 may become a promising candidate as a predictive marker in breast cancer progression through its involvement in regulating cell cycle progression and in transcriptional regulation of MnSOD, and that this molecular mechanism may so have an important clinical interest in the future.

Session 3 : Radiothérapie, radiobiologie, interactions radiations-tissus

Modérateur : Pr Didier Peiffert, Nancy

Transfer translational research in radiobiology

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Ionizing radiations induce DNA strand breaks which ultimately cause cell death. However, there are numerous steps between the first interaction of an ionizing particle with a tissue and cell death. Understanding these mechanisms is the prerequisite to future therapeutic options. To improve the therapeutic ratio, clinicians need radiobiologic data to prevent early and late side-effects or complications. Small modulated and targeted beams produced by a new generation of linear accelerator could recently lead to the development of hypofractionated irradiation schedule. Calculation of equivalent doses according to classical fractionation knowledge is needed. Although mathematic models and algorithms have been already developed, they need some more precise and accurate radiobiological data. Because patients are treated in a multidisciplinary approach, association of radiotherapy and chemotherapy are now the mainly base of treatment. However, best association and best schedule (in terms of timing) are not still well determined, especially with the use of new targeted drugs; this is a way to get better.

Then, some fields of transfer translational research in and for radiotherapy include:

Homeostasis and intrinsic radiosensitivity-based researches: Hypoxia can occur both early and late during the development of all solid tumors. Many studies have demonstrated that the degree of hypoxia correlates with poor prognosis. A more comprehensive approach is based on the new anti-VEGF drugs and imagering facilities (MRI, PET scan…).

The improvement of our knowledge about DNA double-strand and single-strand breaks and DNA repair involves intrinsic biomarkers of homologous and non-homologous recombination and unique DNA repair gene polymorphisms in cancer patients. Increasing the efficiency of DNA breaks can be obtained by the use of new drugs targeting precisely repair proteins or repair pathways.

The role of telomere was extensively studied, but short term and long term consequences of damaged telomeres are not still used to radiosensitize and promote of the transmission of radiation induced damage.

Radiosensitization: Role of viral infection in the radiosensitization was focused these last years with the discovery of the role of Human Papillomavirus in the response to radiation.

Association of radiation and targeted therapy approaches lead to new paradigms of treatment, but these drugs are usely in use in the clinic before any comprehensive studied have been undertaken in the radiobiology field.

Cell death (senescence, apoptosis, autophagy) and intrinsic radiosensitivity are correlated to tumor radiosensibilisation and type of ionizing particle used, however the modalities to stimulate them are not yet well defined and fully clinically used.

Normal tissue radioprotection: Normal tissue damage comprehension in lung, central nervous system and skin have been clinically described but radiobiologic research are in development in the fields of DLCO-diffusion, brain stem-cell and fibrosis processes. These researches are performed in parallel with the analyses of new radio-protective drugs. The results could be integrated in the biological modeling for tumor control probability (TCP) and normal tissue complication probability (NTCP) in the area of dose-volume histograms.

Low-dose radiosensitization and carcinogenesis associated with chromosomal rearrangements could help the prevention of radiation-induced second cancer.

Rehabilitation of irradiated tissues

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Background: Radiotherapy is an efficient carcinological treatment, and nearly two thirds of all cancer patients will receive radiotherapy at some point during their disease treatment. However it may adversely impact on healthy tissues included within the irradiated volume, and the injury mechanism is characterized by ischemia, cell apoptosis and fibrosis. For long-term survivors, the risk of postirradiation sarcoma has been estimated to be up to 0.8% overall after radiotherapy.

Clinical experiences: A benefit of reconstructive surgery on surrounding radiation-induced injuries was observed, especially with Coleman technique that involves fat tissues transplantation into irradiated areas. These clinical improvements were correlated with pathological data. Thus, we hypothesized that the clinical improvement could be induced by an increase in vascularization and a revitalisation of interstitial tissues. A cellular contingency with angiogenic potentiality, in particular adipose and mesenchymal stem cells contained in fatty tissues, could take part in the revascularisation of ischemic irradiated tissues.

Experimental studies: Firstly, we developped an experimental animal model of radio-induced tissue degeneration to better understand the radi-

ation-induced defect. Sequential analysis was based on observational staging recordings and non invasive imaging using planar scintigraphy. Additional radiohistology, cytokines and growth factors analyses, histology and immunohistochemistry were also performed. In this long term 12 months study, development of soft tissue sarcoma was observed in the irradiation field. Secondly, the rehabilitation of radio-induced tissue injury by cellular therapy with autologous bone marrow mesenchymal stem cells (BMSCs), based partly on their angiogenic ability, might represent a new therapeutic approach. This feasibility was investigated in the rat model exposed to radiation with an emphasis on two prerequisites: the quality of engrafted BMSCs and the short term cell retention within the damaged area. Finally, this program will investigate whether BMSC therapy could be a preventive strategy in the development of radio-induced sarcoma.

Multifunctional nanoparticles for vascular photodynamic therapy

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Photodynamic therapy (PDT) is being assessed as an adjuvant therapy for the treatment of malignant gliomas. Despite conventional treatments, local relapses remain higher than 80%. PDT relies on the light activation of a photosensitizer (PS) localized in the tumor site. If tumor destruction can result from direct cytotoxic damage, other indirect mechanisms seem to play a major part. One of them, the « anti-vascular effect », is characterized by the alteration of the tumor vascular network, that induces blood flow reduction, and *in fine* thrombi formation. A large and growing literature shows the essential role played by the anti-vascular effect in tumor eradication by PDT, which is then called *Vascular Targeted Photodynamic therapy* (VTP). This effect (and hence treatment efficacy) is potentiated by the enhanced and more selective accumulation of the PS in the tumor neovessels. A new PS conjugated to a peptide (ATWLPPR) targeting neuropilin-1 (NRP-1), a $VEGF_{165}$ co-receptor, has been synthesized. This peptide binds to a recombinant chimeric form of the NRP-1 protein, as does the peptide-conjugated PS, but with a lower affinity. Intracellular uptake and photodynamic efficacy were improved in endothelial cells over-expressing NRP-1 due to the coupling of the PS to the peptide. In human malignant gliomas-bearing mice, the conjugated PS is taken up actively by tumor-associated endothelial cells, and induces, following treatment, a statistically significant tumor growth delay. Nevertheless, improvements can and need to be brought to our targeting strategy, in order to reduce non-specific phenomena arising from, on one hand, the hydrophobic character leading to the aggregation of the conjugate, which reduces its affinity for its target, and, on the other hand, from a large accumulation in the reticuloendothelial system, resulting in the degradation of the peptide. With the aim at overcoming these limitations, multifunctional furtive nanoparticles, decorated with a targeting peptide or pseudopeptide, can be used as vectors for the PS and can at the same time be detected by MRI.

The principle of nanoparticles that can, at the same time, target angiogenic endothelial cells, allow imaging by MRI and lead to a treatment optimized (drug-light interval) of the targeted tumor is a novel concept. These nanoparticles for imaging and VTP of brain tumors encompass strong arguments necessary to an efficient therapeutic targeting : limiting the aggregation problem by transporting grafted PS molecules, preventing opsonins from adsorbing on the surface of nanoparticles and acting selectively by grafting on their periphery affin and stable peptides.

Session 4 : Tumorothèques – Etudes rétrospectives – Aspects réglementaires

Modérateur : Pr François Plénat, Nancy

Biobanks of the Cancéropôle Grand-Est – Results and prospects

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Introduction: The structuring of the biobanks within the CGE began in 2001 with the support of the INSERM and the DHOS, relayed from the latter by the INCA.

These biobanks keep frozen and paraffin embedded biological samples. As part of research programs, they collect and qualify the samples, look for essential associated clinical, biological and pathological data, and organize the transfer of the samples for analysis. In addition, they ensure the freezing of human tumor samples for molecular analysis improving cancer treatments.

Results: Ten biobanks are structured at an inter-regional level (one in Besançon, one in Dijon, three in Nancy, three in Reims and two in Strasbourg). Since 2003, they are part of a network with biannual coordination meetings in order to standardize the techniques, to collect rare or emergent tumours, and to manage collections through a common database.

Such collections have enabled the biobanks of Dijon, Nancy and Strasbourg to participate in 2008 in two national research programs on fibromatosis and soft tissue sarcoma.

Further to an INCa call for projects in 2005, a regional biobanking networks was organized around platforms of molecular genetics of cancer in Alsace, Bourgogne, Champagne-Ardenne, and Franche-Comté. The aim was to allow each surgeon to have access to biobanking regardless of where he practices.

Since 2006, the biobanks of Dijon, Nancy and Strasbourg are engaged in a process of certification with a financial assistance of the ANR and the INSERM, and the biobanks of Nancy and Strasbourg are participating in the PNES lung project (national project of scientific excellence).

Prospects: In the short term, the CGE is setting up a Regional Virtual Biobank, allowing to browse through the collections. The standardization of all documents necessary for the submission of scientific projects requiring the participation of the biobanks, and for the transfer of samples will be implemented.

Certification of all biobanks should be obtained in the medium-term.

Tumor banks – Retrospective studies – Statutory aspects – FAQ: from the driving of a biomedical research to the establishment of a collection

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[1] BÉVIÈRE B., *La protection des personnes dans la recherche biomédicale*, Les Études Hospitalières, Collection Thèses, 2001, p. 15.
[2] Ibid, p. 22.

According to the article L. 1121-1 of the Public Health Code, any research organized and practised on the Human being with the aim of the development of biological or medical knowledge is a biomedical research. It is thus about researches which "have for object the knowledge of the healthy or sick man by the appreciation of the effects of an equipment or a medicine, its efficiency, its pharmacokinetic security and its optimal dose. They can also allow to work out the reliability of a device or an equipment in real conditions of use"[1]. It has " the objective to identify, to analyze and to explain the diseases, the dysfunctions of the human body and to discover the curative molecules "[2]. The legal framework surrounding the experiment arose from the will to protect the subjects of the research, and several constraints so come to press on whoever decides to operate a study, also it is advisable to bound the outlines of this protection through several questions:
– Why a regulation in biomedical research?
– Who are the actors of the research?
– What are the procedural conditions for the implementation of a biomedical research?
– What are the criteria of appreciation of validity of a research?

Besides, the research activity, as well as the activity of care, implies more and more the collection of elements stemming from the human body. By the law of August 6th, 2004 and the decree of August 10th, 2007, the legislator intervened to clarify the normative frame concerning the collections of biological samples, what also raises several questioning:
– What is a tumor bank in the sense of the law?
– What are the conditions for the constitution of a collection?
– What are the incidences on the shape of the assent that must be given by the person?
– What is the status of the resources so collected? Of the data which are associated with it?

The object of this intervention will be to answer these various questions and others by reminding the legal environment relating to these various aspects of the biomedical research.

Vendredi 20 mars 2009

Session 5 : Diagnostic moléculaire et prédiction de réponse

Modérateur : Dr Jean-Pierre Ghnassia, Strasbourg

Pathologists and the molecular level for diagnosis of cancer as well as prognosis and predictive evaluation

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The diagnosis of tumor malignancy is classically performed on morphology at a macroscopic and/or microscopic level and the prognosis of these tumors are for a long time based on tumor staging and morphologic criteria such as histologic subtype, differentiation, grade, mitotic index, necrosis....Some of these criteria proved to be related to tumor responsiveness to therapy and morphology by itself may be used with efficacy to establish treatment planning. It has been demonstrated, for example, that breast lobular carcinoma is less sensitive to chemotherapy than ductal carcinoma and that patients with such a tumor have no benefice to receive neoadjuvant chemotherapy, and that different chemotherapy regimens are optimal according to differentiation in neuroendocrine tumors.

Since the late 1980's, analyzes of cancer cells at a protein level with immunohistochemistry (IHC) have been extensively used to help pathologists to well classify tumors and sometimes to assess some robust prognostic and predictive factors. It is the case for example with hormone receptors and HER2 in breast cancer, CD117 in gastrointestinal stromal tumor and Ki67 in various malignancies. Like all medical procedures, this IHC may be prone to some inter and intra laboratory variations and, for this reason, has to be supervised by a rigorous internal and external quality control (CQ) program. In France, 2 major programs are proposed to the pathologists, AFAQAP, which is a French program and UK-Nequas, which is an European one.

Pathologists may also analyze tumor cells at a gene level with in situ hybridization techniques (FISH, CISH and SISH) that can be used for diagnostic (for example, specific translocation may be present in Ewing sarcoma, PNET, synovialosarcoma .... and gene amplification may be specific of malignancy like in some subtypes of liposarcomas), for prognosis (for example, MYC amplification in neuroblastoma) and for prediction of therapeutic effects (for example, HER2 amplification and level of HER2 amplification and trastuzumab responsiveness in breast cancers). As IHC, hybridization techniques may be done on tissue micro-array and has to be supervised by rigorous CQ programs.

In case of gene mutation, the abnormality of the gene product may lead sometimes to protein abnormality and analysed by IHC (for example disappearance of E-Cadherin staining in lobular carcinoma of the breast), but in the vast majority of the cases the mutation is analyzed by molecular biology techniques such as PCR, sequencing, ... In these situations, pathologists may acquire these techniques but, above all, have to collect and store tissue samples in optimal conditions (frozen, fixation). They are also essential for sample characterization (are there tumor cells in this sample, are there enough tumor cells in this sample, are there effectively invasive tumor cells in this sample...) in order to give optimal chance to the molecular analysis to give results that represent the reality of the tumor.

Gene expression profiling is the most recent test for tumor investigation. It also needs optimal tissue samples, and has also to be compared with classical morphologic or IHC data. For example, in breast carcinoma, micro-array test as Oncotype analyzed gene expression that can be correctly analyzed by optimal IHC procedures (HR, HER2, proliferation...).

Molecular diagnostic and response prediction: the biologist point of view

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Molecular diagnostic has been increasing in the last decade with the emergence of targeted therapies in major cancer pathologies. Using targeted therapies leads to better clinical results in selected tumors. However the identification of the primary target that help to develop targeted therapy agents, such as HER2 or EGFR, appears to be inadequate to ensure an optimal prediction of the clinical response and additional markers are needed to improve the use of targeted therapies in terms of clinical benefit and pharmacoeconomics..

Because of this absolute need to better classify the tumors at the molecular level i.e. oncogenesis, cell metabolism and signaling markers, combination of techniques are used and the conventional pathology techniques have to be completed by molecular biology techniques. At the hospital level, this leads to the development of bio-pathology platforms with pathologists and clinical molecular biologists. In addition, some « regional » structures has been created. These « tumor molecular genetics platforms » gathering geneticians, pathologists and moleclar biologists enable an easy access to innovative molecular diagnostic to all patients and oncologists.

The example of *KRAS* genotyping in metastatic colorectal cancer (mCRC) clearly illustrate this organisation with a ten-fold increase in genotyping from the beginning till the end of 2008, based on networking of private and public pathology and molecular biology structures.

The future is most probably to tend to develop innovative approaches to better personalize the use of innovative and expensive therapeutic approaches such as combination of targeted therapies aiming at circumventing resistance mechanisms. This requires the evaluation and the validation of techniques that are not commonly used in diagnostic departments in retrospective studies based on tumor bank collections of clinically annotated specimens and prospective clinical trials.

Being at the frontier between experimental studies with research laboratory techniques and clinical diagnostic with certified techniques, a tremendous effort is needed to ensure the clinical relevance of the new diagnostic markers in terms of quantity and quality and in terms of technicity and availability of the technique. One major step is that innovative diagnostic should most probably end at modifying and optimizing the handling procedures of the tumor specimens (fixatives, fixation and /or freezing delays) to warrant the high quality of the molecular species (mRNA, miRNA, phosphorylated signaling proteins) that have to be analyzed. This will be the translational reasearch challenge that bio-pathology platforms will have to face within the near future.

Session 6 : Imagerie diagnostique et fonctionnelle

Modérateur : Dr Philippe Genne, Dijon

PET imaging in oncology

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PET for Positron Emission Tomography is an imaging method based on the use of positron emitting radionuclides such as ^{11}C, ^{15}O or ^{18}F for labelling of organic molecules, whether a simple one like H_20 or a more complex like anti cancer drug. PET is currently the fastest growing imaging modality. ^{18}F-FDG for ^{18}F-Fluorodeoxyglucose which accumulates in tumor cells has been as far the most developed tracer, the main advantage of which lying in its ability to identify with high contrast, tumors and associated lymph nodes or distant metastases. The method has gained large acceptance in clinical oncology for instance before to decide whether or not to refer lung cancer for surgery, to better define radiation field for cervix cancer or to identify the site of an occult CA15.3 secretion. A promising field of application for FDG is assessment of therapy response, especially early in the course of therapy, either in neoadjuvant or metastatic setting; superiority over morphologic imaging lies in the ability to better separate tumoral tissue from inflammatory changes and in the fact that metabolic changes due to therapy can be depicted before morphologic changes occur; the objective is to identify non responded patients for whom another strategy could be considered; such approach could be especially interesting to avoid useless expense and toxicity for some targeted drugs proved to be efficient on a subset of patients only. Thus, empirically defined RECIST criteria might be progressively substituted by PET criteria defined from either visual analysis or quantitative approach with use of Standardized Uptake Value. While still in investigation for therapy response in primaries like breast or esophagus cancers, FDG is of routine use in ongoing trials with lymphoma. To overcome limitations of FDG, other radiopharmaceuticals become progressively available for oncology purpose, like ^{68}Ga-DOTATOC for endocrine tumors, ^{18}F-Choline for prostate cancer or ^{18}F-FLT as a tracer dedicated to treatment response. The recent onset of microPET technology has opened the field of preclinical research to metabolic imaging by allowing to perform high resolution PET imaging on small animals. With concomitant development of animal models, and especially human xenograft models, microTEP is a perfect tool for translational research in oncology. It offers the possibility to use radiotracers which are specific to different factors involved in cancer, like hypoxia or apoptosis, for a better understanding of cancer mechanisms and in a similar way to develop new tracers. MicroPET allows in vivo non invasive determination of biodistribution for any drug and, by permitting a non invasive longitudinal follow-up of animals, is also a way to test investigational new drugs, before initiation of clinical trials.

Vibrational spectral imaging: applications in oncology

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Scientific background: Fourier-Transform Infrared Spectral Imaging (FTIR SI) is a promising medical imaging technique. It provides with a spatially resolved molecular characterization of microscopic areas, while preserving the biochemical integrity of the sample. Developments in the chemometric analysis of the spectra enable the use of fixed paraffin-embedded samples without any chemical dewaxing. Retrospective

studies from archival samples are now possible and permit the development of applications of FTIR SI in cancer detection and prevention.

Material and methods: FTIR SI and chemometrics have been used to aid in the differential diagnosis of skin carcinomas. A databank of spectral markers associated to basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and Bowen's disease was built, and a diagnostic algorithm developed by pattern recognition techniques.

A further exploration of the applications of FTIR SI has been performed by analyzing colon cancers. We have recently demonstrated the presence of intra-tumor heterogeneity in xenografted human colon tumors, which is not detectable by classical histology. We now focus on the *in situ* characterization of the secreted mucus in normal colon and colon adenocarcinomas.

Results: FTIR SI and chemometrics provide with pseudo-color images of skin carcinomas that are more informative than conventional staining. These pseudo-color images can highlight tumor areas and provide with a diagnosis automatically without any staining. IR spectra from BCC, SCC and Bowen's disease reveal molecular modifications (nucleic acids and proteins) associated to the disease.

The comparison of IR spectra from bovine purified mucins and human mucus extracted from benign cysts suggests that the secreted mucus in colon adenocarcinoma is mainly due to mucin glycoproteins. Mucin-specific pseudo-color images were generated, and the apical and basal parts of mucus secreting cells, containing respectively high and low amount of intracellular mucus, could be easily distinguished. The relative comparison of spectral subclusters associated to the secreted mucus permits the discrimination between normal colon and colon adenocarcinoma. These mucus subclusters present significant variations in glycosylation, sialylation and secondary structures of mucins, reflecting alterations in carbohydrate and sialic acid contents and changes in the secondary structure of mucin core proteins between tissue types.

Conclusion: We show the great potential of FTIR SI in oncology. Applications are numerous and the integration of this method to conventional laboratory procedures is feasible and could provide with additional molecular information to conventional stainings.

Session 7 : Essais cliniques précoces

Modérateur : Pr Hervé Curé, Reims

Clinical trials early phase II: new concepts and translationnal research

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As we are entering the era of "molecular medicine", it becomes a high priority to understand why some patients benefit from the therapies and some don't. We now have new and very powerful tools, that should greatly facilitate this task and lead to greatly improved treatment individualisation in a not too distant future. This dream will never become a reality if translational research is not "facilitated". Individual tumor profiles must be obtained in the context of clinical trials, analysed in the laboratory and correlated to clinical outcome. The creation of this link between clinicians and laboratory scientists is essential.

The growing importance of clinical research in France requires physicians who are adequately trained to conduct clinical trials. To realise projects of translational investigation and to participate in interdisciplinary clinical trials, basic knowledge on clinical and pharmaceutical trials is essential. The number of clinical trials has continued to grow by about 20% in the past six months, but there is no corresponding growth in product approval by the food and drug administration. Late-stage clinical failures due to lack of efficacy or toxicity continues to be a challenge. The optimization of absorption, distribution, metabolism and elimination has improved drug candidate selection and reduced early clinical failure. The current challenge is how to avoid late stage clinical failures. Expanded knowledge of drug target distribution, pharmacokinetics and validated biomarkers will allow implementation of appropriate drug delivery and clinical trial designs to reduce drug exposure to off-target organs such as the liver and kidney and could reduce potential untoward effects.

Translational research and biological specimen collection has become a central component of clinical research design in a relatively short period of time.

An optimal application of targeted therapy needs a knowledge for the tumoral status of the target itself. It is also interesting to dispose of biological informations resulting from the interactions between treatment and target (biological proof of the concept). These informations are at the basis of the conception of new clinical protocols in oncology. There are specific methodological aspects in targeted therapies like EPR (early progression rate) or RDD (randomized discontinuation design), along with Adaptive Design such as CRM (continual reassessment method, Eff-TOX (dose-finding based on efficacy-toxicity, PPD (predictive probability design) or ADTT (adaptive randomization for targeted therapy) methods.

The primary goal of translational research is to integrate the advanced

informations of molecular biology in moving forward from phase I and II trials to phase III trials of target-based drugs.

Immunomolecular and cellular therapy protocols: focus on strategies conducted in the Cancéropôle Grand-Est platform

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Allogenic hematopoietic stem cell (AHSC) is an effective treatment for leukemia patients. However, Graft-versus Host Disease is a lethal adverse event leading us to develop several strategies to improve allogenic based immunotherapies: **i)** A phase I/II trial of Herpes-Simplex Virus-thymidine kinase (HSV-tk)-expressing gene modified T cells (GMTC) administration at time of HLA-identical sibling bone marrow transplantation (BMT), **ii)** A phase I/II clinical trial based on allogenic narural killer cell infusion in association with an anti-EGFR monoclonal antibody, **iii)** A preclinical study to develop a vaccination protocol leading to the expansion of specific cytotoxic lymphocytes targeting rituximab resistant B cell lymphoma.

A phase I/II trial of Herpes-Simplex Virus-thymidine kinase (HSV-tk)-expressing gene modified T cells (GMTC) administration at time of HLA-identical sibling bone marrow transplantation (BMT) demonstrated that such an approach could modulate deleterious alloreactivity after BMT (Tiberghien *et al.*, Blood 2001). The Neomycin resistance gene (NeoR) was transferred together with the HSV-tk gene in a retroviral vector to allow for *in vitro* GMTC selection. More than 6 years after BMT, 4 patients are alive in complete remission, off immunosuppression, free of chronic graft-versus-host disease (GvHD). Long-term circulating GMTC are continuously found in all 4 patients, as assessed by quantitative PCR (QPCR) for NeoR gene (6 years post-BMT: 0.031 +/- 0.017% of PBMC). Unexpectedly, presence of NeoR gene was readily detected by single run PCR whereas a nested PCR was necessary for HSV-tk gene detection. As PCR assays for both genes have a similar sensitivity, this result suggested that deletions may be present in GMTC. Junction deletion areas were sequenced and involved, for 3 out of 4 patients, homologue sequence motifs (CCGCCC, AATTC and GATC respectively for pt# 6, 7 & 8), and suggesting recombination mechanisms within the transgene sequences. These sequence motifs were corrected in HSV-tk to propose a phase II study.

Anti-CD20 based immunotherapy is effective in B cell lymphomas. We reported evidence of a novel alternatively spliced transcript of the hCD20 gene, specifically expressed at detectable levels in leukemic, lymphoma, activated or EBV-transformed B cells, but not in normal resting B cells. Further experiments determined the positive correlation between DCD20 mRNA spliced form and resistance to rituximab. Our initial results suggested that DCD20 quantification may be an indicator of minimal residual disease, as a potential predictive marker of relapse, especially in patients with no other molecular marker. Immunogenic peptides related to DCD20 mRNA spliced form were identified and used to expand specific cytotoxic T lymphocytes in HLA-A2/DR1 transgenic mice.

Early clinical trials in pediatric oncology

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Pediatric cancers represent less than 1% of all cancers (approximately 2000 new cases per year in France) but are the first disease-related death cause in children. Approximately 25% of these cancers are specific for children and therefore cannot benefit from clinical research performed in adults. Several reasons can explain that phase I and to a lessen degree phase II trials, are rare in children: the low number of refractory and recurent diseases (related to a high cure rate of 75%); the low number of centers accredited to perform early clinical trials; the difficulty to obtain from pharmaceutical companies new drugs because of the small market of pediatric cancer. Thus, in 2002, among the 217 drugs that were authorized in cancer therapy, only 2 have been evaluated in children (temozolomide and rasburicase). Clinical trials are performed within the framework of the French Pediatric Oncology Society since the end of the 80's in 9 French centers, corresponding to approximately 80% of the total accrual of the children treated for a cancer. Since 1995, the clinical trials are performed in collaboration with the United Kingdom Children Cancer Study Group and presently, most of the trials are performed within the Innovative Therapies for Children with Cancer (ITCC) extended to 30 european centers. Determination of the maximal tolerated dose begins with a dose corresponding to 80% of the start-dose used in adult. Seventeen trials have been performed in France and England, between 1994 and 2002, evaluating 10 cytotoxic agents in 559 patients among whom 64% were included in France. Ten trials have been performed within the ITCC in 327 patients with 62% of the patients coming from France, 18% from England, 9% from Italy and 5% from Germany. Among the 10 molecules that were evaluated, 5 were targeted molecular therapies. In most cases, the trials are promoted by pharmaceutical companies, especially since EMEA proposed extension of the patent duration when the drugs are evaluated in children. Some studies are promoted by academic groups and take place after evaluation of the drugs in vitro and in vivo in pediatric tumor models.

Conclusion: Clinical trials in pediatric oncology have been rare but are progressing, especially since targeted therapies have been proposed and because of the development of a multicentric international structure (ITCC) and the strong incitement of EMEA for the pharmaceutical companies.

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Communications

The methyl cytosine base flipping induced by the UHRF1 SRA domain is involved in the tumor suppressor gene silencing inheritance

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Cytosine methylation of DNA is a major epigenetic hallmark of gene silencing occurring mostly within CpG dinucleotides. Hypermethylation of tumor suppressor gene promoters is a frequent event occurring during tumoral development. Moreover, cancer cell proliferation implies that the DNA methylation patterns of these promoters with an extreme high fidelity throughout cell division. This is achieved by UHRF1, thanks to its SRA domain (Set and Ring Associated) that exhibits high affinity for hemi-methylated DNA, *i.e.* DNA methylated only on one strand. In a first step, the SRA domain of UHRF1 flips the methyl cytosine out of the DNA helix and buries it in a pocket in the protein [1].

The recognition of the hemi-methylated DNA by the UHRF1 SRA domain subsequently allows the maintenance DNA methyltransferase 1 (DNMT1) to be recruited to the target sites as a result of the interaction of the SRA domain of UHRF1 and the SRA-binding domain (SRA-BD) of DNMT1 [2]. This recruitment finally allows the faithful duplication of the DNA methylation patterns.

The aim of the present study was to check whether the base flipping mechanism is involved in the tumor suppressor gene silencing inheritance. Here, we show in the T-lymphocyte leukemic cell line Jurkat, that down-regulation of UHRF1, via specific siRNA for UHRF1, up-regulates tumor suppressor gene expression such as $p16^{INK4A}$ and *p73*. Moreover, we show that over-expression of UHRF1 down-regulates the *p73* expression. We have also observed, by site-directed mutagenesis experiments, that preventing the "NKR Finger" of the SRA domain to flip out the methyl cytosine from the DNA helix, leads to an inability of UHRF1 to down-regulate *p73* expression.

Together, these results show that the SRA domain of UHRF1 is involved in the methylation patterns inheritance of the tumor suppressor gene *p73*. We propose that this property of the SRA domain is important for the "cancer signature" to be perpetuated from cell generation to generation.

References

[1] G.V. Avvakumov, J.R. Walker, S. Xue, Y. Li, S. Duan, C. Bronner, C.H. Arrowsmith and S. Dhe-Paganon. *Nature*, 2008, 455, 822-825.

[2] M. Achour, X. Jacq, P. Rondé, M. Alhosin, C. Charlot, T. Chataigneau, M. Jeanblanc, M. Macaluso, A. Giordano, A.D. Hughes, V.B. Schini-Kerth, and C. Bronner. *Oncogene*, 2008, 27, 2187-2197.

Peripheral blood biomarker correlating with clinical outcome in a randomized phase IIb trial evaluating the therapeutic vaccine TG4010 in NSCLC patients

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Introduction: TG4010 is a recombinant Modified Virus Ankara (MVA) encoding both the tumor-associated antigen MUC1 and the cytokine IL2. It was tested in a randomized, controlled, open label and multicenter study evaluating TG4010 in combination with chemotherapy in patients with advanced (stage IIIb-wet and IV) non-small cell lung cancer (NSCLC).

Patients and methods: Patients were randomized to:

Arm 1: treatment with chemotherapy (Cisplatin 75 mg/m^2 on day1 (d1) and Gemcitabine 1 250mg/m^2 on d1 and day8 (d8) every 3 weeks for up to 6 cycles) combined with 10^8 pfu TG4010 subcutaneously every week for 6 weeks then every 3 weeks until disease progression; or

Arm 2: chemotherapy alone.
Patient blood samples were drawn prior to therapy (d1) as well as at d43 (1 week following the 6th injection of TG4010 in Arm 1) and at d85 (three weeks following the 8th TG4010 injection, in Arm 1). Lymphocytes were assessed by 5-colour flow cytometry for MUC1 tetramer binding and phenotype markers identifying activated Natural Killer (aNK) cells.

The primary endpoint of the study was progression-free survival (PFS) at 6 months; secondary endpoints included overall survival, response rate, safety, and immune parameters.

Intermediate results: 148 patients were randomized. Demographics and characteristics of the disease did not differ between study arms. Efficacy results are provided as an intent to treat analysis after central reading. PFS at 6 months is 44% (33/74) in Arm 1 and 35% (26/74) in Arm 2. Objective response rate is 43% (32/74) in Arm 1 and 27% (20/74) in Arm 2 Overall median survival was 10.7 months in Arm 1 and 10.3 months in Arm 2 (p=0.327).

Most adverse events (AEs) were considered related to chemotherapy or to underlying disease. Analysis of

lymphocyte phenotypes indicates that about one quarter of patients have abnormally high levels of aNKcells prior to therapy, with respect to levels in a healthy population. Of 138 patients evaluated for immunophenotye by 5-color flow cytometry, 101 were considered to have normal levels of aNK cells at d1. An evaluation focussing on these 101 patients (48 in Arm 1 and 53 in Arm 2) shows (in Arms 1 and 2 respectively): PFS at 6 months = 58% and 38% (p = 0.04); response rates = 56%) and 26% (p = 0.007); Time to progression = 6.4 months and 4.4 months (p = 0.006) and overall survival = 18 months and 11.3 months (p = 0.02). CD8+ pheripheral blood mononuclear cells from patients in both study arms stained with MUC1 tetramers. No correlation between tetramer staining and vaccination or with clinical outcome was observed

Conclusion: These results identify levels of aNK cells as a potential predictive biomarker which may be useful for patient selection in future cancer immunotherapy studies.

Clinical grade generationg of human anti-adenovirus-cytotoxic T cells for adoptive immunotherapy

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Adenovirus (ADV) infections represent one of the major cause of morbidity and mortality following Hematopoietic stem cell transplantation. The incidence of ADV infections ranges between 5 to 30%, with pediatric recipients showing the highest rate of infection. The mortality rate is very high in those patients: 60 to 73%, despite new antiviral treatment strategies. Actually, it has been demonstrated that a sufficient host T-cell response is essential to clear the virus.

We describe here a complete clinical grade generation of Human anti-Adenovirus cytotoxic T cells in order to propose an adoptive immunotherapy to the recipient.

Healthy donor mononuclear cells (PBMC), known for their good cellular immunity against ADV, are stimulated during 6 hours with the Peptivator-ADV5 (Miltenyi Biotec) which is a synthetic peptide pool covering the ADV5 Hexon protein. Gamma Interferon (IFNγ) secreting cells are isolated on the CliniMACS device using the Cytokine Capture System (Miltenyi Biotec) (Feuchtinger *et al*, 2007). The results of 4 immunomagnetic selections are presented in the table below.

Isolated T lymphocytes (CTL) are cultured with IL2 and autologous feeder cells (irradiated cells from the negative fraction) in order to perform the functional quality controls.

We first controlled the ability of the amplified CTL to secrete IFNγ when restimulated with the Peptivator ADV. A cytotoxicity of 40% against autologous dendritic cells (DC) as target cells (10/1) loaded with ADV5 or ADV 2 lysates could be observed when a cytotoxicity of 6% was reported with non loaded DC. Finally, we could observe a low allogeneic reaction with CTL against non HLA identical healthy donor PBMC, decreased of more than one log compared to the autologous PBMC.

Good Manufacturing Practice-grade generation of ADV-specific T cells for adoptive immunotherapy could be achieved with a synthetic antigen. This technology presents the advantages to be fast, without any *in vitro* amplification before infusion, and to allow a good reactivity to propose immunotherapy in case of anti-viral treatment failure.

Before selection			*After selection*				
N°	*TNC (10^6)*	*TNC (10^6)*	*CD4-IFNγ+ Purity (%)*	*CD4-IFNγ+ Yield (%)*	*CD8-IFNγ+ Purity (%)*	*CD8-IFNγ+ Yield (%)*	*Viability (%)*
1	484	0.384	66	48	45	60	58
2	52.6	0.5	58.6	119	22.8	10	79
3	140	2.5	31	> 100	28	> 100	65
4	65	0.45	47.4	100	37.7	87	83

Arsthinol for the treatment of leukaemia: a new look at an old treatment

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Scientific background: Arsenic compounds have been used as medicinal agents for many centuries for the treatment of diseases such as psoriasis, syphilis, rheumatosis.

From the 1700's until the introduction of modern chemotherapy and radiation therapy in the mid 1900's, arsenic was a mainstay in the treatment of leukemia. The discovery in the 1980's that arsenic trioxide induces complete remission in a high percentage of patients with acute promyelocytic leukemia (APL) has awakened interest in this metalloid for the treatment of human diseases. In particular, arsthinol, which was used in the treatment of amebiasis and in dermatology, has showed a striking effectiveness on the U937 myelomonocytic cells and on the K562 erythroleukemic cells as compared with As_2O_3 and other dithiarsolanes.

This work aims to detail the potentiality of arsthinol on APL. It includes both an *in vitro* study (on APL NB4 cell line) and a pharmacokinetics study in mice. Arsthinol has been compared with As_2O_3.

Material and Methods: The cytotoxic activity of each compound (arsthinol or As_2O_3) was estimated using the NB4 promyelocytic leukemia cell line. Briefly, exponential growing cells were seeded into a 96-well plate at a final density of 1.10^5/well using different concentrations of arsthinol (0.01 µM to 1 mM). Cells were incubated for 1 or 2 days at 37 °C in a humidified 5% CO_2 atmosphere. Viabilities were determined using the classical MTT test.

Pharmacokinetics studies were done in female CD1 mice (0.2 mmol/kg dose level via the caudal vein). Blood and tissue samples (liver, kidney and brain) were removed at various time points (5, 30 min, 1, 5, 8, 18, 24, 48 h). The amounts of total arsenic in the samples were performed using a colorimetric method (Elliott and Loper, 1974) after digestion with nitric acid (HNO_3; 65%) and hydrogen peroxide (H_2O_2; 30%).

Results: As tested *in vitro* on NB4 cell line, the cytotoxic activity of arsthinol was significantly better (IC_{50} = 0.78 ± 0.08 µM) than that of arsenic trioxide (IC_{50} = 1.60 ± 0.23 µM). Moreover this very good activity was associated (*in vivo*) with very high concentrations of arsenic in the bone marrow (Cmax = 155 µg/g) and lower concentration in the brain (Cmax = 11 µg/g) and other organs.

Conclusion: Arsthinol could potentially be considered as a good candidate for the treatment of APL. It offers 2 advantages compared to As_2O_3: a better *in vitro* activity and higher bone marrow concentrations. Moreover it has been already used in human with an acceptable toxicity profile.

Reference

Elliott, S.C., Loper, B.R., 1974. *Anal Chem.* 46, 2256-2257.

Cell death induced by highlet radiation combined with oxaliplatin in two human cancer cell lines

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Background: Concomitant chemoradiation therapy has improved treatment outcomes in several types of human cancers. However, in some cases, malignant cells can continue to resist to their destruction by ionising radiation. Irradiation with linear energy transfer (LET) accelerated particles (fast neutrons and carbon ions) offers a possibility to overcome radioresistance against standard low-LET radiation. Combining these two approaches is therefore an interesting strategy to kill radioresistant cancer cells. We previously reported that fast neutrons could induce autophagy, an alternative mode of programmed cell death, when combined with oxaliplatin (Ox) in U-87 glioblastoma cells. We further investigated the effects of fast neutron irradiation combined with Ox in SK-Hep1 (human hepatocellular carcinoma) and SQ-20B (human squamous head and neck carcinoma), with the aim of comparing the occurrence of autophagy with the number and persistence of DNA double strand breaks (DSBs). PJ-34, a poly(ADP-ribose)polymerase (PARP) inhibitor, was also added in order to assess its ability to amplify DNA damages caused by the co-treatment.

Material/Methods: SK-Hep-1 and SQ-20B cells were irradiated at 4 and 8 Gy by (p(65)+Be) neutrons (produced at the Cyclotron Research Center, Louvain-la-Neuve, Belgium) in the presence or absence of Ox (3µM) and/or PJ34 (5µM). The incidences of apoptosis, autophagy and DSBs were determined at various times post-irradiation. The percentages of apoptotic and autophagic cells were measured by flow cytometry after propidium iodide and acridine orange staining, and foci corresponding to DSBs were scored after an anti-γ-H2AX labelling. Autophagic vacuoles were visualized by fluorescence microscopy after GFP-LC3 transfection.

Results: The induction of a large number of DSBs at 24 hr, persisting until 72 hours after irradiation, in Ox-treated, neutrons-irradiated cells, was obtained. At 24 hr, this number was highly elevated compared to non-irradiated untreated control cells and was further increased when PJ34 was added to the co-treatment. The highest percentage of autophagic cells was also obtained at this time point under the same conditions. Thus, the existence of a correlation between the persistence of DSBs and the induction of autophagy after high-LET irradiation is strongly suggested. In contrast, levels of apoptosis remained low in co-treated cells as well as in irradiated cells only.

Conclusions: Together, these results point out the role of autophagic cell death as an important mode of cancer cells destruction by radiation. Future combinations will incorporate new platinum analogues and other PARP inhibitors.

Development of microemulsion of mitotane for improvement of oral bioavailabity

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Introduction: Mitotane (o, p'-DDD) is an hydrophobic organochlorine used in the treatment of metastatic or inoperable adrenocortical carcinoma [1]. In therapy, oral doses of 1300 g of mitotane administered for 3 to 5 months are needed to achieve the target concentrations of 14 mg/l [2]. Moreover many gatrointestinal side effects may limit its compliance. To improve its bioavailability, we developped a self microemulsifying drug delivery system (SMEDDS). These SMEDDS can be described as isotropic mixtures of lipid, surfactant, co-surfactant and mitotane, that rapidly form a microemulsion after mixing in water.

Material and method: The influence of oils, surfactants and co-surfactants on forming SMEDDS was investigated by constructing ternary phase diagrams. A series of mixtures was prepared with various amounts of Capryol®, Tween® 20 and Cremo-

phor®. SMEDDS were characterized by morphological observations and droplet size measurements.

The intestinal drug permeation of SMEDDS of mitotane (3mM) was assessed in a Ussing-type apparatus.

The prepared mitotane SMEDDS was compared to the conventional tablets (Lysodren®) after single oral of 100 mg/kg in rabbits. Pharmacokinetics parameters were determined from the plasma.

Results discussion: The optimum formulation consisted of a mixture of Capryol® as the oily phase (33%), Tween® 20 as the surfactant (33%) and Cremophor® EL as the second surfactant (33%). This formulation formed spontaneous microemulsion with mean globule size < 50 nm. The intestinal drug permeation studies showed that the SMEDDS formulation was found to pass through the intestinal barrier much faster than a solution of mitotane (14.85 ± 0.8 µmol.cm-2 vs. 3.03 ± 0.2 µmol.cm^{-2}). Moreover in rabbits, the absorption of mitotane from the SMEDDS formulation, showed a 3.4-fold increase in relative bioavailability compared with that of the conventional form.

Conclusion: This study shows that microemulsions are an interesting form to improve the bioavailability of mitotane. Microemulsions could reduce oral dosing regimens and the gastrointestinal side effects of mitotane in the treatment of adrenocortical carcinoma in humans.

References

[1] S.Hahner and M. Fassnacht, *Curr Opin Investig Drugs*. 6 (2005), pp. 386-394.

[2] M. Terzolo, A. Pia A. Berruti, G. Osella, A. Ali, V. Carbone, E. Testa, L. Dogliotti and A. Angeli, *Journal of Clinical Endocrinology and Metabolism*. 85 (2000), pp. 2234-2238.

Discovery of new PPAR direct target genes in the glioblastoma cell line T98G

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Scientific background: We recently showed the down regulation of the gene encoding semaphorin 6B an axonal guidance protein, in T98G glioma cells after in vitro treatment with PPARβ or α ligands. Since PPARs have been shown to be involved in the regulation of the development of some cancers we were interested in elucidating the role of semaphorin 6B and started a transcriptomic analysis of T98G cells to discover more genes regulated by PPARs in brain tumor cells.

PPAR could regulate directly after binding to a PPRE element in the promoter region of target genes or indirectly by interference with other transduction pathways in the cell (eg NFκB or BCL6, STAT3 etc). We localized potential PPRE in the promoter region of PPAR regulated genes and validate them as new target genes by Chromatin immunoprecipitation (ChIP) analysis of T98G cells treated with a PPARβ ligand.

Material and Methods: T98G cells were treated for 24h with L165041, a PPARβ ligand before RNA isolation for transcriptomic analysis or chromatin preparation for ChIP analysis.

Transcriptomic analysis was done at the IGBMC (Strasbourg) on home made DNA arrays. For genes which exhibited an expression fold change >+4 or <-4 PCR primers were designed in the proximity of potential PPRE in their promoter region which was analysed with our program PPREfinder.

ChIP analysis was done with a commercial kit of Active Motif but only for PPRE with a calculated binding strength to PPAR ≥1% of that of the consensus PPRE sequence AGGTCANAGGTCA.

Results: The transcriptomic analysis of T98G cells revealed 150 up and 100 down regulated genes with a threshold of 4 time fold change.

52 of these genes were chosen for their likely implication in the tumorogenicity. PPREfinder_Knime identified 71 potential PPRE elements within the ≥1% chosen range of presumptive strength.

The usage of 10 of these PPRE has already been validated by ChIP analysis using an antibody against PPARα or γ. Interestingly, the theoretically weaker PPRE elements are sometimes stronger PPAR binder than theoretically stronger PPRE elements.

Conclusion: Many new identified PPAR regulated genes are involved in cell adhesion and motility, in cell division control. This raises the possibility of usefulness of PPAR ligands to modulate the growth potential of glioblastoma cells.

The discrepancy between calculated strength of a PPRE and its usage according to ChIP results demonstrates the necessity of direct identification of ChIP retrieved fragments with CGH arrays instead of targeted PCR identification in order to formally identify PPAR target genes and true PPRE.

Vascular photodynamic treatment by targeting neuropilin-1 in human malignant glioma bearing nude mice

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A new approach of photodynamic therapy (PDT), the VTP (vascular-targeted PDT) was studied. The purpose is to promote the anti-vascular effect of PDT by targeting the tumor vasculature. This strategy was considered by coupling a photosensitizer to an heptapeptide targeting neuropilin-1. We previously demonstrated by binding and competition

experiments that the conjugated photosensitizer displaced $VEGF_{165}$ binding to neuropilin-1. This new targeted photosensitizer proved to be very efficient *in vitro* compared to its non-conjugated counterpart [1]. This study compares the peptide-conjugated photosensitizer *versus* the non-conjugated photosensitizer to highlight the anti-vascular effect *in vivo* after VTP.

The time between the administration of the photosensitizer and the light irradiation (drug light interval) was fixed at 4 hours, corresponding to relevant conjugated photosensitizer levels in tumor, low photosensitizer levels in skin and low degradation of the peptidic moiety [2, 3]. A Doehlert experimental design was selected to model effects and interactions of the conjugate dose, fluence and fluence rate on the growth of U87 human malignant glioma cells xenografted in *nude* mice [4]. The anti-vascular effect with the conjugated photosensitizer was characterized by a decrease in tumor blood flow at about 50% baseline during the treatment, endothelial cells became rounded without alteration of their ultrastructural characteristics, associated with an induction of the protein expression of the tissue factor. The tissue factor-initiated *thrombi* formation was also related with a reduction in fibrinogen diffusion as soon as 4 hours post-VTP. Treatment of glioma-bearing mice with the peptide-conjugated photosensitizer followed by the optimized VTP condition showed a statistically significant tumor growth delay compared with animals who received this treatment with the non-conjugated photosensitizer. This strategy of targeting of neuropilin-1 improves the potential of VTP. It allows to consider the use of multifunctional nanoparticles [5].

References

[1] Tirand L. *et al., J. Control Release*, 2006
[2] Tirand L. *et al., Drug Metabol Disp*, 2007
[3] Thomas N. *et al., Photochem Photobiol Sci*, 2008
[4] Tirand L. *et al., Int J Radiat Oncol Biol Phys*, 2009
[5] Bechet D. *et al., Trends Biotechnol*, 2008

A novel role for STAT3 in NK-cell immunosurveillance subversion

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Natural Killer (NK) cells represent one of the main effectors of the immunosurveillance against tumors, by exerting two major effector functions, cytolysis of target cells and production of cytokines and chemokines. Evasion of cancer immunosurveillance is a hallmark of many malignancies. In this work, we aimed to assess the role of Signal Transducer and Activator of Transcription 3 (STAT3) in NK cell-immunosurveillance subversion. STAT proteins are a family of latent cytoplasmic transcription factors considered as oncogenic proteins. STAT3 signaling affects the expression and functions of a variety of genes involved in cell survival, proliferation, invasion and angiogenesis.

To initiate this study, we selected a colorectal cancer cell line (HT29) poorly able to activate NK cells. STAT3 is constitutively phosphorylated in HT29. STAT3 neutralization in this cell line decreased the tumor growth in a xenograft model, supporting an oncogenic role of STAT3 in this model. STAT3 neutralization in HT29, using pharmacological inhibitors or siRNA technology, resulted in an increase of NK cell cytotoxicity and IFN-g production. This ability was partially restored in the presence of NKG2D neutralizing antibodies, suggesting that STAT3 activation in HT29 prevents NKG2D-mediated NK cell activation. Therefore we investigated the expression of NKG2D ligands according to STAT3 activation in HT29. Using quantitative real-time PCR, western blotting and flow-cytometry analysis, we observed that MHC class-I-chain-related protein A (MICA) was up-regulated following STAT3 neutralization.

The link between oncogenesis and NKG2D ligands expression has been showed previously by Raulet et al. implicating a cross-talk with DNA damage repair mechanism. Here we show another pathway that is part of NK immunoediting of colorectal cancer implicating STAT3.

Regulation of Cdc25 activity by the thioredoxin system during oxidative stress in breast cancer cells

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The CDC25 phosphatases are key regulators of cell cycle progression. Three isoforms (A, B and C) have been identified in mammalian cells. Recent *in vitro* studies have shown that an intramolecular disulfide bond is formed in CDC25 active site under mild oxidative stress conditions, leading to inactivation. However, CDC25 activity can be restored *in vitro* by the thioredoxin/thioredoxin reductase system (Trx/TrxR). Since CDC25 and the Trx/TrxR system may be upregulated in breast cancer and since ROS signals drive proliferation and other events required for tumor progression, we postulated that the Trx/TrxR system is essential for tumor progression.

Thus, to decipher the role of Trx/TrxR in the regulation of CDC25 activity in cancer cells, we have studied the *in vitro* inhibition of the three CDC25 isoforms by hydrogen peroxide (H_2O_2). CDC25 A, B and C showed a similar sensitivity towards H_2O_2 (CI_{50}: CDC25A, 100 µM; CDC25B, 75 µM; CDC25C, 300 µM respectively). Moreover, we have tested CDC25A reactivation by the Trx/TrxR system and by a reducing agent, TCEP and we observed that CDC25A activity could be been totally recovered in both case.

Next, the interaction between CDC25 phosphatases and Trx/TrxR system has been studied in breast cancer MCF7 cells. CDC25 activity was evaluated in cells treated with H_2O_2 while Trx/TrxR activity was modulated. For this purpose, we used a novel and powerful TrxR

inhibitor, *ie* GoPI-Sugar (G-S). The optimal conditions for TrxR inhibition were established and Trx redox state was evaluated under these conditions.

We then observed that CDC25 could be inactivated in a dose-dependent manner by H_2O_2 in MCF7 cells. However, this inhibition was not total, even in the presence of high H_2O_2 concentrations. This suggests the presence of a reactivating system which acts in oxidative conditions to restore CDC25 activity. This system could be Trx/TrxR system since we found that CDC25 inhibition by H_2O_2 is higher when the Trx/TrxR system is inactivated.

This study should enable us to better understand the contribution of the Trx/TrxR system in the preservation of CDC25 activity, including in the case of treatment with anticancer agents generating oxidative stress such as Doxorubicin, which is frequently used for the treatment of breast cancer.

Peripheral blast cell decrease during induction therapy in acute myeloblastic leudemia: a new prognostic tool

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Scientific background: In acute myeloblastic leukemia (AML), peripheral blasts are often present at diagnosis, that could be used to appreciate the chemosensitivity of individual patients. Morphological identification of these cells, easy at diagnosis and when their frequency is higher than 5%, becomes difficult below this thresholds and when chemotherapy impairs cell morphology.

The development of multiparametric flow cytometry allows to define accurately concomitantly in one sample the characteristics of normal mature cell subsets in the peripheral blood, and could be used to perform an accurate evaluation of the blastic cell compartment.

Material and methods: A technique developed in the French Groupe d'Etude Immunoologique des Leucémies (GEIL) has been devised to perform such an evaluation in a single-tube panel. The combination of morphometric parameters of forward and side scatter, CD45, CD11b, CD14 and CD16 labelling allows to properly identify monocytes, granulocytes and lymphocytes in the peripheral blood. At AML diagnosis, the other significant subset that can be identified is that of peripheral blasts. Applying this combination to sequential samples obtained every day during induction therapy allows to measure blast cell decrease.

We have demonstrated that the slope of blast cell percentage decrease is strongly correlated to prognosis. The sharper the slope the best the prognosis, indicating that efficient chemosensitivity indeed is a strong prognostic parameter. An alternative way of expressing data is to evaluate daily the differential in peripheral blasts percentage with reference to the first day of induction therapy. The day when this analysis demonstrates a one log decrease has been shown to also carry strong prognostic value in terms of complete remission achievement and maintenance.

This method is applied routinely in our laboratory in the follow-up of AML patients during induction therapy.

Conclusion: The pilot study performed in a small number of laboratories according our own suggest that peripheral blast cell decrease measurement in flow cytometry allows for an early appreciation of chemosensitivity. This method is less invasive and more accurate than the classical and often inconclusive day 15 bone marrow aspiration. The strongly standardized measurement provided by flow cytometry provides strong reproducibility and an objective assessment of the peripheral blast load. Upon further validation, this new tool could prove useful for an individualized management of AML patients.

Detection of minimal residual disease in leukemia: the alternative of flow cytometry

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Scientific background: The challenge of induction, consolidation and maintenance chemotherapy in leukemia is to eradicate the malignant clone and avoid the re-emergence of the initial blastic population or of a chemoresistant subset of it. Molecular techniques (MT), when applicable, are highly sensitive for the detection of very low levels of remaining cells and assessment of minimal residual disease (MRD). However, they are only applicable to the fraction of patients with molecular markers, such as Ig or TCR rearrangements, mutations or fusion transcripts. The sensitivity currently recognized is around 10^{-6}, yet several preanalytical hindrances and the time-consuming techniques they involve are responsible, together with a relatively high cost, for a limitation of their clinical applicability. With the development of multiparametrtic flow cytometry (FCM), new approaches have been developed that could harmoniously complement MT.

Material and methods: FCM detection of MRD relies on an extensive and comprehensive immunophenotypic diagnosis. This allows, in both lymphoblastic and myeloblastic acute leukemia, to identify the combination patterns most useful to identify the malignant clone among regenerating and then normal hematopoiesis in the course of therapy. Two parameters can be taken into account in measuring MRD in acute leukemias, i)leukemia associated immunophenotypic patterns (LAIP), at variance with what can be seen on normal cells and ii)increased amounts of subnormal populations, clearly more clustered and abnormally positioned in flow cytometry scattergrams. In some cases specific panels must thus be adapted to a given patient but another approach is the use of a lim-

ited set of antibody combinations, both at diagnosis and during follow-up, characteristizing the malignant population within a given frame of differentiation patterns. This approach is even more easily applied to peripheral blood samples, especially in acute myeloblastic leukemia. This more standardized approach can be developed for patients with chronic lymphocytic leukemia, taking advantage on the differences between normal and leukemic B-cells.

Conclusions: FCM can be successfully applied to the detection of MRD in both acute and chronic leukemia, and could complement molecular approaches. Versatile in its application to peripheral blood and bone marrow, FCM analysis of MRD, with a limited patient-tailored panel, can be performed more frequently than MT. Algorithms will develop from ongoing multicenter studies for an optimal follow up and early detection of relapse.

Effects of microalgae extracts on the prevention of pain linked to chemotherapy in rats

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The improvement of palliative care is part of current medical priorities. Empirical information reported that patients under chemotherapy and receiving a diet containing microalgae, including chlorella, better bear the pain caused by side effects of such treatment (lack of appetite, gastrointestinal problems and urinary disorders, articular, muscle and visceral pain). This research program is part of a European Project started in September 2008 and called Algohub™.

Groups of female rats will be treated daily with oral administration of different strains of microalgae before the induction of visceral pain with cyclophosphamide. The behavior of rats (cachexia, aspect of the body, aspect of the fur…), the observation of faeces and urine, their body weight change and their food and water consumption will be followed daily before and after induction of visceral pain.

Microalgae, in addition to their detoxifying properties, could be effective in resistance to pain during heavy medical treatment such as chemotherapy. The role they could play in palliative care is important and must be clarified for more effective treatment of pain.

Protein chip engineering leading to a revisited concept of BIA-MS: Toward a new SPR-MS µarray platform

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Plasma or serum are very attractive biological samples in the clinical context to find new biomarkers. The advantages of these biological samples are the possibility to perform repeatedly the sampling for the kinetic studies in the therapeutic proteomic studies. But the advantage of plasma to proteomic analysis is in balance with the high level of complexity of this one. The challenge today is to find methodologies to analysis low-abundance proteins in a sample with high dynamic range of concentration of protein, where few high abundance proteins represent the majority of proteins.

The challenge of our approach is to capture, identify and characterize some specific proteins and their variants. In fact, most of the time, the detection of proteins or interactions of proteins, by imunnoaffinity approaches could be biased by non specific adsorption or the capture of modified proteins without any possibility to discriminate the various species. To solve this drawback in detection and diagnosis, we have developed an approach combining Biacore™ analysis with homemade chips and Mass Spectrometry identification directly onto the chips without elution step. Using this technique, identification of protein complexes were routinely obtained giving the opportunity to the "on-a-chip" processing to complete the BIA-MS approach in the discovery and analysis of protein complexes.

This recent technological advance should enable us to continue our investigations into sensitivity and characterization of protein biomarkers captured in biological fluids. At present time, we carried on the analysis on a human disease model in capturing small amounts of LAG-3 protein in plasma. Complete BIA-MS analysis has been realized leading to the detection, identification and characterization of 100 ng/ml of LAG-3 protein spiked in plasma. The last results demonstrate the possibility to analyse directly the plasma without step of fractionation or depletion.

Finally, these developments have been transposed to µarrays and multiplexed analysis was performed through the Flexchip apparatus from Biacore™. The first results of tracking of biomarkers in plasma provided by our "SPR-MS µarray platform" will be presented.

Characterization of the role of human nucleolar protein p120 and ITS yeast homolog Nop2p

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Human p120 is a nucleolar proliferation-associated antigen, and has been detected in the nucleoli of a variety of malignant cells, from breast, liver, blood, lung and brain cancers. p120 is expressed in the G1 phase and its level reaches the maximum during S phase. Expression of p120 is increased 60- to 80-fold in several tumor cells as compared to normal resting cells and correlates with the tumor growth rate. Growth of NIH/3T3 cells was stimulated by transfection with p120 cDNA and inhibited by a p120 antisense construct. A clinical study showed a correlation between the decreased survival of patients with breast and the increased amount of p120 expression. This proliferation marker has

also emerged as one of the targets for cancer therapy using specific antibodies.

A *Saccharomyces cerevisiae* protein homologue to human p120, Nop2p, is required for yeast viability and seems to play a direct role in pre-25S rRNA processing. Alignment of Nop2p with human p120 points to the evolutionary conservation of a large domain that contains a SAM-binding motif and a sequence homologous to protein domain having 5-methylcytosine transferase activity. One goal of our project is to identify the targets of the possible m5C activity of Nop2p and p120. To this end, we had first to develop methods for m5C identification in RNA. The selected approach is based on [^{3}H] S-adenosylmethionine utilization. We also had to develop the production of recombinant Nop2p and p120 proteins in a soluble form.

In addition, as Nop2p was proposed to be involved in pre-25S rRNA maturation and since p120 has a nucleolar localization and was shown to interact with rRNA, a second goal of our work is to establish the possible role of Nop2p/P120 in rRNA maturation. By RNA interference, we are trying to abolish p120 expression in human cells and to test the effect on rRNA processing by Northern blot experiment.

For a better delineation of the Nop2p/P120 functional domains, we also tested the possible complementation of a ΔNOP2 yeast strain by the full-length human p120. The complementation efficiency was very poor. Therefore, we presently test the complementation efficiency of hybrid Nop2p/p120 proteins.

Altogether, we expect to bring several new insights on protein p120 and therefore on its role in carcinogenesis.

Study of MMP inhibitors, analogues of ilomastat with a sulfonylhydrazide as zinc binding group

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Matrix metalloprotease inhibitors (MMPIs) are investigated for their anti-cancer potential as well as for their implication in the treatment of other pathologies. All MMPIs are zinc chelators but sought-after selective MMP inhibition can be achieved through zinc binding modulation. Thus, the development of synthetic MMPIs possessing a good potency and selectivity constitutes an important synthetic target [1].

The aim of our investigation is to synthesize structural analogues of Ilomastat focusing on different modifications of P_1' and P_2' sites and developing new ZBG (zinc binding group) function [2, 3].

We have synthesized and evaluated the MMP inhibitory activity of Ilomastat derivatives having a sulphonylhydrazide group as ZBG. Interaction with the S_2 enzyme subsite is mainly responsible for the inhibitory properties of this derivative as confirmed by molecular docking computation [4, 5].

Sulfonylhydrazides are a new type of MMPIs whose zinc chelating properties are unknown, yet. DFT calculations of the binding modes and free energies of binding for several sulfonylhydrazides are described. Binding in a tridentate fashion is generally observed for uncharged derivatives, deprotonated species bind in a bi- or tridentate mode [6].

References

[1] Bourguet, E. *et al.* Anti-cancer *Agents Med. Chem.* (Submitted)
[2] Augé, F. *et al. Bioorg. Med. Chem. Lett.* 2003, 13, 1783-1786.
[3] Marcq, V. *et al. Bioorg. Med. Chem. Lett.* 2003, 13, 2843-2846.
[4] Moroy, G. *et al. Bioorg. Med. Chem.* 2007, 15(14), 4753-4766.
[5] LeDour, G. *et al. Bioorg. Med. Chem.* 2008, 16, 8745–8759
[6] Rouffet M. *et al. J. Org. Chem.* (Submitted)

Lumican inhibits B16F1 melanoma cell-induced lung metastasis

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Background: Lumican is a small leucine-rich proteoglycan (SLRP) of the extracellular matrix (ECM). We previously showed that lumican is involved in the control of melanoma growth and invasion and may be considered as an anti-tumour factor from the extracellular matrix (Vuillermoz et al, 2004). Moreover, we recently demonstrated that lumican inhibits the migration of melanoma cells by increasing their adhesion to the ECM and identified b1 integrin as mediator of this effect (D'Onofrio *et al*, 2008). The aim of the present study was to analyse the role of lumican in the regulation of the development of lung metastasis

Methods: After 14 days of intracaudal injection of B16F1 melanoma cells in syngenic mice, the lung metastasis were compared in control mice (n=5) injected with mock-transfected B16F1 cells (transfected with pcDNA3 vector alone) to HLum-transfected B16F1 cells (transfected by pcDNA3-HLum construct) "lumican" mice (n=5). The distribution of lumican, cyclin D1, as well as apoptotic markers (cleaved caspase 3, cleaved poly-(ADP-ribose) polymerase (cPARP)) but also Vascular Endothelium Growth Factor (VEGF) and Von Willebrand Factor (vWF) within lung metastasis nodules was investigated by immunohistochemistry. In parallel, the expression of total caspase 3 and cPARP proteins was evaluated by Western immunoblotting.

Results: Expression of the human recombinant lumican core protein by tumour cells significantly dec-

reased of the number and the size of lung metastasis nodules in comparison to the control mice group. This lumican-induced inhibition of lung metastasis might be explained by an increase of tumour cell apoptosis within the metastasis nodules, as revealed by the immunostaining of specific markers such as cPARP and cleaved caspase 3. *In vitro*, Western immunoblotting analysis demonstrated a significant decrease of caspase 3 expression and an increased expression of cPARP in wild type B16F1 cells incubated with increasing amounts of lumican core proteins for 16h. The cell proliferation rate evaluated by the percentage of immunostained cyclin D1-positive tumour cells within the nodules remained constant in the two mice groups. In contrast, the VEGF imunostaining and the number of blood vessels within the lung metastasis nodules was decreased in the lumican-expressing tumours. *In vitro*, pseudotubes formation on Matrigel® by human endothelial cells (HUVEC) was inhibited by lumican. Moreover, the migration of endothelial cells clearly showed an alteration on lumican coating compared to type I collagen or plastic. Altogether, our results suggest that lumican decreased lung metastasis development by inducing tumour cell apoptosis and inhibiting angiogenesis.

Evidence for PPARγ-independent events induced by thiazolidinediones in breast cancer cells

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Scientific background: Peroxisome proliferator-activated receptor gamma (PPARγ) can be activated by natural ligands such as 15-deoxy-delta(12,14)-prostaglandin J2 (15d-PGJ(2)) as well as synthetic drugs such as thiazolidinediones. Agonists of this nuclear receptor display antiproliferative effects on breast cancer cells *in vitro* and some of them have been used in clinical trials. Interestingly, the mode of action of these ligands is not limited to the activation of PPAR and the antiproliferative pathways of thiazolidinediones have not been clearly identified. Therefore, we have investigated early and delayed signaling responses of various PPARg ligands *in vitro* and their potential involvement in cell apoptosis.

Material/Methods: We used four PPARγ agonists, Rosiglitazone (RGZ), Ciglitazone (CGZ), Troglitazone (TGZ) and the natural agonist 15d-PGJ(2), on the hormone-dependent breast cancer cell line MCF-7. Gene expression was studied by RT-PCR, western blotting, immunocytochemistry. RNA interference was used for gene silencing. TGZ derivatives were obtained by chemical synthesis.

Results: After 3 hours of incubation with 25 µM TGZ, CGZ or 15d-PGJ(2), the early gene EGR1 (Early Growth Response gene 1) mRNA level peaked and then gradually decreased. This effect was not observed with RGZ, the most potent activator of PPARγ. The increase in EGR1 protein level in MCF7 cells was observed by western blot and immunofluorescence. After 24 hours, 25 µM TGZ, CGZ or 15d-PGJ(2) induced an inhibition of the estrogen receptor ERα signaling associated with the proteasomal degradation of ERα. Treatments that induced ERα degradation also inhibited cell proliferation. In contrast, 24 hours exposure to RGZ, disrupted neither ERα signaling nor cell proliferation. PPARγ antagonists such as GW 9662 did not block EGR1 expression or ERα proteasomal degradation, which still occurred in case of PPARγ silencing as well as in case of treatment with the PPARγ-inactive compound Delta2-TGZ. However, EGR1 silencing did not inhibit the induction of ERα proteolysis, suggesting that we are pointing out two independent but complementary induced-signaling pathways.

Conclusion: Taken together, these results demonstrate PPARγ-independent mechanisms that could be interesting for the application of new thiazolidinedione derivatives to breast cancer therapy.

Bio-Plex® phosphoprotein array assay for characterization of human tyrosine kinase receptors downstream signaling functionality as clinical response predictive marker to targeted therapy

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Background: Human epidermal growth factor receptors (HER) downstream signaling kinases have major consequences on tumor response to anti-HER monoclonal antibodies and tyrosine kinase inhibitors. In previous work, we demonstrated that expression of phosphoproteins from RAS/RAF/MAPK and PI3K/AKT signaling pathways correlated with cellular response to anti-EGFR targeted therapy in preclinical models. The present study was designed to validate the use of Bio-Plex® phosphoprotein array (BPA) to investigate HER downstream signaling functionality in human tumor frozen biopsies taken at diagnosis from patients bearing breast, head and neck and colorectal cancer. The 2nd point (collab P Laurent-Puig, Paris, France) was to validate the relationship between signaling functionality and response to cetuximab, explored through BPA analyses in metastatic colorectal cancer (CRC).

Methods: Standard operating procedures were optimized regarding sample size, homogenicity, tumor content, protein extraction and detection monoclonal antibodies. HER downstream signaling phos-

phoproteins expression (p-MEK, p-ERK1/2, p-P38MAPK, p-AKT, p-GSK3β, p-P70S6K) were analyzed using BPA. 103 breast, 22 head and neck as well as 75 colon cancer specimens were analyzed. BPA was cross-validated with western blot (WB) analysis in 49 breast cancer specimens. In 42 CRC, BPA results were then confronted to *KRAS* mutation previously analyzed and evaluated for response prediction to cetuximab.

Results: BPA and WB results were statistically correlated and the two methods showed clinical comparability. Great variations of phosphoprotein expression, up to several hundred-folds, were observed among the different tumor specimens and did not correlate with EGFR or HER2 expression levels as determined using immunohistochemistry. Moreover, in CRC, *KRAS* and p-MEK were identified as two independent prognostic markers of patients treated by cetuximab. In wild type *KRAS* tumor, p-MEK overexpression was associated with lower progression-free survival.

Conclusion: These results validate the use of BPA and demonstrates its interest for single-step analysis of signaling pathways functionality in human clinical tumor specimens. Before any treatment initiation, RAS/RAF/MAPK and PI3K/AKT functionality could probably explain discrepancies in clinical response to targeted therapy. In CRC, adding functional data to *KRAS* mutation analysis could be helpful to predict response to cetuximab. From these data BPA could be proposed for evaluation of predictive and/or surrogate markers for clinical response to kinase inhibitors.

KRAS genotyping in metastatic colorectal cancer as molecular diagnostic marker for prescription of anti-EGFR targeted therapies in Alexis Vautrin cancer center

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Background: *KRAS* mutational status was shown to be a highly predictive marker of tumour response regarding anti-epidermal growth factor receptor receptor (EGFR) monoclonal antibodies (Erbitux®, Vectibix®). *KRAS* mutations are also associated with a worse prognosis. Moreover, cetuximab would have a deleterious effect in *KRAS* mutated patients. Therefore, EMEA restricted the presciption of Erbitux® and Vectibix® in metastatic colorectal cancer with nonmutated (wild-type) *KRAS* genes.

Patients and Methods: *KRAS* genotyping is performed weekly at the Alexis Vautrin Center within the framework of the regional "plateforme de génétique moléculaire des cancers INCa". Between may and December 2008, 273 tumors (colon, rectum, duodenum, epiploon, peritonea, and endometrial, hepatic, and lung metastases) were genotyped for *KRAS* mutations. Tumor samples, collected as paraffin-embedded tissue specimens, were macrodissected after selection by a pathologist to ensure a minimum of 50% tumor tissue content. DNA was extracted and controlled. *KRAS* mutations in codons 12 and 13 were analyzed using PCR-based RFLP and Taqman technology.

Results: Among the 273 tumor specimens, 39% (97/273) had *KRAS* mutations with 85% (82/97) in codon 12 and 15% (15/97) in codon 13. 3% (9/273) results remained un-interpretable because of a lack tumor material, DNA degradation or Bouin fixation. No case of discordance between the PCR-RFLP and Taqman techniques was found. These percentages are fully consistent with those reported in the literature. The average time between genotyping prescription by the oncologists and tumor reception was 6.8 ± 4.6 days. The average time between tumor reception sending of the result was 11.3 ± 3.6 days. This activity is currently being in expansion and prospective for 2009 is about 750 cases.

Conclusion: *KRAS* is a crucial molecular parameter to predict anti-EGFR therapy efficacy. *KRAS* genotyping is additionally interesting for diagnostic, pronostic, and medico-legal and pharmaco-economic aspects and represents a first steps towards individualized therapy for cancer. Different technical approaches are currently evaluated through a multicentric assay. Further additional markers of resistance are investigated such as lack of EGFR amplification, PTEN loss, BRAF, PIK3CA and NFkB mutations as well as EGFR downstream signalling phosphoproteins overexpression (Merlin et al. Proc ASCO, 2008). All these parameters require prospective evaluation before integration into clinical decision making, to further increase the power of patient selection for anti-EGFR therapy.

Gene expression profile and response to trastuzumab-docetaxel-based treatment in breast carcinoma

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Background: Resistance to trastuzumab is often observed in women with human epidermal growth factor receptor 2 (HER2)-positive breast cancer and has been shown to involve multiple potential mechanisms. We examined the ability of 30 genes involved in key cellular processes to predict response to trastuzumab, and used microarray analyses to determine potential markers of pathological complete response (pCR).

Patients and Methods: We conducted a retrospective analysis of tumor samples from 38 patients with locally advanced HER2-positive breast cancer who had received trastuzumab combined with docetaxel. Quantitative reverse transcriptase polymerase chain reaction (RT-PCR) was used to assess expression of 30 key genes in all

tumor samples; microarray analyses were performed on 25 samples to identify a prognostic gene expression profile, with 13 blinded samples used to validate the identified profile.

Results: *Bcl-xL* was the only gene found to correlate with response by RT-PCR, being underexpressed in the pCR group and overexpressed in the non-pCR group ($p<0.0001$). The microarray analysis identified a gene expression profile of 28 genes, with 12 upregulated in the pCR group and 16 upregulated in non-pCR group. The 28-gene expression profile classified the 13 validation samples with 92% accuracy, 89% specificity, and 100% sensitivity.

Conclusion: Although *bcl-xL* appears to be a prognostic marker for response to trastuzumab-docetaxel, our results suggest that genes not involved in classical cancer pathways such as apoptosis or DNA repair could also be involved in responses to a trastuzumab-docetaxel-based regimen. The prognostic value of the identified gene profile will be confirmed by using a larger number of cases.

Incidence and prognostic value of FLT-3 and NPM1 gene abnormalities in acute myeloid leukemia in the population of Côte-d'Or

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Context: In Acute Myeloid Leukemia (AML), abnormalities of FLT-3 and NPM1 genes were recently described and their prognostic value established in particular in the forms with normal karyotype in which they condition therapeutic approaches.

Objective: To determine the incidence of the abnormalities of these two genes in AML cases diagnosed in the population of Côte-d'Or and to study their prognostic value in a well defined population.

Materials and Methods: AML diagnosed according to the WHO classification between 01/01/2001 and the 31/12/2006 were included. The data of the karyotype are obtained in 137 cases. The study of the D835 mutation, of the FLT3 internal duplication (ITD) and of the NPM1 gene mutation were realized on the biological material of the diagnosis kept in the Biobank Ferdinand Cabanne of Dijon. The vital status of the patients was collected on 31/10/2007. The calculation of the relative survival was realized by means of the software STATA (V9).

Results: 100 *de novo* AML and 51 secondary AML were registered (73 women and 78 men). The rate of world population standardized incidence rate of *de novo* AML is 2,4 in men and 1,5 in women while it is respectively 1,1 and 0,6 in secondary LAM. The urban predominance is present in both LAM's types. The karyotype is normal in 34% of cases (46/137) (35% of *de novo* AML and 22% of secondary LAM). It is abnormal in 54% of cases (51% of *de novo* AML and 45% of secondary LAM). The molecular analyses were performed in 106 cases: 97 cases of *de novo* AML and 9 cases of secondary LAM. FLT-3-ITD was found in 15 cases, the D835 mutation in 7 cases and the NPM1 mutation in 21 cases. The absence of the D835 mutation and the NPM1 mutation is found in 78 cases while in 18 cases NPM1 mutation is isolated. In 7 cases presenting the D835 mutation, 3 have also NPM1 mutation. FLT- 3-ITD was statistically associated with white blood cell count and peripheral blast cells count. Overall survival and relative survival were lower in cases with a FLT-3-ITD and were not influenced by isolated NPM1 abnormalities.

Conclusion: These molecular data in AML are the first allowing estimating the population-based prognostic value of these genetic abnormalities in AML. They confirm the bad impact on survival of FLT-3ITD and the absence of prognostic value of NPM1 abnormalities.

Search OF serum and/or plasma biomarkers diagnostic, prognostic and/or predictive of invasive fungal disease in neutropenic patients.

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The frequency of invasive fungal infections has risen dramatically in the last twenty years, owing to the development of new medical practices such as the use of cytotoxic drugs and immunosuppressive generators neutropenia. In addition, the mortality of these invasive infections varies from 50 to 90% depending on the underlying disease and the causative agent.

The main agents responsible for fungal infections nosocomial are *Candida spp.* and *Aspergillus spp.* Given the high incidence of these invasive fungal infections, their severity, and the absolute necessity of early treatment, it is urgent to develop new strategies improving early and specific diagnosis. In this context, this study consists to compare the protein expression profiles of patients neutropenia with or without invasive fungal disease.

Since three years, serum / plasma samples were taken of prospectively in patients diagnosed for acute leukemia and programmed to receive several treatments of chemotherapy aplasia. For each period of aplasia, each patient was taken 5 days per week. In total, 182 patients were included and 2500 samples was collected and transferred to liquid nitrogen CRB CHU Dijon. Among these 182 patients, 34 (26 invasive fungal infections filamentous fungus, 8 invasive candidiasis) have submitted at least 1 episode of invasive fungal infection (during chemotherapy treatment for aplasia). For each of these patients, we have also collected blood samples during period(s) of aplasia(s) free(s)

from infectious complications. Finally, we have witnessed patients, ie had not developed infectious episode documented during their periods profound neutropenia, or who developed infectious episodes of different origins.

The proteomic analysis consists to simplify the blood samples in using some processing such as purification and/or fractionation and then analyzed by mass spectrometry (analysis by MALDI-TOF) sub samples generated. The data generated in the form of profiles of peptides mass were pretreated (subtraction base line, standardization, alignment ..) in view of the application of methods for detection of peaks.

Statistical analysis of data generated has focused on three objectives: 1 - the study of proteome variability in patients during one or several phases of aplasia. 2- a longitudinal study that will identify specific markers the emergence of the fungal infection. 3 - a cross-sectional study of comparison between patients at a given time to validate the specific markers of fungal infection compared to data obtained from patients without infection or infection with a different nature.

The descriptive statistics preliminary analysis by principal component analysis, suggests that the variability in peptidome studied periods of aplasia in patients without infection is lower than the variability in peptidome studied periods of aplasia a patient with infection. The validation of this preliminary result will be conducted on the remaining samples available.

HRMAS-NMR metabolomics in a medical environment: applications in cancerology

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Introduction: High Resolution Magic Angle Spinning (HRMAS) – NMR is a technique that allows the study of the metabolic content of intact biopsies. In order to exploit the full potential of HRMAS for the medical analysis of human tumor biopsies, we have recently implanted a dedicated research team and a HRMAS spectrometer in a University Hospitals of Strasbourg (CARMeN project).

Materials and methods: HRMAS spectra were recorded on a Bruker Avance III 500 spectrometer operated at 3°C. Spectra were data reduced into integral regions of 0.02 ppm using the AMIX program and exported into SIMCA P, where a range of multivariate statistical analyses were conducted (PCA, PLS-DA).

Results:

1. Gliomas: We have studied 10 low-grade oligodendrogliomas (WHO grade II), 24 high-grade oligodendrogliomas (WHO grade III), 6 intermediate oligodendrogliomas (WHO grade II/III) and 30 glioblastomas (WHO grade IV). The results suggest that the metabolic information obtained by HRMAS-RMN (the degree of tumor hypoxia) could be better correlated with the patient prognosis than the morphological classification criteria.

2. Colorectal Cancers: The metabolic content of adenocarcinoma colorectal biopsies from 35 patients was studied by HRMAS-NMR. Using a PLS-DA analysis, it is possible to separate the two groups of biopsies (cancerous versus healthy) solely on the metabolic content, principally taurine and myo-inositol.

3. Hodgkin's lymphoma: HRMAS-NMR analysis of Hodgkin's lymphoma biopsies issued from 17 patients was correlated to glucose uptake obtained by ^{18}F-FDG PET/CT in vivo imaging performed before any treatment. The preliminary results suggest that intense in vivo glucose cellular uptake shows high cellular proliferation mainly related with anaerobic metabolism.

4. Ovarian Cancers: We have studied 3 of the major histological types of epithelial ovarian cancers (endometrïoid, mucinous, and serous), including 4 healthy samples. The two first classes, have a fully different metabolic pattern. For the serous case, the heterogeneity is such that we had to mixt serous and healthy prior to PCA. The first factor serves to discriminate the cases of grade 3 (FIGO classification) with the grade 1 and healthy tissue all together, without prior knowledge.

Conclusion: This presentation demonstrates the ability of HRMAS-NMR based metabolomics to clearly discriminate cancerous and healthy tissues and also overcomes the lack of reproducibly inherent of traditional malignity classification. A next step will be to introduce new data into the model used as an unsupervised grading approach to validate it with a large number of samples.

A cytomics core facility dedicated to clinical research in Nancy

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The cytomics concept covers the diverse means allowing for an extensive analysis of cell subsets characteristics down to the level of individual cells. Cytomics covers a broad array of multiparametric (from 5 to at least 20) definition of various immunophenotypic and functional parameters of cell subpopulations.

It allows to assign cells to a well defined cell lineage, characterize their differentiation stage and evaluate their functional capacities, such as activation, ageing, pathological status. Cytomics through the combination of morphological and immunological markers, can be applied to normal or pathological cells (lymphocytes, monocytes, endothelial

cells, platelets, microparticles...) from peripheral blood, bone marrow, biological fluids, and from cells eluted from tissues.

Cytomic studies of discrete and rare cells can now be used as a biomarker approach for diagnosis, follow-up and prognostic studies of patients with various chronic or acute pathologies of inflammatory or neoplastic origin.

Rare events detection and quantification is the future challenge of clinical research which can now be conceivable with the new array of commercially available equipments.

This cytomics core facility will include three complementary equipments for multiparametric characterization of rare events:
– A multilaser multicolour flow cytometer allowing to use new fluorochromes and develop new techniques for the definition of discrete cells (Treg, NKT, dendritic cell subpopulations, minimal residual disease, platelets...) and microparticles.
– The Cell Search Veridex allowing to detect and quantify up to 10-7 cells in a quasi automated fashion. This equipment has been validated as a very powerful tool to detect circulating tumoral epithelial cells (CTC) in metastatic breast, prostate, colorectal cancer and thus establish CTCs as a new prognostic biomarker for adjuvant therapy protocols. It can also quantify endothelial cells in inflammatory disorders and for follow-up of anti-angiogenic therapies. A preparative methodology opens new fields of cell isolation for fundamental research
– The AMNIS system combining flow cytometry and image analysis techniques with a powerful software allows a direct observation of cell markers in individual cells, at a membrane, cytoplasmic and nuclear levels and more efficient functional analysis of cells and even cell interactions.

These equipments will be accessible in a mutualized fashion for the developments of clinical research projects according to good practice procedures of biological and clinical research from various medical structures of the interregional area.

KRAS and *EGFR* mutation in non small cell lung carcinoma patients confer resistance to first line platin based chemotherapy

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Scientific background: Lung cancer remains the most common cause of cancer death worldwide. Upon metastatic disease the use of platinum-based combination chemotherapeutics offer modest efficacy. Therefore, it is important and necessary to identify patients who are highly sensitive for particular anticancer agents and who thus demonstrate a good prognosis.

Somatically acquired mutations in the epidermal growth factor receptor gene (*EGFR*) in NSCLC have been reported to be associated with a significant clinical response to tyrosine kinase inhibitors that targets the EGFR. In contrast some mutations of an EGFR downstream target, *KRAS* may render tumor cells independent of EGFR signaling and thereby resistant to EGFR tyrosine kinase therapy. As both *EGFR* and *KRAS* mutation resume in a constitution activation of the either MAP Kinase pathway and mTor/AKT pathway. Such activation could lead in vitro to resistant to platin derivated, although only few data analysis the impact of K-ras mutation on the efficacy of a first line chemotherapy. So we decide to prospectively determine the response and the progression free survival to first line chemotherapy in metastatic non small cell lung cancer patient upon *KRAS* and *EGFR* genotype.

Results: Eighteen had *EGFR* and/or *KRAS* mutations, 6 *EGFR* mutation and 12 *KRAS* mutation. Response rates were 42% for wild-type and 2% for mutant KRAS (P = 0.022). Significant progression free survival were observed after front line chemotherapy in the wild type group: Median PFS: 7.4 months (95% CI, 5.3–9.4) versus 4.3 months (95% CI, 3.6–5) (p=0.02). *In vitro*, adenocarcinoma cell line with *EGFR* or *KRAS* mutation present a higher level of resistance to cisplatin that could be abrogated by erlotinib and sorafenib concomitant treatment.

Conclusion: *EGFR* and *KRAS* mutation confer resistant to platin based chemotherapy in non small cell lung adenocarcinoma. Interestingly, this resistance could be abrogated by erlotinib and sorafenib *in vitro*. These data give a strong new insight for the design of new clinical trial using *EGFR* and *KRAS* genotyping.

Interest of supramolecular complexes for busulfan-based chemotherapy: a preliminary study

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Scientific Background: Busulfan belongs to a subclass of alkylating agents known as alkyl sulfonates. Its main uses are in bone marrow transplantation, especially in Chronic Myelogenous Leukemia (CML), where it is used as a conditioning drug. Busulfan can control tumour burden but cannot prevent transformation or correct cytogenic abnormalities. Though not as common, it may also be used for Chronic Lymphocytic Leukemia (CLL). Regrettably, its use is also associated with an important cytotoxic response which includes interstitial pulmonary fibrosis, hyperpigmentation, seizures, hepatic (veno-occlusive disease) and wasting syndrome...That is why, in spite of the fact that Busulfan was for a very long time the standard of treatment for CML, it is now replaced by the new gold standard, Imatinib.

Our objectives are to evaluate the complexation of busulfan with a cyclodextrin-based molecular receptor (i.e. Bis-β-Cyclodextrinyl-bis-ureido-diazacrown pseudo-cryptand) in order to (i) increase water solubility (x100), (ii) the biodisponibility, and (ii) decrease the cytotoxicity of the drug.

Material and Methods: First, viability as well as cytotoxic impacts of our complex [Bis-β-Cyclodextrinyl bis-ureido-diazacrown pseudo-cryptand/Busulfan] were determined by performing MTT and Neutral Red assays, respectively, on MRC-5 cells (ATCC CCL-171, human pulmonary embryonic fibroblasts).

Second, the anti-cancerous activity of the complex was evaluated on both K-562 (ATCC CCL-243, lymphoblast/bone marrow/chronic myelogenous leukaemia) and K-562 Doxo^R cell lines. Finally, the Maximal Tolerate Dose (MTD) was evaluated by testing two different concentrations of the vector (i.e. 5 and 7 g/L), on two groups (n = 5, each) of Sprague-Dawley rats (single injection of 500 µL at D0; observance: 30 days).

Results: First, we demonstrated that our molecular receptor (i.e. Bis-β-Cyclodextrinyl bis-ureido-diazacrown pseudo-cryptand) has no impact both in terms of viability and cytotoxicity on MRC-5 cells. Moreover, complexation of busulfan with our molecular receptor permits us to diminish from about 50% the cytotoxicity compared to the tested drug (i.e. busulfan) alone.

Second, we observed a conserved anti-oncogenic activity of our molecular receptor, on both cancerous cell lines (i.e. K-562 and K-562 Doxo^R) comparable with that of busulfan alone.

Finally, when we tried to evaluate the toxicity of our molecular receptor *in vivo* (single injection in rats, see Materials & Methods above); we clearly did not observe any toxicity of our molecular receptor, with no induction of any lethality during the 30 days of the experiment.

Conclusion: As a preliminary study, our results were very encouraging and prompt us to go ahead, and for the near future we have in project the realization of a pharmacokinetic/pharmacodynamic (PK/PD) study on the animal with our complex (collaboration with R. Gref, UMR 8612 CNRS-Paris 11 (Dir P. Couvreur) & A. Paci, UPRES EA 3535 IGR-Paris 11).

Toward the understanding of the H/ACA RNP domain of human telomerase and human dyskeratosis pathology

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Telomerase is a ribonucleoprotein complex (RNP) with reverse transcriptase activity which is responsible for *de novo* synthesis and maintenance of chromosomal termini, i.e. telomeres. Its activity is crucial since loss of telomeres leads to end-to-end chromosomal fusions, facilitates genetic recombination and triggers cell death through apoptosis. In addition, telomerase is highly active in tumoral cells. Its activation in tumoral cells is largely responsible for cell immortality. Telomerase is therefore used as a biomarker of carcinogenesis. This RNP enzyme contains an RNA component TR (Telomerase RNA) and a catalytic protein TERT (Telomerase Reverse Transcriptase). In vertebrates, the telomere template used for DNA synthesis, is present within the 5′ half of TR and the 3′ half forms an H/ACA-like domain essential for telomerase stability. This 3′ domain is structurally similar to domains contained in small nucleolar RNAs (snoRNAs), which act as guides for the conversion of uridines into pseudouridines (Ψ) in ribosomal RNAs. A common set of proteins named NOP10, NHP2, GAR1, and dyskerin, a pseudouridine synthase, are assembled on snoRNA and TR H/ACA motifs to form H/ACA RNP domains. Numerous mutations in TR, TERT, and in dyskerin, NOP10, or NHP2 lead to human Dyskeratosis Congenita (DC), a clinically and genetically heterogeneous, rare and fatal bone marrow failure syndrome. Mutations result in a lower stability of TR and shorter telomeres. Hence, telomeres shortening as well as snoRNP deficiency likely account for the pathology.

Homologues of H/ACA snoRNPs exist in archaeal species. Whereas no *in vitro* assembly system exists for eukaryal H/ACA RNP domains, we developed conditions for *in vitro* reconstitution of active H/ACA RNPs from the purified recombinant core proteins and *in vitro* transcribed RNA. This allowed us and others to progress in understanding the role of each H/ACA RNP protein for H/ACA RNP structure and activity. We also participated to determination of the 3D structures of some of the H/ACA sRNP components, and recently the 3D structure of a fully assembled RNP was determined by another group. Altogether the data brought important insight on the architecture of the human telomerase H/ACA domain. We also studied the impact of the NOP10 mutation R34W identified in some DC patients on the assembly and activity of archaeal H/ACA RNPs. Our data indicate that it affects RNP structure but not Ψ-synthase activity. These results strongly suggest that the DC symptoms are due to an unusual and unstable structure of the H/ACA domains of H/ACA RNPs in particular telomerase.

Study of the expression of cytokines and growth factors in a murin model of tissular radio-induced degeneration

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Background: The failure of angiogenesis plays a central role in radiation-induced tissue degeneration, especially for the genesis of late effects and can be explained by two interdependent mechanisms: hypocellularity and unfavourable microenvironment. The objective of this study was to quantify tissular

changes (skin and muscle) in the key mediators involved, after irradiation, in order to determine their respective role in the failure of angiogenesis and tissue degeneration.

Material and methods: We used a rat model of unilateral irradiation of hindlimb, with sequential sacrifices after 6 weeks, 6 months and one year. The expression of selected key mediators (IL-1α, IL-2, IL-6, IL-10, IFN-γ, TNF-α, VEGF, GM-CSF, TGF-β1) was measured by immunoanalysis and Western Blot in samples taken at sacrifice of the animal. Values were compared to macroscopic and histological observations.

Results: Macroscopic and histological changes were very similar to those observed after radiotherapy for human. Lesions were more important in skin than in muscle. Expression of GM-CSF was increased. A proangiogenic signal (VEGF) was found especially in skin, and particularly at 6 weeks. Irradiated tissues displayed chronic inflammation with significant increase in expression of IL-2, IL-1α and TNF-α. IL-10 and TGF-β1 were found to be correlated with the fibrosis scoring: IL-6 and IFN-γ showed no major variation.

Conclusion: This study led to the establishment of a reliable model of tissular degeneration. Pro-angiogenic signals were evidenced, but less intensively than expected. VEGF did not appear to have a substantial impact on fibrosis. Irradiated tissues displayed chronic inflammation, but its implication in late effects was found to be limited, which is in agreement with usual clinical observations in humans. On the contrary, IL-10 and TGF-β1 seemed to play a key role in the genesis of fibrosis. IL-10 exerted a protective role that seemed independent of the control of the inflammatory reaction. The high levels in GM-CSF expression validates the theoretical interest of tissular rehabilitation by stem cells. This model offers the opportunity to study molecular mechanisms of rehabilitation techniques, and their effectiveness.

Optimization of a new non viral vector for DNA transfection

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The development of new vectors, allowing the delivery of DNA into cells, has received major interest for many years to treat cancers or genetic illnesses. However, the clinical application of such vectors requires the development of efficient, steady and sterile vectors enabling the transfer of gene *in vivo*. Non viral, polymer or lipid based vectors offer a new impetus to gene therapy because they are less toxic than viral vectors (no endogenous recombination, no immunological reactions, delivery of large plasmid size and easy production).

The aim of this study is to develop a new "vector tool for DNA delivery" made of methacrylic polymeric nanoparticles (Eudragit® RS and RL). These nanoparticles were prepared by nanoprecipitation method then fully characterized. Nanoparticles without DNA have a size of about 50 nm and a zeta potential of +50 to +60 mV with Eudragit® RS and RL, respectively. After mixing nanoparticles and DNA during 30 min at room temperature, we observed that particle size increased and zeta potential decreased confirming the adsorption of the DNA. We have also estimated the adsorption yield of DNA onto nanoparticles by gel retardation assay (semi quantitative method). We mixed together 4 µg DNA and different quantities of nanoparticles. We obtained a total DNA adsorption when using the ratio 4 µg DNA / 500 µg of nanoparticles.

Cytotoxicity assays based on mitochondrial activity (MTT Test) revealed that the nanoparticles (without DNA) were determined on different cellular types and depends on nanoparticles concentration. After optimisation of nanoparticle manufacturing, the first tests of transfection have been performed on cells with plasmid coding for the Green Fluorescence Protein (GFP). The transfection was evaluated by flow cytometry, measuring the quantity of fluorescent cells. Preliminary results showed interesting transfection rates of the protein (6-7% of transfection, similar of the lipofectamine).

However these vectors, aimed to be used for the transfection of tumor suppressing genes (PTEN) need to be optimized. The development of such vectors could offer encouraging perspectives in therapeutic innovation against cancer, allowing us to foresee stable forms for efficient and direct treatment of patients.

Mass spectrometry molecular imaging technology to help classify ovary cancer

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Ovarian cancer concern more often women older than 50 years old, and patients younger than 35 years old with personal or familiarly breast, colon or ovary cancers or that have been known to have mutation in BRCA2 genes. The severity of the disease is principally due to the extension of the tumor at the time of the diagnosis. Ovarian cancer is responsible of more death than all other gynaecological cancers. It is non only the 4th world cause of woman death in developed countries but it is responsible of 6,1% of all woman death by cancer. The rate of this cancer is in France 11,6 for 100000 per year and at the world scale is 5,4 for 100000.

Mass spectrometry (MS) is now a major tool in protein analyses

from the post-translational modification characterization until the biomarkers hunting in biological fluids. Recently, MS studies have demonstrated that this technique can also be used in molecular imaging.

The project is base on the use of different approaches in order to find correlations between plasmatic markers and/or histological and *MS* imaging. The results obtained in previous Inca innovation project demonstrate the contribution of mass spectrometry (MALDI-TOF) technology, for the molecular imaging, to find news intra tumours specifics biomarkers specific of some ovarian cancers. The objectives of this project are 1) in collaboration with physicians and pathologists, the validation of these news biomarkers in terms of diagnosis, prognosis and predictive responsiveness to therapy and 2) how the news data generated by these technologies, added to the classical approached could be assist the clinician to give a more specific diagnosis and/or prognostic information.

Part of the project is on the statistical tools analyses for the treatment and the data analyses obtained by MS molecular imaging in order to defined the modality of management, treatment and data analyses allowing a comparative study between the spectra obtained on the same tissue slices and on the whole of the slice.

Biomarkers found, are fragments of proteins such as alpha Reg. Information on the presence or the kinetics of appearance of these biomarkers in tumors and plasma, will be associated with imaging and clinical data to validate their interests to support the diagnosis and monitoring of the patient.

Identification of resistance biomarkers to neo-adjuvant therapies in patients with early breast cancer

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One of the approaches for treatment of extensive breast cancer is pre-operative (neo-adjuvant) therapy, which is currently applied to about 10% of patients with early breast cancer. This approach 1) reduces the requirement for extensive surgery (mastectomy), 2) is currently as efficient as post-surgery (adjuvant) therapy, 3) facilitates identification of resistance and surrogate markers for long-term outcome and 4) could facilitate treatment tailoring to individual patients in the next future. The main objective is to determine whether a peptide signature, identified by MALDI-TOF mass spectrometry, in the serum or plasma of these patients could be predictive of partial or complete resistance (defined on pathological criteria) to neo-adjuvant therapies including either an anthracycline or a taxane.

Today, a biological collection composed of 204 blood samples (2040 aliquots) from 70 patients is available, taking blood in kinetic before and after each chemotherapy cycle (1 injection every three weeks. This blood collection is completed with Clinical data (TEP imaging, conventional markers, histological response of each patient after six chemotherapy cycles, and for some patients frozen tumors and ARN Array studies). For the discovery phase we will use the samples of 50 patients and one other set of samples of 70 patients will be used for validation phase. To increase the number of patients included in this study some others cancer centers will participate at the recruitment.

In parallel, the team statistician CLIPP developing new statistical models to take into account the technical, biological and clinical variability but also the link between samples from the same patients (longitudinal analysis).

We know today that the detection of new biomarkers using only the magnetic bead chromatography technology is not possible by a lack of sensitivity. For this, we have set up in routine a procedure of depletion in order to remove 99% of the 20 protein majority (fplc Sigma column). This process generates 3 fractions by blood sample, the peptides in each of these fractions are purified using three types of magnetic beads chromatography (RPC 18, WCX, SAX), then analyzed by MALDI-MS/MS analyze. Today the blood samples of 30 patients are analyzed and the statistical analyze is in current.

Oncogenic role of neuropilin-2: development of a new therapeutic strategy to promote p53 expression and tumor cell apoptosis

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Neuropilin-2 (NRP-2) is multifunctional glycoprotein originally described in the nervous system, capable of mediating axon retraction and guidance by binding class III semaphorins. Interestingly, NRP-2 was shown to support an angiogenic activity by binding several growth factors including VEGF and by interacting with the family of VEGF tyrosine kinase receptors. In the current study, we investigate the role of NRP2 in colorectal cancer progression. We produced colon cancer cell lines transfected with NRP2 transgene or siRNA to confirm the role of NRP2 in cancer oncogenesis. MTT test and cell cycle analysis revealed that NRP2 promotes tumor proliferation in vitro. In vivo, NRP2 inhibition using specific siRNA prevented xenograft formation in all mice, whereas NRP2 transfection enhances tumor growth.

The ability of NRP2 to orchestrate Epithelial to Mesenchymatal Transition was investigated by

immunohistochemical analyses. Expression of NRP2 was correlated with loss of epithelial markers such as cytokeratin-20 and E-cadherin and with acquisition of mesenchymal molecules such as vimentin. Furthermore, we showed that NRP2 can positively regulate TGF-B1 pathway, by enhancing phosphorylation of Smad2/3. Moreover, expression of p53 and NRP2 were found to be inversely correlated. Transfection of NRP2 led to a reduced p53 expression whereas downregulation of NRP2 by siRNA enhanced p53 expression.

The oncogenic properties of NRP2 and its direct involvement in EMT prompted us to generate therapeutic monoclonal antibodies targeting this molecule. We developed a monoclonal antibody (ITAC-B1) capable to reverse the oncogenic phenotype conferred by NRP2 and demonstrated that NRP2 inhibition restores p53 expression and synergizes with conventional chemotherapy to induce apoptosis and to prevent tumor growth in vivo.

Altered apoptotic profiles as intrinsic marker for radiation-induced sequelae in patients overexposed for a prostate adenocarcinoma (cohorte Epopa)

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Background: Low percentage of CD4 and CD8 lymphocyte radiation-induced apoptosis has been shown to be correlated with high grade of sequelae. In addition, recent data suggest that patients with severe radiation-induced late side effects possess low radiation-induced CD8 lymphocyte apoptosis in vitro. Between 2000 and 2006, 433 patients were overexposed (8% to 10%) during a course of conformal radiotherapy for a prostate adenocarcinoma in Jean Monnet hospital (CJHM), Epinal, France due to an inappropriate use of the treatment planning system. This study is performed within a national research program proposed to increase the scientific knowledge on iatrogenic effects related to overexposure of ionizing radiation, by studying their relationship with dosimetric, clinical, biologic and genetic characteristics. The aim of this study is to determine whether radiation-induced apoptosis rate in CD4 and CD8 lyphocytes could be a marker for intrinsic hypersensitivity to radiation.

Patients and Methods: This study was initiated in October 2008. About 400 patients should be enrolled. The inclusion criteria are established as follows: consecutive patients treated for a prostate adenocarcinoma in the radiation department of the CJHM between 2000 and 2006. Fresh heparinized blood samples were irradiated (8 Gy monodose, 3 Gy/min). Following irradiation, the PBMC were collected and prepared for flow cytometric analysis and cell sorting. DNA was stained with propidium iodide. Following the separation of CD4 and CD8 lymphocyte types, radiation-induced cell death was quantified through cell size reduction and DNA-fragmentation.

Results and conclusion: We expect to correlate the received doses, the volume of irradiated normal tissues, the events, with biologic, phenotypic and genetic data. The primary study endpoint is to evaluate incidence and severity of adverse events related to radiotherapy (according to SOMA-LENT and CTCAE scales). The secondary endpoint is the evaluation of T-lymphocyte radiation-induced apoptosis to predict individual intrinsic hypersensitivity due radiation due to genetic predisposition.

Study on human telomerase assembly

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Telomerase is a ribonucleoprotein (RNP) enzyme responsible for synthesis of repeated sequences located at the extremities of chromosomes. These repeated sequences are required for chromosome stability and cell viability. The telomerase RNP contains the hTR RNA, and several proteins, including the reverse transcriptase hTERT, hTR RNA contains two domains. The 5' domain forms a pseudoknot structure and binds hTERT. The H/ACA 3' domain is required for stability. H/ACA domains are also found in H/ACA RNPs that guide and catalyze RNA pseudouridylations. Like these guide RNA, the hTR H/ACA domain associates with 4 proteins (Dyskerine, GAR1, NOP10 and NHP2 proteins). Assembly of telomerase is highly relevant for carcinogenesis, since activation of telomerase is one major event leading to cell immortality. However, only limited information is available on the mechanisms of assembly of telomerase. Like H/ACA snoRNP assembly, it seems to be a multistep-ordered process. A pre-complex containing Dyskerine, NOP10, NHP2 and an assembly factor, called NAF1, is likely formed in the cytoplasm. This complex is transported to the nucleus, where it is associated with the H/ACA domain of nascent RNAs. Exchange of NAF1 for GAR1 allows the production of functional H/ACA RNP domains. In collaboration with the E Bertrand (Montpellier), we showed that several factors are implicated in H/ACA RNP assembly: NUFIP, the R2TP complex, and HSP90. Another protein complex may be involved in H/ACA RNP assembly, the Survival of Motoneurons complex (SMN). This complex which contains the SMN, Gemin2 to Gemin8 and Unrip proteins, was already shown to play a crucial for spliceosomal UsnRNP assembly and the SMN protein can interact with the GAR1 protein of H/ACA RNPs.

For further study on the role of the SMN complex in H/ACA RNP assembly, we tested by yeast two hybrid assays whether other pro-

teins of the SMN complex may interact with H/ACA RNP proteins and with the assembly factors mentioned above. By this approach, we discovered several possible interactions, especially, between NAF1, Gemin 3 and Gemin8. These data suggests that the SMN complex is involved in the replacement of NAF1 by GAR1 during the assembly of H/ACA RNP domain. One may imagine that this complex associates with GAR1, through interactions mediated by SMN and GAR1 and with the pre- H/ACA RNP complex, through interactions between NAF1 and Gemin3 and 8, allowing the replacement of NAF1 by GAR1. We are testing this hypothesis by biochemical and cellular experiments. The SMN complex may also be involved in hTERT binding to the 5' domain of hTR and we will also explore this possibility.

Direct or indirect anti-angiogenic effects of cetuximab in human endothelial cell cultures

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Background: Cetuximab, a recombinant chimeric antibody targeting the extra-cellular domain of EGFR (Epidermal Growth Factor Receptor) overexpressed by tumor cells, is indicated in the treatment of locally and regionally advanced head and neck squamous cell carcinoma (HNSCC) in combination with radiation, and as monotherapy for recurrent and metastatic HNSCC. The anti-tumoral effects of cetuximab are known to be due to an interaction with direct tumor cellular growth (proliferation, migration and apoptosis) and to an anti-angiogenic effect attributed to the inhibition of VEGF secretion by tumor cells. In addition, it has been reported that not only tumor cells but tumor endothelial cells (ECs) expressed EGFR and are responsive to EGF. Indeed, cetuximab could act directly on tumor endothelial cells. Netherless, mechanisms of EGFR overexpression by tumor ECs are still unknown.

Direct (via EGFR) or indirect (via VEGF secretion) targeting of tumor blood vessel with anti-EGFR may be a viable strategy for tumor growth inhibition. In this study, direct or indirect anti-angiogenic effects of cetuximab were investigated in ECs culture.

Methods: Direct effects of cetuximab: Human Umbilical Vein ECs (HUVECs) were allowed to grow in presence of cetuximab (2 – 400 µg/ml) in the culture medium.

Indirect effect of cetuximab: Conditioned media (CM) were collected from culture of HNSCC cell line (CAL27 or FaDu) after 48 hours with or without exposure to cetuximab and EGF (10 ng/ml). Then, HUVEC were allowed to grow in these CMs.

Metabolic activity and proliferation of HUVECs were analyzed using MTT and Hoechst assays respectively.

In vitro angiogenesis assay: HUVEC were seeded and cultured in CM during 5 hours in Matrigel®-coated wells. Cells were then fixed in paraformaldehyde. Capillary structures were analyzed after phalloïdin-Texas Red staining. The global expression of two markers (VEGFR-2 and Notch-4) was analyzed by immunofluorescence.

Results: Direct exposure of HUVEC to low concentrations of cetuximab (2 to 20 µg/mL) had no effect on proliferation, while inducing a significant decrease in tumor cells proliferation. Above 25 µg/mL cetuximab, induced a slight (approx. 20%) decrease in ECs proliferation. CM collected from culture of FaDu or Cal27 tumor cells in presence of cetuximab and EGF influenced HUVECs metabolic activity, HUVECs expression of angiogenesis markers (VEGFR-2, Notch-4) and formation of capillary structures.

Conclusion: Cetuximab had not direct antiangiogenic effects on non-tumor ECs. Tumor cells treatment by EGF and/or cetuximab modified the angiogenic signaling agents secreted into the CM. This effect induced changes in HUVECs behavior and modifications of capillary structures.

A genomic and transcriptional analysis of HPV-infected oropharyngeal head and neck squamous cell carcinoma

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Scientific background: The infection by High Risk Human Papillomavirus (HR-HPV) is linked to the onset and development of about 25% of head and neck squamous cell carcinoma (HNSCC) and defines a distinct clinical subpopulation, whose molecular features are loosely defined.

Material and Methods: In order to get more insights in the molecular bases of HPV-driven head and neck carcinogenesis, we have performed a statistical as well as a transcriptomic and genomic analysis of infected and non-infected HNSCC by using an Affymetrix GeneChip® and Comparative Genomic Hybridation (CGH) approach. Results from the microarray experiments were validated *in vivo* by quantitative real-time RT-PCR.

Reults: A supervised differential gene expression analysis using SAM approach resulted in a total of 645 up- and down-regulated genes in HPV-positive samples. We performed an unsupervised Statistical Analysis of Microarray (SAM) comparing 30 HPV-negative to 8 HPV-positive lesions from the oropharynx, 5/8 infected HNSCC were recovered in the same tumor cluster, suggesting that HPV-driven carcinogenesis correlates with a specific deregulated gene expression pattern. The analysis of the CGH data

showed that HPV-positive oropharyngeal lesions did not display the genomic aberrations that are classically found in oral lesions (loss of the 3p and 9p regions, amplification of the 11q13 locus). However, the loss of the 16q chromosomic regions was found to be a HPV-specific genetic event. Strikingly, genes clustered in the 16q22-24 locus displayed a decreased expression in HPV-infected HNSCC. Among them, the locus encoding the Amyloid Beta Precursor Protein Binding Protein 1 (APPBP1) showed a low mRNA expression specifically in HPV RNA+ HNSCC. APPBP1 is the regulatory subunit of the Uba3-APPBP1 complex, which is an E1 Ubiquitine-like conjugating enzyme involved in p53 transcriptional activity inhibition via p53 NEDDylation.

Conclusions: Loss of the 16q22-24 region was the major genetic event we observed in HPV-infected HNSCC. The loss of the *APPBP1* gene, located in 16q22, would result in a higher p53 activity and participate to the increased radiosensitivity of HPV-positive tumours. APPBP1 is therefore an interesting candidate to predict the tumor response to radiation therapy.

Low Foxp3 cells infiltrate is a marker of pathological complete response to neoadjuvant chemotherapy in breast carcinoma

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Background: T cell infiltration is associated with tumor prognosis in many cancer types. To assess the capacity of neoadjuvant chemotherapy to impact on T cell infiltration in breast cancer, we investigated if pathological complete response (pCR) to neoadjuvant chemotherapy correlated with T cell infiltration.

Methods: CD3, CD8 and Foxp3 positive cells infiltrate were detected by immunohistochemistry in a series of 56 breast cancer patients treated by neoadjuvant chemotherapy between June 2003 and May 2005. Infiltrates were analysed before and after the end of the treatment. Cytotoxic cells were stained using anti-Granzyme b and TiA1 antibodies.

Results: Poor prognosis factors (negative hormonal receptors, high tumor grade and nodal involvement) correlated with a significant higher number of CD3, CD8 and Foxp3 cells infiltrates before chemotherapy. Chemotherapy only induced a decrease in the number of Foxp3 cells ($p < 0.01$) while the level of CD8 and CD3 cells were maintained ($p = 0.8$). pCR were observed in 12 patients. These patients had a drastic decrease of Foxp3 positive cells (mean: 1.6 ± 1.4 versus 0.08 ± 0.2; $p < 0.001$) while these cells remained elevated in non responders (mean: 1.5±1 versus 1 ± 1 $p = 0.2$). Furthermore, using a cutoff criterion that combined high CD8 infiltration and no Foxp3 cell infiltration on surgical specimens we could predict pCR with a sensitivity of 75% and a specificity of 93%. Cytotoxic effector cells infiltrate was only enhanced in pCR. In multivariate analysis, only high tumor grade and low Foxp3 cell infiltration on final histological specimens were independent predictors of a pCR (HR (95% CI): 17,7394 (1,1 to 272,5) $p = 0.04$ and 0,0132 (0,0002 to 0,9) $p = 0.04$ respectively). The Kaplan Meier estimate of metastasis-free survival indicated a trend toward higher percentage of metastases-free patients in pCR and combined immunological criterion (Log rank test, p=0.1 and $p = 0.12$ respectively).

Conclusion: These findings indicate that pathological complete response to neoadjuvant chemotherapy is associated with an immunological profile consisting in absence of immunosuppressive Foxp3 cells, presence of high number of CD8 T cells, and an increased number of cytotoxic cells. These results argue for the induction of an antitumor immune response by chemotherapy in this group of patients.

Intratumoral localization kinetics of Foslip® using two photon confocal microscopy: corelation with PDT efficiency

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Background: Photodynamic therapy (PDT) requires a combination of photosensitizer (PS), light and oxygen to produce cytotoxic species. The tumor vasculature and neoplastic cells in the parenchyma are two potential targets of PDT. The distribution of PS in different compartments can be adjusted by changing the interval between the administration of drugs and irradiation (DLI). The efficiency of PDT is closely correlated with the distribution of PS in each compartment at the time of treatment.

Objective: The objective of this work is to determine the Foslip® localization kinetics within the tumor then correlate this localization to the treatment efficacy.

Method: The model used was a xenografted nude mice (EMT6 cells). The PS intra-tumor localization has been achieved by two-photon laser scanning confocal microscope (TPLSCM). The evaluation of the efficiency of PDT was performed by measuring tumor regrowth after treatment.

Results: TPLSCM enables the ex-vivo localization of PS in the tumor. A localization kinetic was made from 15 min to 24 h after injection. For short intervals of 15 minutes and 1 hour, the photosensitiser was strictly confined to the vessels. For 3 hours DLI, the drug fluorescence starts to diffuse into the nearby tissues. mTHPC fluorescence distribution pattern at six hours after administration was characterized by its localization in tumour and endothelial cells. For 15 and 24 h post injec-

tion, the fluorescence arises from the tumour cells and is most pronounced at a remote distance from the vessels. The best PDT efficiency was obtained for DLI of 6 hours, time for which the localization of PS is clearly seen in tumor and endothelial cells. This highlights the destruction of tumors by Foslip®-PDT by a combination of direct effect on cancer cells and by a vascular effect.

Conclusion: These results demonstrates the significance of the spatial and temporal intratumor distribution of PDT so that better informed clinical protocols can be initiated. The two photon confocal fluorescence imaging technique used here provides an excellent tool to study the intratumor distribution of not only other PS but also of chemotherapy agents, which share common physiological barriers in their transport from the vessels through the extravascular space to reach the tumor cells.

Simulation of lung motions by morphing for external radiation treatments

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Introduction: The morphing is a process of image transformation. The goal of the study is to prove the feasibility of this original solution of the lung motion simulation. The aim of the implementation of this process is to reduce significantly the patient's dose in treatments by radiation from diagnosis to therapy.

Materials and methods: For one patient, two images issue to a 4D-CT was taken into account: the end of the expiration (considered as our source image) and the end of the inspiration (target image). A threshold was applied on the images that were then segmented. Several algorithms had been elaborated in two and three dimensions. The general idea is to control the deformation of the lungs and to compare each intermediate image with the final one. Currently, all the algorithms were used using only one patient's data. Other data will be available soon through a partnership with the University Hospital of Besançon. Four morphing algorithms have been designed, implemented and evaluated.

Results and conclusion: To quantify the results, a comparison between morphing images and 4D-CT images has been made. Whatever the algorithm used is, the result is interesting: indeed, we have a maximum of 11% difference between lung surfaces. In future prospects, a best management of kinetic will be implemented in order to have a deformation closer to the real motions.

The alpha5beta1 integrin is implicated in human glioblastoma tumorigenicity and chemoresistance

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Scientific Background: Of solid tumors, glioblastoma remains the most resistant to therapy and new therapeutic approaches are needed. The potential role of $\alpha5\beta1$ integrins in cancer has recently attracted much interest. Its over-expression in tumoral neovessels and in glioblastoma cells makes it a potential interesting therapeutic target. We investigated its role in glioblastoma carcinogenesis and chemoresistance.

Methods: The $\alpha5$ integrin subunit was stably overexpressed or knocked down in the U87MG glioblastoma cell line. Effects of the genetic manipulations were analysed on cell cycle, proliferation, clonogenicity, protein phosphorylation, in basal conditions as well as in the presence of chemotherapeutic agents. Ellipticine (a DNA intercalating agent and topoisomerase II inhibitor), which demonstrated specificity towards brain tumor cell lines, as well as Temozolomide (currently used in the clinic) were investigated as chemotherapeutic agents.

Results: U87MG cells depleted in the $\alpha5$ subunit were less aggressive than control cells; they arrested in the G0/G1 phase of the cell cycle and presented the characteristics of senescent cells (as shown by acidic β-galactosidase staining). Accordingly they were less able to develop colonies. In basal conditions, they showed a decrease in AKT and p42/44 MAPK phosphorylation. Conversely, the forced expression of the $\alpha5$ subunit shifted the cells towards a more aggressive phenotype as shown by proliferation and clonogenicity assays. Ellipticine and Temozolomide both triggered premature senescence in U87MG control cells by activation of the p53/p21 pathways. The reduction of $\alpha5$ level increased the cell chemo-sensitivity towards the drugs. In these cells, a higher level of ser15-phosphorylated p53 (active p53) and p21 mRNA was noted after Temozolomide treatment leading to an increase in senescent cells as compared to treated U87MG control cells. Inversely, the forced expression of the $\alpha5$ subunit rendered the cells less sensitive to the drugs through an inhibition of the p53 pathway activation.

Conclusions: Our results highlight a new role of the $\alpha5\beta1$ integrin in the control of glioblastoma aggressiveness. The human glioblastoma responsiveness to chemotherapy may be dependent on the $\alpha5\beta1$ integrin expression level. Our data may have a crucial impact in the clinical management of brain tumours.

In vivo bimodal spectroscopic study of human skin types classification

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Background/aims: Autofluorescence and Elastic Scattering Spectroscopic methods, applied yet to *in vivo* method for characterization of living tissue, are of great interest in non-invasive cancer diagnosis. In this study, we created a method for human skin classification, based on bimodal spectroscopic system developed by our research team and data processing. In addition, ANN (Artificial Neural Network) and LDA (Linear Discriminant Analysis) methods are respectively implemented for the data classification, so as to compare their efficiency.

Method & Material: The spatially resolved autofluorescence and elastic scattering spectra were obtained *in vivo* at 180 sites of normal skin from 9 healthy volunteers, using excitation wavelengths at 360, 368, 390, 400, 410, 420 and 430 nm. The multiple emission spectra were collected by an imaging spectrograph. A series of pre-processing, including background subtraction, filtering, power normalization and system response calibration, was performed on directly acquired spectra. Then, several spectral characteristics (peak intensity, slopes, etc) considered as useful for distinguishing between different skin types were calculated. Then, Wilcoxon Tests and correlation analysis were implemented for character selection according to their statistical significance and redundancy. Finally, ANN (Artificial Neural Network) and LDA (Linear Discriminant Analysis) methods were respectively applied to the data classification for comparison of their performance on this.

Results & Discussion: In accordance with Fitzpatrick Test, 5 persons in 9 were classified as skin type IV. This would be a standard for evaluating the precision of our classification methods. For spectra measured on one site at 6 fiber optical channels, total 120 characters had initially been found significant for differentiation of skin type between III and IV. After Wilcoxon Tests and correlation analysis, 34 characters were held for data classification. The most of these was relative to elastic scattering spectra. It seems to us very logical; because the skin type is mostly defined by melanin concentration which can be seen directly on the Diffuse Reflectance. The tow adopted classification methods didn't reveal much efficiency difference. The obtained result could both coincide perfectly with that by Fitzpatrick Test (ANN: correctness at about 99.56% for type III and 100% for type IV; LDA: 98.25% for type III and 100% for type IV). Nevertheless, it must be noted that among the volunteers of skin type III, one individual has also some skin characteristics belonging to skin type II. Even though the final marks of Fitzpatrick Test decided him as type III, spectra collected on this individual is still different from those of others in the same group. So, it would very likely interfere at learning step of our classification method, so that we had relatively less satisfactory classification result for type III.

Conclusion: Throughout our study, the bimodal spectroscopy, combined with suitable spectral processing method, was proved to be a useful and simple way to classify human skin non-invasively. Our result would also be of importance for, afterward, how to better diagnose cutaneous disease in people of different skin type with spectroscopy.

Assessment of Foscan®-photoinduced apoptosis by immunohistochemistry to caspase-3, active caspase-7 or cleaved PARP

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Scientific background: Photodynamic treatment (PDT) with Foscan® induced apoptosis through an intrinsic pathway involving caspase-3 and/or caspase-7 activation. Cleavage of Poly-ADP-ribose polymerase 1 (PARP-1) a major substrate of both caspases is therefore a valuable marker of apoptosis. The aim of the study was to discriminate caspase-dependent apoptotic pathways by applying immunohistochemistry to cleaved PARP (c-PARP), active caspase-3 or -7 on tissue section from preclinical models subjected to Foscan®-PDT.

Material and Methods: Antibodies to c-PARP, active caspase-3 or -7 were applied on deparaffinized sections from three monolayer cell lines, HT29, KB and MDA-MB231, from HT29 spheroids and tumors 24h after treatment with Foscan®-PDT. Control samples were submitted to Foscan® but not irradiated. Each antibody was then applied on frozen tissue sections (control or photosensitized tumor) for immunofluorescence analysis. The overlay of immunofluorescence images determined the co-localisation between active caspase-3 and c-PARP or active caspase-7.

Results: In control specimen, the antibody to c-PARP failed to detect apoptosis as efficiently as active caspase-3 or -7 immunostaining. Although c-PARP was found perfectly co-localized with active caspase-3, it was poorly expressed in many caspase-3 expressing cells. After treatment by Foscan®-PDT, a higher number of cells displayed large fluorescent spots from both caspase-3 and c-PARP labelling. In addition c-PARP yielded close percentage of labelled cells as compared to active caspase-3 and -7 labelling in photosensitized KB and HT29 monolayer cells or spheroids. These results suggest that the extend of PARP cleavage could be limited in case of spontaneous apoptosis whereas it could be dependent on treatment-induced apoptosis. However, a significant higher number of active caspase-3 labelled cells was registered in MDA-MB231 monolayer cells and in HT29 xenografts submitted to Foscan®-PDT. Co-localization analysis showed the absence of active caspase-7 labelling in some caspase-3 expressing cells suggesting that Foscan®-PDT-induced apoptosis was mainly processed through the activation of caspase-3 in HT29 tumor. Likewise, MDA-MB231 monolayer cells could be more susceptible to caspase-3 activation after Foscan®-PDT than HT29 or KB cell lines.

Conclusion: c-PARP could be an useful indicator of treatment-induced apoptosis. The different pattern of expression of active caspase-3 and -7 in Foscan® photosensitized tumors demonstrates the relevance of using antibodies which can discriminate caspase-dependent apoptotic pathways.

Discussion about new alternatives to Monte-Carlo calculations for clinical radiotherapy and radiation protection

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Monte Carlo codes (MC), precise but slow, are very important tools in the large majority of specialties connected to radiation physics, radiation protection and dosimetry. A reflection is led there about the opportunity of the other computer solutions, not only based on the unique allowing increasing computer power or on the "biasing" used for relative acceleration of these codes in the case of photons, but on more sensible methods (techniques of learning), already used successfully for a long time in other scientific or industrial applications often taken away from the radiation protection or from the medical dosimetry.

Two alternatives techniques has been carried out by IRMA/ENISYS/ FEMTO-ST: Artificical Neural Network Calculation (ANN) and Case Based Reasoning (CBR) .

The main goal of ANN is to combine some MC calculations (offline calculation) with an ANN code (NEURAD, developed by our crew) in order to drastically reduce time calculation without compromise regarding precision.

During a calibration phase (training phase), some MC calculation for homogeneous media are used in order to build our neural network. Within a second phase, clinical dosimetric calculations (for unknown heterogeneous media) can be performed by using the trained network. This approach permits us to obtain results in less that one minute (by using a low end PC) without exceeding an error of few percents, comparing with non-biased MC calculations (several days of calculation using a multi-processors workstation).

CBR is a learning tool which enables the resolution of problems. It is based on the retrieval of similar problems. By presenting several anatomical cases, we used CBR to enable a closer reconstruction of phantoms which will be used for radiation protection purposes.

Our current study involving CBR is about radiation protection but we are studying the use of that method to construct an helping tool for dosimetry.

All of these studies constitute a first step for improving radiotherapy and radiation protection calculations and demonstrate some alternative to MC-only calculation, very accurate but quite time consuming.

alpha5beta1 integrin expression level and p53 protein status both determine the chemotherapy outcome of human glioblastoma.

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Scientific background: Gliomas are the most abundant adult brain tumors nowadays treated with conventional therapies (surgery, radiotherapy and/or chemotherapy). These therapeutic strategies have demonstrated only modest survival benefits. Recent progress in the field of glioma molecular biology helped to identify new potential therapeutic targets. Among them, $\alpha5\beta1$ integrin has recently attracted interest as it appears overexpressed in Glioblastoma (GBM). We have shown recently that SJ749, a specific $\alpha5\beta1$ integrin antagonist, inhibits proliferation of GBM cell lines. We studied here the implication of this integrin in the chemotherapy answer of GBM in relation with the p53 protein status of the tumors.

Material and Methods: U87MG (p53WT) and U373 (non functional mutated p53) cell lines were treated with ellipticine (a DNA intercalating agent and topoisomerase II inhibitor). $\alpha5\beta1$ integrin was inhibited by SJ749. Apoptosis and senescence were quantified by FACS analysis/ PARP cleavage and acidic β galactosidase staining respectively. P53 activation was assessed by western blot with anti phosphor-ser15 p53 specific antibodies and by quantification of p53-targeted gene transcription. U373 cells were genetically manipulated to over-express the $\alpha5$ integrin subunit.

Results: Ellipticine mainly induced premature senescence in U87MG cells through activation of p53 pathway. In contrast, ellipticine triggered p53-independent apoptosis in U373 cells. Inhibition of $\alpha5\beta1$ integrin by SJ749 antagonist reversed the ellipticine-induced senescent phenotype in U87MG cells and led to cell apoptosis by inhibiting the p53 activation. In contrast, no increase in apoptosis after ellipticine and SJ749 cotreatment was detected in U373 cells. Intriguingly, we observed that overexpression of the $\alpha5$ subunit in U373 cells restored the cell capability to senesce either in absence or in presence of ellipticine and inhibited apoptosis. In these conditions, SJ749 was unable to cause apoptosis but rather increased the senescent cell population.

Conclusions: Our data reveal a crucial role of the $\alpha5\beta1$ integrin in the control of the balance between apoptosis and senescence after chemotherapy in glioblastoma. The impact of the integrin is clearly dependent of the p53 status of the tumour. Although p53 status has hardly been demonstrated as a predictor of chemotherapeutic answer in glioblastoma, concomitant screening of tumors for $\alpha5\beta1$ integrin and p53 status may be warranted in patients with brain cancer resistant to chemotherapy.

Caveolin-1 regulates glioblastoma aggressiveness through the control of $\alpha_5\beta_1$ integrin expression and modulates glioblastoma responsiveness to SJ749, an $\alpha_5\beta_1$ integrin antagonist

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Scientific background: Gliomas are the most common deadly brain tumors. Despite advances in neurosurgery, radiation and medical oncology, the prognosis for patient with glioblastoma (GBM) did not improve in the last 30 years. Caveolin-1 plays a checkpoint function in the regulation of processes often altered in cancer. Although increased expression of caveolin-1 is the norm in glioma, populations of caveolin-1 positive and negative cells coexist among GBM specimens. We studied the contribution of such cells to the phenotype of GBM by manipulating caveolin-1 in U87MG.

Material and Methods: Caveolin-1 was overexpressed or knocked down in U87MG and proliferation, clonogenicity and invasion were examined. PCR Arrays were undertaken to determine pathways altered after caveolin-1 manipulation. The involvement of $\alpha_5\beta_1$ and $\alpha_2\beta_1$ integrins was studied by overexpressing or knocking down α_5 or α_2. Sensitivity of cells to an $\alpha_5\beta_1$ integrin antagonist was determined using SJ749. Finally, the expression levels of both caveolin-1 and $\alpha_5\beta_1$ integrin were analyzed in 24 glioma patient samples and normal brain by qPCR.

Results: The reduction of caveolin-1 levels shifted cells towards a more aggressive phenotype as conversely the forced expression of caveolin-1 slowed down proliferation, clonogenicity and invasion. Using PCR array strategies, we identified integrins as the main set of genes affected by caveolin-1. Their expression was inversely correlated to caveolin-1. The phenotypic changes observed after caveolin-1 modulation were mediated specifically by $\alpha_5\beta_1$ integrins. As a consequence of the regulation of $\alpha_5\beta_1$ levels by caveolin-1, the sensitivity of cells to SJ749 was affected. p44/42 MAPK were identified as the regulator of $\alpha_5\beta_1$ integrin by caveolin-1. The inverse correlation between caveolin-1 and $\alpha_5\beta_1$ integrin was observed in human brain tumor biopsies of various grade.

Conclusions: Caveolin-1 regulates GBM cells aggressiveness. $\alpha_5\beta_1$ integrin was identified as the principal mediator of caveolin-1 effects. The status of caveolin-1/$\alpha_5\beta_1$ integrin might be a useful marker of the tumor behavior with caveolin-$1_{low}/\alpha_5\beta_{1high}$ levels indicating a highly proliferative and infiltrative tumor exerting sensitivity to SJ749. Conversely, caveolin-$1_{high}/\alpha_5\beta_{1low}$ levels could indicate a delay in tumor progression and reduced response to the drug. Our data open new aspects in the understanding of the caveolin-1/$\alpha_5\beta_1$ integrin partnership in fundamental cell biology and its contribution to glioma biology.

56-MESS metallointercalator: pharmacological evaluation of a new potentiel antitumor platinum complexe

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Scientific background: Platinum compounds are chemotherapeutic agents widely used in clinic. Their cytotoxicity is mainly due to DNA adduct formation triggering cell death. However, their therapeutic efficacy is restricted because of toxicity and acquired or intrinsic resistance. Recent elucidations on cell signaling and DNA-targeting allowed the synthesis of many compounds, including metallointercalators, active on resistant cell lines. A screening of several metallointercalators with substituted 1,10-phenanthroline highlighted the significant higher cytotoxicity of the [(5,6-dimethyl-1,10-phenanthroline)(1S,2S-diaminocyclohexane) platinum(II)] (56-MESS) complex in vitro. The aim of this study is to precisely assess the pharmacological potential of this compound.

Material and Methods: In vitro investigations were performed on following cell lines: HT29 (human colic cancer), IGROV1 (human ovarian cancer), PROb and CP33 (rat colic cancer respectively sensitive and resistant to cisplatin). 56-MESS cytotoxicity was evaluated versus cisplatin and oxaliplatin by clonogenic assay whereas cellular penetration of 56-MESS was determinated versus cisplatin by intracellular platinum quantification. In vivo investigations performed in BD-IX rat combined toxicological analyses and antitumor effect study.

Results: 56-MESS treatment induced a significant higher cytotoxic effect in all cell lines studied. Moreover, much higher and faster cellular accumulation of platinum occurred. Thus, the high cytotoxicity of 56-MESS resulted in part from its higher ability to accumulate into the cell, probably linked to the hydrophobicity of the phenanthroline ligand. In vivo, 56-MESS displayed tubulopathy with lymphocyte infiltrates and interstitial fibrous development. And, contrary to cisplatin, 56-MESS treatment did not cure BD-IX rats bearing peritoneal carcinomatosis, after both systemic and local administration, probably because of an inactivating metabolism or a too low accumulation in tumor nodules.

Conclusion: Also 56-MESS is cytotoxic in vitro, its in vivo behaviour suggests that 56-MESS is not an appropriate drug for later development. Thus, in vitro/in vivo correlation is not so obvious, and in vivo screening may be useful to select best compounds. Future investigations will examine the in vivo screening of metallointercalators which exhibited a cytotoxic effect at least equivalent to cisplatin in vitro. Identification of nucleotidic targets and cellular mechanisms involved in cytotoxicity also remain major objectives.

Alteration of PTEN could explain cetuximab resistance in head and neck squamous cell carcinoma (HNSCC): effect on angiogenesis

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Background: Anti-EGFR monoclonal antibodies and tyrosine kinase inhibitors have been shown to be very potent for treatment of many cancers. However, several resistance mechanisms to anti-EGFR targeted therapies has been reported among which the implication of PTEN phosphatase, main regulator of PI3K/AKT signalling pathway, has been found to correlate with resistance to cetuximab (Erbitux®) in colorectal cancer. Because PI3K/AKT signalling pathway is involved in angiogenesis control, we believe that a loss of PTEN expression can lead to an overproduction of vascular endothelial growth factor (VEGF) and contribute to cetuximab resistance.

Methods: Human CAL27 HNSCC cell line, sensitive to cetuximab, was transfected by PTEN-siRNA and exposed to cetuximab during the period of inhibition of PTEN expression ("siPTEN CAL27" cells). Cell proliferation and cytotoxicity were evaluated using MTT assays and cells counting. Western blot and Bioplex proteins array (BPA) were used to check PTEN and p-AKT expression and to evaluate the activation of signalling pathways. 48 hours after exposure to cetuximab, the culture media were collected to serve as conditioned medium (CM) for aortic ring culture. Aortic ring morphology (sprouting and vascular structures) was investigated by microscopy. VEGF expression was quantified in CM using BPA.

Results: In cells exposed to cetuximab, a significant increase in cell proliferation and metabolic activity, was observed in siPTEN-CAL27 and correlated with a significant increase in AKT phosphorylation. In the aortic ring cultures, changes in vascular morphology were observed with CM from siPTEN-CAL27 versus CM from control CAL27 after exposure to cetuximab. In CM collected from siPTEN-CAL27, an increase in VEGF expression was mesured as compared to CM from untransfected cells. Exposure of siPTEN-CAL27 to cetuximab had no effect on VEGF secretion as opposed to untransfected CAL27.

Conclusions: Exposure of siPTEN-CAL27 to Cetuximab did not decrease cell viability as observed in untransfected cells. Loss of PTEN expression was found to alter PI3K/AKT signalling, to induce an over-secretion of VEGF and to lead to changes in aortic ring morphology. This phenotype could explain the resistance to cetuximab in PTEN-null tumors.

Classification of prostate magnetic resonance spectra

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Diagnosis of Prostate cancer (PCa) usually requires histology of samples obtained invasively during transrectal ultrasound-guided biopsy. With the arrival of high field MR, new imaging methods have been developed such as Magnetic Resonance Spectroscopic Imaging without the use of an endorectal coil. This technique is non-invasive and gives us information on metabolite content. Some recent studies have shown that in the case of PCa, an elevation of the Choline/Citrate ratio is observed. Choline/Citrate ratios are extracted using the LCModel (3) software and we have defined five classes characterising pathology: from Likely Benign (I) to Likely Malignant (V) (2). Our aim was to retrieve this classification without quantifying spectra and to highlight other potential biomarkers. Classification is made by SVM [1] and a boosting method. When looking for new biomarkers, we used a Sequential Forward Select algorithm with SVM as the classifier and boosting feature selection capabilities.

We had at our disposal two databases: the first, made up of 99 patient data sets (4197 spectra 3D CSI), the second, from preclinical data on 6 rats (3 controls and 3 PCa) with single voxel MRS performed at 4 different times.

Results: Initially, we tried to classify human prostate LCModel-treated-spectra: In the central and peripheral glands, we obtained error rates of 6.3% and 9.2%, respectively. When we used the magnitude of spectra without any other processing, we obtained an error rate of 20.4% in central gland and 30.5% in peripheral zone. By applying feature selection algorithm on human data, the main features selected corresponded to the choline and citrate peaks. Other positions within the spectra were also selected, but these data must be confirmed. Due to small dataset for animals, we could not use a classification algorithm and we only tried feature selection methods. As for human, a set of features was selected and must be validated.

Conclusion: These results reinforce our initial idea that we may attempt a diagnosis of prostate cancer by automatically processing magnetic resonance spectra. However, LCModel time processing is quite long (about 6 seconds by spectrum) and we do not have control over the procedure, and so, we are currently working on a solution in order to work with raw data.

Feature selection methods comfort the idea of using choline and citrate as biomarkers. In the case of preclinical studies, the position defined in the spectrum as a significant feature still needs to be validated.

Reference

[1] Vapnik V, ed. The Nature of Statistical Learning Theory: Springer-Verlag, 1995; 2 Walker PM, Créhange G, Parfait S, et al. Relationship between human prostate cancer ADC measurements and 3D-MRSI at 3T without endorectal coils. In:ESMRMB annual congress, Valence, 2008; 3 Provencher S.W.: Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magn Reson Med 30, 672 (1993).

Rational design of effective therapy combining irinotecan and rapamycin to target mTOR/HIF-1 alpha axis in colon cancer

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Scientific background: Despite recent progress, colon cancer is often resistant to combination chemotherapy, highlighting the need for development of novel therapeutic approaches. Here we investigated how colon tumor response to irinotecan could be improved by combination with mTOR inhibitors. This association was rationally designed based on gene-expression profiling of patient-derived colon xenograft tumors following treatment with irinotecan alone.

Material and Methods: Irinotecan was first tested on 7 patient-derived subcutaneous colon tumor xenografts and its gene expression signature was determined using Affymetrix micro arrays. Based on these results, xenografts were treated with low doses of irinotecan alone, rapamycin alone or combination of both drugs. Cellular effects of irinotecan and rapamycin were further characterized for HT-29 and HCT-116 colon cancer cells in vitro in hypoxic conditions.

Results: Gene expression analysis in xenografted tumors showed that two-third of the down-regulated genes after treatment with irinotecan alone are HIF-1 alpha target genes. This signature correlated with a complete inhibition of HIF-1 alpha protein accumulation. Since this effect appeared independent of mTOR pathway inhibition, we rationally hypothesized that rapamycin, a potent mTOR inhibitor, could synergize at low doses with irinotecan.

Xenografted tumors treated with the combined treatment showed a dramatic reduction in tumor volume which was accompanied by a synergistic inhibition of the mTOR/HIF-1 alpha axis. In vitro experiments showed that exposure to both drugs at low concentrations resulted in massive HT-29 cell death under hypoxic conditions and pointed to a cytotoxic effect mediated through HIF-1 alpha inhibition. Experiments using siRNA targeting specifically HIF-1 alpha confirm this result. HCT-116 cells were less sensitive to the combined treatment due to constitutive activation of PI3K/Akt and Ras/MAPK pathway. However, sensitivity in these cells could be restored by combining irinotecan with selective agents targeting activated Akt (LY294002) or K-Ras (salirasib).

Conclusions: These results identify HIF-1 alpha as a promising target and provide a rationale for the clinical trials combining irinotecan and mTOR inhibitors in colon cancer that we are currently setting up. Given that HIF-1 alpha regulates the expression of many genes implicated in epithelial-mesenchymal transition and metastasis, we are now investigating the impact of HIF-1 alpha modulation on cancer cell invasion.

Computation of realistic virtual phantom images for the assessment of lesion detection in digital mammography

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In mammography, image quality refers to the ability of a mammograms to transmit the necessary information to determine the presence of lesions which could lead to breast cancer . Mammograms are analyzed to search for abnormalities such as microcalcifications or masses. As radiographic representations of microcalcifications are small and well contrasted points and the spots related to masses are bigger but less contrasted, digital mammograms should provide images with both high spatial resolution and high detection at low contrast.

In order to estimate image quality, two approaches currently exist. The first is widely used by manufacturers to compare the performance of digital detectors under laboratory conditions. These parameters are the Modulation Transfer Function (MTF) and the Noise Power Spectrum (NPS), relating to the spatial resolution and the noise properties of detectors. However, these parameters present the disadvantage of having no direct relation with lesions detection in clinical conditions.

The second approach for image quality assessment uses contrast-detail phantoms allowing subjective evaluations based on multiple-forced choice experiments. Contrast-detail phantoms consist of a support with inserts of varying size and thickness simulating microcalcifications and masses. The quality of the acquired phantom images is usually represented by contrast-detail curves determined with the true and false positives (inserts) detected by human observers. The advantage of this subjective approach is that the assessment estimates the detection of lesions in

a realistic way. However, subjective tests have the drawback of large inter-observers variability. Furthermore, they consume a significant amount of time and work.

The aim of this work is the development of a method leading to image quality assessment which is both human independent, and directly related to lesion detection. This method is based on the computation of realistic virtual images of contrast-detail phantoms, which are generated by taking into account the physical parameters of detector (spatial resolution and noise under clinical conditions). Then, virtual images are evaluated by a mathematical observer in an automatic way.

Results show that the virtual and real images exhibit a high similarity which is validated by the high correlation between the contrast-detail curves.

The proposed methodology allows for lesion detection by eliminating the disadvantages of the two existing approaches. This method also provides an optimization tool to find the exposure conditions that gives the best image quality by reducing the exposure of patients.

Experimental rat model of osteoradionecrosis

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Background: Radiotherapy has been proved to successfully treat local and regional neoplasic lesions but it may adversely impact on normal tissues within the irradiated volume, especially on the bone tissue. Bone tissue necrosis, termed as osteoradionecrosis (ORN) is the most devastating radiotherapy-induced complications.

The aim of the present study was to develop an experimental animal model of hindlimb osteoradionecrosis, to better understand its irradiation induced defect.

Methods: In this long term study, rats were irradiated bilaterally on the hindlimb with a single dose of 30 or 50 Gy. Sequential analysis was based on observational staging recordings and non invasive imaging using planar scintigraphy. Additional radiography, radiohistology and histology works were also done to describe the ORN-induced histological alteration.

Results: Following irradiation, all animals developed acute and late effects, yet the severity of this effect were much stronger in the 50 Gy irradiated group. Animal developed foot necrosis, which was very aggressive and gradually extended to the entire lower extremity. The bone uptake of 99mTC-HDP was found to significantly decrease. Radiographic and histological studies evidenced bone lysis, with lesions characteristic of ORN.

Conclusions: After 50 Gy-irradiation, our rat model highlighted tissue degenerescence similar to those occurring during human osteoradionecrosis, both in vivo and in vitro. Because of the increased use of radiation therapy, it is likely that osteoradionecrosis will become an increasing clinical problem. The development of an animal model is an essential step for exploring pathogenesis of osteoradionecrosis and it may be used for testing the efficiency of treatments.

Rehabilitation of head and neck irradiated tissues by autologous fat transplantation

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Background: Treatment of head and neck cancers allows good carcinological results but induces aesthetic and functional sequelae. Autologous fat transplants have been used to correct aesthetic defect since the past century and exhibit many of the qualities of the ideal filler. Results reported here stem from experiences from 2000 with abdominal fat grafting in 11 patients who were referred to our center for aesthetic subcutaneous or submucous head and neck reconstruction after radiotherapy.

Methods: Abdominal fat tissues were harvested and injection in host site was performed similarly to the Lipostructure technique described by Coleman. The postoperative follow-up periods ranged from 2 months to 88 months (mean: 39.9). Clinical monitoring of the patients has been carried out. Additional pathological study was performed on irradiated tissues surrounding the scar, as well as abdominal fat and treated tissues.

Results: No surgical procedure complications occurred. For all cases, except for one patient, the rehabilitation was aesthetic and functional. The quality of life of the patients was improved. The pathological data highlighted a decrease in irradiated morphologic patterns characterized by an absence of necrotic areas and a high vascular network density associated with a normal histological structure.

Conclusions: Fat tissues can be successfully transplanted into irradiated areas, inducing both aesthetic and functional improvement. The cellular and/or tissular mechanisms underlying these changes need further investigation.

Quantum dots in axillary lymph node mapping: biodistribution in healthy mice

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Background: Breast cancer is the first cause of cancer death among women and its incidence doubled in the last two decades. Several approaches for the treatment of these cancers have been developed. The axillary lymph node dissection (ALND) leads to numerous morbidity complications and is now advantageously replaced by the dissection and the biopsy of the sentinel lymph node. Although this approach has strong advantages, it has its own limitations which are manipulation of radioactive products and possible anaphylactic reactions to the dye. As recently proposed, these limitations could in principle be by-passed if semiconductor nanoparticles (quantum dots or QDs) were used as fluorescent contrast agents for the *in vivo* imaging of sentinel lymph nodes. QDs are fluorescent nanoparticles with unique optical properties like strong resistance to photobleaching, size dependent emission wavelength, large molar extinction coefficient, and good quantum yield.

Materiel and Methods: CdSe/ZnS core/shell QDs emitting around 655 nm were used in our studies. 20 µL of 1 µM (20 pmol) QDs solution were injected subcutaneously in the anterior paw of healthy nude mice and the axillary lymph node (ALN) was identified visually after injection of a blue dye. *In vivo* fluorescence spectroscopy was performed on ALN before the mice were sacrificed at 5, 15, 30, 60 min and 24 h after QDs injection. ALN and all other organs were removed, cryosectioned and observed in fluorescence microscopy. The organs were then chemically made soluble to extract QDs. Plasmatic, urinary and fecal fluorescence levels were measured.

Results: QDs were detected in ALN as soon as 5 min and up to 24 h after the injection. The maximum amount of QDs in the ALN was detected 60 min after the injection and corresponds to 2.42% of the injected dose. Most of the injected QDs remained at the injection site. No QDs were detected in other tissues, plasma, urine and feces.

Conclusion: Effective and rapid (few minutes) detection of sentinel lymph node using fluorescent imaging of QDs was demonstrated. This work was done using very low doses of injected QDs and the detection was done using a minimally invasive method.

Biodistribution of near-infrared emitting quantum dots by mass spectroscopy and fluorescence imaging of axillary lymph node in healthy mice

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Background: Breast cancer is the first cause of cancer death among women and its incidence doubled in the last two decades. Several approaches for the treatment of these cancers have been developed. The axillary lymph node dissection (ALND) leads to numerous morbidity complications and is now advantageously replaced by the dissection and the biopsy of the sentinel lymph node. Although this approach has strong advantages, it has its own limitations which are manipulation of radioactive products and possible anaphylactic reactions to the dye. As recently proposed, these limitations could in principle be by-passed if semiconductor nanoparticles (quantum dots or QDs) were used as fluorescent contrast agents for the *in vivo* imaging of sentinel lymph nodes. QDs are fluorescent nanoparticles with unique optical properties like strong resistance to photobleaching, size dependent emission wavelength, large molar extinction coefficient, and good quantum yield.

Materiel and Methods: CdTe/ZnS core/shell QDs emitting around 800 nm were used in our studies. 4 µL of 5 µM (20 pmol) QDs solution were injected subcutaneously in the right anterior paw of healthy balb/c mice. Animals were sacrificed at 5, 15, 30 min; 1, 4 h and 1, 2, 4, 10 days after QDs injection. Organs, blood and excretions were collected and analysed by Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS). Moreover, after injection of 20 pmol of QDs by the same method previously described, i*n vivo* fluorescence imaging was performed in mice on right ALN during 10 days.

Results: QDs were observed in right ALN as soon as 5 min and up to 10 days after the injection by ICP-MS and *in vivo* fluorescence imaging. The maximum amount of QDs in the ALN was detected 4 h after the injection and corresponds to 1.36% of the injected dose. QDs were also detected in studied organs and blood by ICP-MS for all the period of the study. However, QDs were not excreted nor by urine or feces for up to 10 days.

Conclusion: Effective and rapid (few minutes) detection of ALN using fluorescence imaging of near-infrared emitting QDs in living animals was demonstrated. However, the use of QDs without heavy metals and the improvement of their surface chemistry are required to allow their excretion, and therefore, to eliminate any risk of toxicity.

Recombinant IL-24 induces apoptosis in cells engaged into the cell cycle, through dephosphorylation of pSTAT3 and derepression of p53

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A gene was discovered by substractive hybridization following in vitro differentiation of melanocytes, and named melanoma differentiation antigen-7 mda7. Later it was found that the MDA7 molecule can be secreted and renamed IL-24. This molecule is repressed in metastatic melanomas and it was suggested that it is a tumour suppressor, although direct evidence for this is

still lacking. Herein we show that IL-24 is an inhibitor of the cell cycle and protects terminally differentiated cells such as B-cells from chronic lymphocytic leukemia [1-3]. In contrast if CLL cells enter the cell cycle following culture with IL-2, they undergo apoptosis following secondary addition of IL-24 to the culture. Apoptosis is limited to cells in G2/M phases and it is reversed by pifithrin alpha, an inhibitor of p53, and to a lesser extent by the caspase inhibitor zvad-fmk. IL-2 induces STAT3 phosphorylation in CLL and IL-24 rapidly dephosphorylates STAT3; this effect of IL-24 is abolished if cells are pretreated with Na pervanadate indicating that IL-24 activated a tyrosine phosphatase [4]. As STAT3 is a repressor of p53 transcription, we propose the following molecular mechanisms to explain the paradoxical effect of IL-24. In resting cells IL-24 activates a survival program whereas it is sensed as a stress factor in cycling cells in which p53 is repressed. By derepressing p53 expression through the dephosphorylation of pSTAT3, IL-24 may deliver a stop signal to cells thereby leading them to exit the cell cycle which triggers apoptosis.

Our results are in keeping with recent data demonstrating that induction of p53 triggers the death of malignant cells in several in vivo models [5, 6].

References

[1] Gary H, et al. Blood. 2002.100:4537.
[2] Gary H, et al. J Immunol. 2007. 179:4335.
[3] Sainz-Perez A, et al. Leukemia. 2006. 20:498.
[4] Sainz-Perez A, et al. J Immunol. 2008. 181:6051.
[5] Ventura A, et al. Nature. 2007. 445:661.
[6] Xue W, et al. Nature. 2007. 445:656.

Cluster validity and fuzziness index analysis for fuzzy clustering of IR images of cancerous skin samples

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Scientific background: Infrared (IR) micro-spectral imaging is an efficient method to analyze the molecular composition of biomedical samples. Recent studies have shown its potential to detect and characterize cancerous tissues in their early stages, independently of visual morphology. IR micro-imaging could thus be developed as a sensitive, non-invasive and objective diagnostic tool in clinical oncology.

A final step in the processing of the spectral dataset acquired on biomedical samples is generally provided by conventional "hard" clustering algorithms (such as K-means). These clustering techniques assign each recorded spectrum to one cluster automatically. The final result is a false-colour image of the tissue section which is comparable to the conventional histology. K-means of IR data was proved to be very efficient to characterize tumoral tissue, but its main drawback is that it cannot differentiate the nuances in the assignment of pixels, particularly those located at the interfaces between different tissue structures.

Methods: In this study, we will focus on fuzzy clustering techniques to classify data from IR images acquired on different cancerous skin samples. The fuzzy clustering extends the traditional clustering concept by allowing each recorded spectrum to be assigned to every cluster with an associated membership value. Therefore, for unclear cluster boundaries, fuzzy clustering may obtain more reasonable results. However, as with the K-means algorithm, fuzzy c-means needs the number of clusters to be pre-specified in advance as an input parameter to the algorithm. This algorithm also needs a second input parameter called "fuzziness index" which controls the fuzziness of the membership.

We analyzed and developed various cluster validity indices, which can generate the optimal number of clusters automatically without *a priori* knowledge about the specificity of the sample. In addition, these indices indicate also the optimal "fuzziness index" to be used in order to obtain the best fuzzy partition.

Results and conclusion: To assess the efficiency of the analyzed validity indices, we compare the false-colour images reconstructed after fuzzy c-means clustering techniques with conventional histology (Hematoxylin-Eosin staining). Our preliminary results suggest that fuzzy c-means is a more suitable method than K-means to classify IR spectral data. It is very promising in clinics as an innovative tool dedicated to the direct histopathological analysis of cancerous tissues routinely manipulated in anatomopathology.

Predictive study of hepatocellular carcinoma in cirrhotic patients

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Project context: The Circe_Predict project is associated at the Circe project (**Ci**rrhose et **R**isque de **C**arcinome hépatocellulaire dans le grand Est). The circe project is a ethiological and physiopathologic study from Hepatocellular carcinoma (HCC) which have been initiated in the five French Great East regions. This project relies on networks of clinicians, gastroenterologists, epidemiologists, biostatisticians, chemometricians, and biologists. It involves six INSERM, CNRS, and University research units and two platforms of the Great East Canceropole (Biological Resources Centres, Registries). This case-control study provides nutritional, metabolic, biological and radiological studies.

Scientific context: Hepatocellular carcinoma (HCC) is the second cause of death due to cancer worldwide. This cancer generally arises in alcoholic and viral cirrhotic patients. Its extremely poor prognosis is due to late diagnosis. Emerging technologies for the evaluation of gene expression, especially at the molecular level, are opening new opportunities for the identification of biomarkers of HCC in cirrhotic patients. Development of new diagnostic tools in biological samples easily accessible may contribute to improve the prognosis of HCC. The goal of this project is to study predictive markers of HCC taking into account environmental, nutritional, and metabolic factors which may be identified by the CiRCE multi-centric study in progress in Great Eastern France.

Description of the project: The main objective of the study is to identify markers of cancer risk among patients with cirrhosis. The search for biomarkers predictive CHC will be made by two different approaches, proteomics and vibrational spectroscopy, whose results will be compared and / or combination of these and associated risk factors environmental, nutritional and metabolic. .The project is based on a case-control study including cirrhotic patients with (600 cases) and without (600 controls) HCC. The biological analysis will be conducted by teams CNRS UMR 6237 of Reims and Proteomics platform CLIPP of Dijon. The correlations between the spectra and clinical data, nutritional and metabolic will perform using the method of PLSR (partial least square regression) based on calculations of co-variance. The statistical studies of Circe_Predict project will involve teams Ducoroy Patrick (Clipper, Dijon), Pascal Roy (Department of Biostatistics HCL, UMR CNRS 5558, Lyon) and Achim Kohler (Matforsk Institute, Norway) and Juergen Schmitt (Synthon GmBH, Germany).

Expected results: The innovative aspect of this project is to develop a large scale epidemiological, biochemical and molecular study in research fields of hepatic carcinogenesis. Practical implications are expected for a better HCC prevention among cirrhotic patients and for early diagnosis and treatment of HCC.

Control of tumor progression by basement membrane collagen-derived matrikines

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Tumor progression is characterized by different steps involving interactions between tumor or endothelial cells and the extracellular matrix macromolecules of the tumor microenvironment. Among them, several domains of basement membrane associated collagens (collagens IV, XV, XVIII or XIX), generated by a limited proteolysis and termed matrikines, are able to control the different steps of tumor progression by inhibiting proteolytic cascades involved in local tumor invasion or in tumor angiogenesis. We previously demonstrated that the C-terminal fragment of tumstatin, the NC1 domain of the α3(IV) collagen chain, inhibits tumor progression in an experimental *in vivo* murine melanoma model. The inhibitory activity concerns proliferation and migration of tumor cells as well as their invasive properties by inducing a down-regulation of the expression and activation of matrix metalloproteinases (MMPs) and plasmin system.

Now, we demonstrate that the NC1 domain of the α4(IV) collagen chain also exerts a strong anti-tumor activity through the induction of an anti-proliferative effect on melanoma cells and a down-regulation of their invasive properties such as MMP-14 expression and activation. The overexpression of matrikines, induced *in vivo* in mice by DNA electrotransfer, leads to a large inhibition of melanoma tumor growth and an increase of mice survival.

Collectively, our results show that matrikines are able to limit tumor progression *in vivo* and that these matrikines, or structural derived analogues, constitute a new family of potent anti-tumor pharmaceutical therapies.

Analysis of mucus by IR spectral imaging in colon adenocarcinomas

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Scientific background: FTIR spectral imaging (FTIR SI) is emerging as a promising tool for generating a spectral histology based on the whole biochemical composition of tissues. We reported previously that FTIR SI of colon carcinomas is highly efficient to reproduce histology directly from paraffin-embedded (PE) tissue sections and to provide relevant information on the intra-tumor heterogeneity that cannot be revealed by classical histology. Our study aimed at a further exploration of the possibilities of the FTIR SI approach for histological recognition, with a particular emphasis on secreted mucus.

Materiel and methods: Spectral acquisitions (Spotlight 300, Perkin-Elmer) of human normal colon (n=3) and colon adenocarcinomas (n=10) PE tissue sections.

Results: By referring to IR spectra originated from bovine purified mucins and human mucus extracted from benign cysts, we show that secreted mucus of the tissue sections exhibit the spectral fingerprint of mucin glycoproteins. This permitted an *in situ* localization of mucus-rich areas and of tissular structures devoid of mucus by constructing color-coded images using a k-means clustering of spectral data. In addition, a clear distinction can be achieved between the apical and basal parts of mucus secreting cells,

containing respectively high and low amount of intracellular mucus. To discriminate normal from adenocarcinoma colon tissues, a k-means subclustering was performed exclusively on secreted mucus spectra. These subclusters are present in both tissue types but the samples present significant differences of subcluster percentages that can be used to discriminate normal from tumoral tissues. Analysis of mean spectra of the clusters highlighted variations in glycosylation, sialylation and amide bands between tissue types. These results reflect alterations in carbohydrate and sialic acid contents as well as changes in secondary structure of mucin core proteins between tissue types. Interestingly, k-means subclustering, performed on the amide bands only, indicates that changes in mucin secondary structure represent the most relevant spectral marker of mucus to discriminate colon adenocarcinomas from normal tissues.

Conclusion: This study shows the possibility to use FTIR-SI to study the mucin biochemical composition directly on PE tissue sections. Furthermore, the mucin biochemical differences revealed by FTIR-SI permit to discriminate all the normal tissues from adenocarcinomas samples. Finally, we suggest that discrimination between tissue types is principally due to variations in mucin core proteins.

FL3, a synthetic analogue of rocaglaol displays a potent and selective cytotoxicity toward cancer cells in an AIF-mediated manner

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Scientific background: Flavaglines, exemplified by rocaglaol, constitute a family of natural compounds that display promising anticancer properties.

Results: We present here the first synthetic analogue displaying cytotoxicity against human cancer cells superior to that of rocaglaol, and without any sign of cardiotoxicity. This analogue, FL3, inhibited cell proliferation and viability (IC50 ≈ 1 nM) at lower doses than did the parent compound, rocaglaol (FL1), or the anticancer drugs doxorubicin, camptothecin, and vinblastine. In HL60 cells, FL3 treatment induced cell cycle arrest at the G2-M transition followed by progressive induction of apoptosis. Concentrations of FL3 that blocked HL60 cells in G2/M phase had limited effects on caspase 3/7 activities, and the broad-spectrum caspase inhibitor Z-VAD-FMK did not affect FL3 cytotoxicity on HL60 cells, indicating that in these cells FL3 induced apoptosis in a caspase-independent manner. Indeed, FL3 induced the translocation of apoptosis inducing factor (AIF) from the mitochondria to the nucleus. Interestingly, FL3 enhanced doxorubicin cytotoxicity in Hep G2 cells and retained its potency against adriamycin-resistant cells, comparable to adriamycin-sensitive MCF7 cells. FL3 and FL1 displayed no cardiotoxic effects in H9c2 cardiomyocytes.

Conclusion: Together our data suggest that flavaglines, and in particular FL3, is a promising new class of antineoplastic agents with the capacity to induce growth arrest and/or apoptosis of cancer cells without compromising the viability of cardiomyocytes and therefore potentially preventing heart failure, a well known adverse effect of conventional cytostatic agents.

Identification of potential protein markers and molecular actors of conventional chondrogenic tumor malignant transformation

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Chondrogenic neoplasms are a group of tumours that form cartilage, because their cells share much of a chondrocyte phenotype. "Chondrosarcoma" is a general term used to describe, among chondrogenic neoplasms, those that exhibit malignancy. Their early diagnosis after histological analysis is very difficult, due to their similarity to benign tumours, which are 100-times more frequent. Distinction between the two is important, however, because chondrosarcoma, unlike benign chondroma, should be removed surgically before metastasis occurs. It is thus critical to identify unequivocal molecular markers of chondrosarcoma. Chondrogenic neoplasia share major biochemical compounds of hyaline cartilage, collagens and proteoglycans (PGs). Therefore, their detailed two-dimensional electrophoresis-based proteomic analysis was undoable until recently. We have developed a biochemical method to extract the cartilage proteome, allowing exclusion of collagens and PGs. Using a recently developed biochemical method for cartilage proteome extraction, we have performed proteomic analysis of multiple human conventional chondrogenic tumours in the search for recurrent, specific markers of their malignancy. We have identified 2 polypeptides, which exhibit malignity-related, inverse expression variation: the carboxyl-terminal propeptide of procollagen II (C2CP) is primarily found in chondromas, while its procollagen I counterpart (C1CP) is restricted to chondrosarcomas. Strikingly, recombinant C1CP exerts chemotactic activity toward endothelial cells *in vitro*, while C2CP does not. Therefore, C1CP might participate in the vascularization of chondrogenic tumours *in vivo*. In conclusion, C1CP and C2CP might be useful markers for clinical, early diagnosis of chondrosarcomas and they might be important determi-

nants of tumour angiogenesis. Statistical validation of these proteins as markers of conventional chondrogenic tumours is currently investigated by immunohistochemistry and their detailed activities regarding angiogenesis are being investigated.

Grape polyphenols intake prevents C26 adenocarcinoma growth and neovascularization

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Introduction: The growth of a tumor and its ability to develop metastases is angiogenesis-dependent. In Europe, colon cancer is the second and the third cause of mortality due to cancer in women and men, respectively. It has been estimated that an effective nutritional prevention, namely to eat more fruits and vegetables, would allow to avoid the arisen of more than 60 to 80% of colon cancer. Since epidemiological studies have indicated that moderate and regular consumption of wine is associated with a reduced risk of cancer, the aim of the present study was to determine whether *Red Wine Polyphenols* (RWPs) prevent tumor growth, in part, by controlling angiogenesis.

Methods: C26 cells, derived from colon carcinomas (chemically induced in BALB/c mice), were subcutaneously injected in each flank of 9 weeks-old BALB/c mice. Two days after the injection, RWPs or vehicle were given in the drinking water at the dose of 100 mg/kg/day for the following 26 days. At the end of the treatment period, we investigated the macrovessel density in tumors by high definition microCT system using a radio opaque silicon rubber and the microvessel density by immunohistochemistry (anti-CD31) on frozen sections. In parallel, we measured an index of proliferation (Ki67) and apoptosis (TUNEL, activated caspase-3) and we determined the expression level of pro-angiogenic factors (*Vascular Endothelial Growth Factor (*VEGF), *Matrix Metalloproteinases* (MMP)-2, MMP-9) and tumor suppressor genes (p21, p16, p53 and p73) on paraffin sections.

Results: After one month of treatment with RWPs, tumor size was significantly reduced by 30% compared to the control group. On the one hand, the vessel density assessed by microCT and immunohistochemistry, was reduced by 40% and by 47%, respectively in the RWPs-treated group. We observed a concomitant decreased expression level of the major pro-angiogenic factors VEGF, MMP-2 and MMP-9 in tumor cells. On the other hand, RWPs treatment reduced by 61% the proliferating index (Ki67) of tumor cells and increased by 81% the apoptotic index (TUNEL) associated with high caspase-3 activity in tumor cells. RWPs also induced the expression of tumor suppressor genes such as p16, p21, p53 and p73 in tumor cells.

Conclusions: RWPs reduce tumor growth by preventing tumor neovascularisation and by inducing tumor cells apoptosis through the expression of tumor suppressor genes. Altogether, these findings indicate that RWPs have potent anti-cancerous properties. As this syngenic model is very remote from the human pathophysiology, further studies are necessary to highlight the anti-cancer properties of RWPs in more relevant models of colon cancer.

A correlation between G to mutations of the *K-RAS* gene and the hypermethylation of the MGMT gene promoter is tumor specific in colorectal cancer

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Background: Mutations in the K-ras gene seem to have an effect on the survival rate of CRC patients and the G to A mutations in codon 12 might play a role in the progression of this neoplasia. The O^6-methylguanine DNA methyltransferase (MGMT) gene repairs guanine methyl adducts leading to a G to A transition, its transcription level being regulated by CpGs methylation level. It has been shown in two studies (American and Japanese cohort) that a large majority of tumors with G to A mutation in K-ras showed MGMT promoter hypermethylation. Hence, in order to generalize this correlation, we decided to study a European population.

Patients and Methods: Tumor samples and their corresponding blood were obtained from CRC patients from Luxembourg at the adenocarcinoma stage. K-ras mutations in codon 12 and 13 were detected by direct sequencing. The MGMT promoter methylation status was analyzed by methylation-specific PCR (MSP) after bisulfite treatment.

Results: In a previous study, we sequenced 234 tumor samples and found 57 K-ras mutations with 39 G to A transitions. In order to check if these mutations were tumor specific, we sequenced the DNA extracted from their corresponding blood. None of them was mutated, suggesting that the mutations occur during the progression of the disease. Hence in order to correlate the level of the MGMT gene promoter methylation with the occurrence of these mutations, we performed MSP experiments on these 39 tumors and their corresponding blood DNA. Three CpGs methylation status could be distinguished: unmethylated (U), fully methylated (M) and partially methylated (U/M). The level of methylation remains the same for the U status (11 cases), and is switched to a U status for the U/M and M (except one case).

Conclusions: We showed (1) that K-ras G to A transitions and the hypermethylation of the MGMT promoter

are correlated in our European cohort (2) these mutations are specific of the tumor as they are not observed in their corresponding blood (3), this absence of mutation is correlated with a low methylation level of the MGMT promoter CpGs. The deregulation of the promoter methylation may potentially be the first step towards the mutation of the K-ras gene leading to the progression of the disease.

Lumcorin: a leucine-rich repeat 9-derived peptide from human lumican inhibiting melanoma cell migration

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Malignant melanoma is the second cancer in mortality rate in Caucasian population due to its high metastatic potential. Lumican is an extracellular matrix (ECM) protein present in normal adult skin as a glycoprotein with a 37 kDa core protein. Lumican belongs to the small leucine-rich proteoglycan (SLRP) family. Its primary structure consits of 11 leucine-rich repeat (LRR). These repeats contain the 11-amino acid hallmark motif LxxLxLxxNxL. Lumican is known to decrease melanoma progression *in vivo* and to retard cell migration *in vitro*. The aim of the present study was to determine the active sequence of lumican core protein involved in the inhibition of melanoma cell migration. We showed that the anti-migratory effect of lumican is mediated by its core protein. Using different peptides, we localized an active site in the LRR9 of lumican core protein. We propose to name lumcorin (fragment of lumican core protein) the derived-peptide from this site. Lumcorin was able to inhibit the migration of melanoma cell in vitro as well as the complete lumican core protein. Moreover, we showed that lumican and lumcorin inhibits migration through the α2 integrin.

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Plateformes

Innovative immune targeting of cancer (ITAC)

Coordination: Christophe Borg, John Wijdenes, David Chalmers, Christophe Ferrand

EFS Bourgogne Franche Comté/société Diaclone, UMR 645 INSERM, EFS BFC, Université de Franche Comté, Besançon, France
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Objectives: Biological therapies provided a critical improvement in the management of cancer patients. Over the years Diaclone has developed many unique therapeutic monoclonal antibodies such as B-E2 (CD2), B-F5 (CD4), B-B10 (CD25 / Leukotac), B-C7 (anti-TNF-a), B-E8 (anti-IL-6) and B-B4 (anti-CD138) with a specific interest in inflammatory diseases. The ITAC platform gathered the technological and scientific expertise of UMR 645 INSERM/EFS/UFC and the Diaclone company to support the development of innovative immunomolecular treatments in oncological or hematological malignancies.

Facilities And Equipment: The ITAC platform technical facilities include: monoclonal antibody production (cloning and purification of antigen, immunization, production), pre-clinical assessment (cell culture, molecular biology, apoptosis, xenografts, vaccination study in human HLA transgenic mice, in vivo imaging with the Nightowl luciferase detection system).

Ongoing Research Programs:
– Development of therapeutic mAb targeting neuropilin 2 to promote colon cancer cell apoptosis.
– Production of therapeutic mAb to trigger immune co-stimulatory receptors.
– Development of a vaccination strategy to expand cytotoxic T cell lymphocytes directed against rituximab resistant B cell lymphoma.

Transfected cell array platform

Laurent BRINO, scientific management, Benoît FISCHER, operations management

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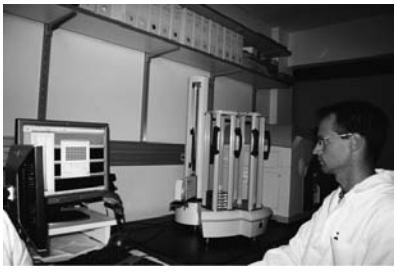
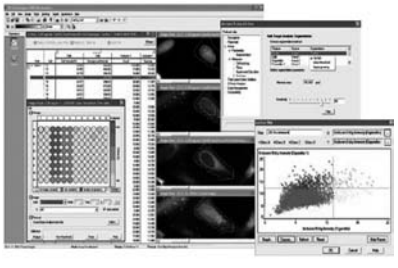


The Transfected Cell Array Platform is a **post-genomic facility** allowing the identification of genes responsible for a particular **cellular phenotype.** It is based on **high throughput** screens combined to **high content** phenotypic analysis. Member of a network of other genomic facilities including Transcriptomics, Genomics, ChIP sequencing, Proteomics and Bioinformatics, and located within the IGBMC institute, our perspectives are oriented towards Integrative Biology and Systems Biology.

Core applications are based on the use of **siRNA thematic libraries** (kinases, phosphatases, plasma membrane receptors, druggable genes) and chemical transfection to create **transient gene knock-down** in mammalian cells. The phenotypic analysis exploits an integrated infrastructure composed of an automated optical microscope – *InCell1000 analyzer* –, standard image analysis protocols as well as in house developed and customized statistical data analysis software (*RReportGenerator*).



High throughput screens are achieved owing to a *TECAN* robotic station for cell transfection and post-transfection processes (staining, immuno-cytochemistry) and to a *Caliper Twister II* robotic arm coupling microplate stacks to the *InCELL1000* microscope.

The Transfected Cell Array Platform is open to **academic and industrial** users.

Main publications

Lupberger J., Brino L., Baumert T.F. RNAi - A powerful tool to unravel hepatitis C virus-host interactions within the infectious life cycle. J. Hepatol., 2008, 48, 523-525.

Raffelsberger W., Krause Y., Moulinier L., Kieffer D., Morand A.L., Brino L., Poch O. RReport Generator: automatic reports from routine statistical analysis using R. Bioinformatics, 2008, 24, 276-278.

Papait R, Pistore C, Grazini U, Babbio F, Cogliati S, Pecoraro D, Brino L, Morand AL, Dechampesme AM, Spada F, Leonhardt H, McBlane F, Oudet P, Bonapace IM. 2008 The PHD domain of Np95 (mUHRF1) is involved in large-scale reorganization of pericentromeric heterochromatin. Mol Biol Cell. 19(8):3554-63

Xenograft platform for colon cancers and brain tumors in Strasbourg

Eric Guérin[1,2], Erwan Pencreach[1,2], Céline Nicolet[2], Laetitia Ruck[2], Nathalie Hamelin[2], Madeleine Jaillet[2], Natacha Entz-Werlé[1,2], Marie-Pierre Gaub[1,2], Dominique Guenot[2]

[1] Laboratoire de Biochimie et Biologie Moléculaire, Hôpitaux Universitaires de Strasbourg, Hôpital de Hautepierre, Strasbourg, France; [2] Equipe d'Accueil de l'Université de Strasbourg, Faculté de Médecine, Bâtiment Inserm U682, Hôpital de Hautepierre, Strasbourg, France
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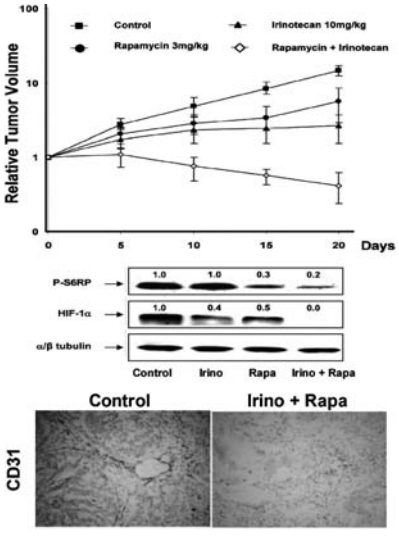
Description of the platform: Preclinical models of human cancer are needed to prioritize and select potential drug candidates for investigations in man. Human tumor xenografts established directly from patient biopsy material represent such valuable tools for *in vivo* drug testing. Within the framework of the Cancéropôle Grand-Est, we developed a panel of well-characterized human primary tumor xenografts, focusing initially on human colon cancers and that we recently extended to human brain tumors. 31 xenograft models of human colon cancers of different stages (from stage I to stage IV including liver metastases) and 6 xenografts models of human brain tumors (4 glioblastoma and 2 oligodendroglioma of grade B) are now available. We showed that xenografting does not alter the genetic or the histological profiles of the original tumors even after multiple passages (ref. 1). In particular, intra-tumoral heterogeneity was maintained over time, suggesting that no clonal selection occurred in the nude mice. In addition, because only tumor cells from the implanted tumor will survive in xenograft, xenografting in nude mice provide an efficient way to remove contaminating non-neoplastic cells, thus facilitating the establishment of molecular signature in cancer cells.

Case study: These properties have been exploited in our laboratory to define the gene expression signature elicited *in vivo* by irinotecan in colon cancer xenografts, with the aim to rationally design further innovative therapeutic combinations that could improve colon tumor response to irinotecan.

Gene expression analysis in xenografted tumors showed that two-third of the down-regulated genes after treatment with irinotecan alone are HIF-1 alpha target genes (ref. 2). Since this effect appeared independent of mTOR pathway inhibition, we rationally hypothesized that rapamycin, a potent mTOR inhibitor, could synergize at low doses with irinotecan.



Xenografted tumors treated with the combined treatment showed a dramatic reduction in tumor volume which was accompanied by a synergistic inhibition of the mTOR/HIF-1 alpha axis and an important inhibition of tumor angiogenesis (see figure and ref. 3). These results identify HIF-1 alpha as a promising target and provide a rationale for the clinical trials combining irinotecan and mTOR inhibitors that we are currently setting up within the Cancéropôle Grand-Est (CGE).

Other applications: Several other successful applications of our xenografts models include Infra-Red spectral imaging for histopathological characterization of colon carcinomas (Axe IV of CGE, ref. 4), tissue and serum proteomics for the identification of novel biomarkers in colon cancer (Réseau Structurant INCa, Axe V of CGE), discovery and functional validation of new druggable targets for the treatment of colorectal cancer (Transnational EurotransBio Project), study of the molecular and functional interaction between EGFR-mediated oncogenic signaling and topoisomerase I in colorectal cancer (INCa Inter-Cancéropôle Project), study of integrin $\alpha 5\beta 1$ as a therapeutic target in human glioblastoma (ARC and National Ligue against Cancer Projects).

References

[1] Guenot *et al., J Pathol*, 2006, 208, 643-652
[2] Guérin *et al.*, 2009, manuscript in preparation
[3] Pencreach *et al., Clin Cancer Res*, 2009, 15, in press
[4] Wolthuis *et al., Anal Chem*, 2008, 80,8461-8469

Human tumor xenograft platform for translational oncology and radiobiology in Nancy

Jean-Louis Merlin, Pascal Chastagner, Sophie Pinel, Béatrice Faivre, Philippe Becuwe, Gilles Karcher, François Plénat

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Platform:The platform has been mainly devoted to the development of human malignant glioma xenografts models but is now also working in head and neck, breast and colon carcinomas. Human tumor specimens are xenografted hetero- or orthotopically in nude mice. These models are stored as grafts or frozen. Among 14 malignant glioma models 7 appears are near-perfect phenocopies of clinical human malignant gliomas with infiltrating properties, microvascular density lower than in normal cortex, dense and homogenous glial component etc...). Morphological and functional characteristics of the models are controlled regularly using standard histology, immunohistochemistry, genotyping, proliferation and survival phenotyping, microvascular density, therapeutic targets expression.

Several models have been used for preclinical evaluation of the response to cancer therapies including chemotherapy, targeted therapy, alone or combined with radiotherapy.

Morphology and molecular markers: Evaluation of response to treatment of the xenografts is performed using macroscopic evaluation of tumor progression as well as vascular imaging using intravital microscopy and morphological and biological determination of molecular markers using IHC, ISH, western blot, Q-PCR, as well as phosphoprotein and cytokine array or functional markers such as pO2.

Imaging: Non invasive techniques such as MRI (collab Pr D Canet, Nancy Université) or PET- and gamma-imaging (NanCyclo-TEP) are used and proved interesting in orthotopic models, especially in malignant gliomas, allowing long term follow-up of grafting and tumor response.

Radiobiology: In the domain of experimental radiotherapy, attention is focused on the representativity of the radiation scheduling and fractionated scheduling is applied to remain as close as possible to the clinical scheduling.

References: The platform infrastructure has been used for the evaluation of modulators of radiation response being conventional chemotherapy agents [1] as well as liposomes [2], oxygenating-agents [3] and proteasome inhibitors [4]. On-going projects concern tyrosine kinase receptors and signaling kinases targeted therapies, and angiogenic agents.

References

[1] Pinel S, et al. Topotecan can compensate for protracted radiation treatment time effects in high grade glioma xenografts. J Neurooncol. 2006
[2] Labussière M, et al. Interest of liposomal doxorubicin as a radiosensitizer in malignant glioma xenografts. Anticancer Drugs. 2008
[3] Scigliano S, et al. Measurement of hypoxia using invasive oxygen-sensitive electrode, pimonidazole binding and 18F-FDG uptake in anaemic or erythropoietin-treated mice bearing human glioma xenografts. Int J Oncol. 2008
[4] Labussiere M, et al. Proteasome inhibition by bortezomib does not translate into efficacy on two malignant glioma xenografts. Oncol Rep. 2008

Biobanks platform of the Cancéropôle Grand-Est

Agnès Neuville

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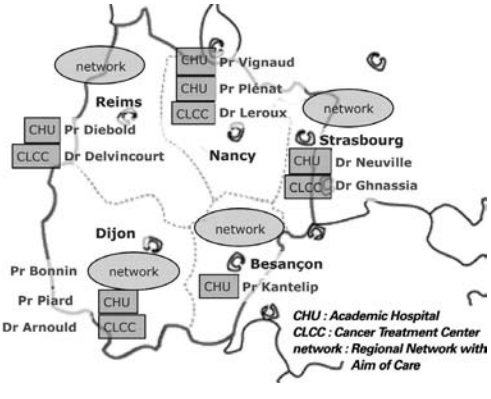
agnes.mechine@chru-strasbourg.fr

What is it?: Banks of frozen and/or embedded biological samples:
– In aim of care for the improvement of the diagnosis, prognosis and treatment of patients.
– In research aim to deliver biological material together with full pathological, biological and clinical annotations to be used in research program.

Who and where is it?: See plan opposite.

How does it work?:
– Submission of a research project requiring biological resources to the person in charge of the Biobank.
– Survey of the project by a scientific committee.
– Establishment of a contract between the research team and the Biobank.
– Transfer of the material according to a MTA (material transfer agreement).
– Information feedback on the use of the biological material.
– Biobank aknowledgements in publications.



How much does it cost?:The cost of banking and use of biological samples must be taken into account and integrated into grant applications.

A standard cost evaluation is currently conducted by INCa (French National Cancer Institute) at the national level. A total cost of 100 € per sample was estimated by the Biobank of the CHU of Strasbourg.

Virtual Biobank: Biobanks of the CHU of Nancy and Strasbourg contribute since 2006 to the experimental National Virtual Bank within the framework of the PNES lung (integrated national programme of excellence).

The Biobanks platform of the CGE is setting up a TVR (Regional Virtual Bank), allowing to locate and browse the collections of biological samples available to research programs.

Tissue and cell engineering platform: development in oncology and haematology

Coordination: Fabienne Pouthier, Jérôme Vacheron, Christophe Borg, Pierre Tiberghien.
Institutional partnership: Cancéropôle Grand-Est, INCa, INSERM, university hospital of Besançon

Etablissement Français du Sang Bourgogne Franche Comté (EFS BFC). UMR 645 Inserm, EFS BFC, Université de Franche-Comté, Besançon, France
Pierre.tiberghien@efs.sante.fr

Objectives: Cell therapy can be defined as the use of living, functional cells, administered to treat diseases. Hematological malignancies demonstrate susceptibility to T cell and natural killer cell (NK) mediated killing as evidenced by the potent graft versus disease effect mediated by alloreactive lymphocytes following allogeneic haematopoietic transplantation (AHT). Our field of investigation concerns the development of adoptive immunotherapy that would more selectively target and eliminate tumor cells.

Facilities: The cellular therapy platform is located in the blood bank of Besançon and includes facilities for T lymphocyte and NK lymphocyte isolation and culture. Genetic modulations of lymphocyte functions are studied. The EFS Bourgogne Franche Comté has promoted a cord blood cell bank for AHT (1000 in 2008) and to provide researchers with cord blood derived lymphocytes and stem cells.

Another objective is to place at the disposal of biotechnological companies all the biological and reglementary facilities to sustain the emergence of innovative cellular products. Our French blood bank platform supported the cellular production of the Txcell company.

Research program in onco-hematology:
– Phase I/II trial of Herpes-Simplex Virus-thymidine kinase (HSV-tk)-expressing gene modified T cells administration at time of HLA-identical sibling bone marrow transplantation (BMT). Principal Investigator: Pr Eric Deconinck and Pr Pierre Tiberghien.
– Phase I/II trial of Allogenic NK cell therapy in association to cetuximab in EGFR expressing gastrointestinal malignancies. Principal Investigator: Dr Christophe Borg.

Biomonitoring platform of the clinical investigation center of Franche-Comté

Coordination: Philippe Saas, Marina Deschamps

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http://www.chu-besancon.fr/biomonitoring/

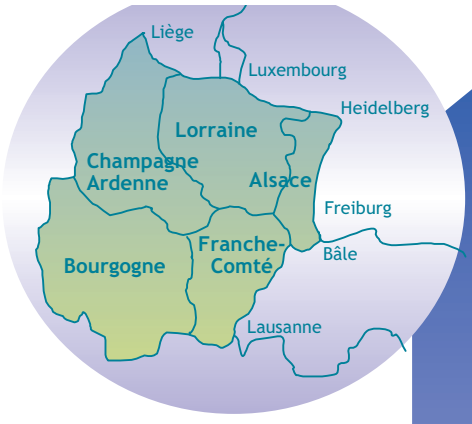
Objectives: The main objective of the *BioMonitoring platform* is the biological follow-up of patients included in biotherapy studies or receiving organ transplantation. We are also interested in the qualification of cell and tissue therapy products (i.e. haematopoietic graft, NK cells, regulatory T cells, gene-modified T cells...). In addition, the platform participates in clinical research programmes that require biological analyses (e.g. immunological and haematological analyses). The platform also includes an "innovative pole" that conducts research activities and develops new biological assays.

Facilities And Equipment: The *BioMonitoring Platform* is located in the *French Blood Bank Centre* (EFS B/FC, Besançon, France) and is working in closed collaboration with Inserm unit UMR645. Thus, the *BioMonitoring Platform* benefits from all the equipments of the Institute, in particular the Molecular and Cell Biology facilities. The *BioMonitoring Platform* has 3 biologists (PhD) specialized in immunology, haematology and molecular biology respectively, a research assistant and 3 lab technicians.

Ongoing Research:
– Identification of relevant biomarkers to assess the clinical interest of bevacizumab in rectal cancer patients (INOVA study, investigator: Dr Christophe Borg).
– Biological monitoring of several phase II clinical trials of patients treated with metronomic chemotherapies (prostate cancer) or biotherapies (targeting of EGFR).
– Identification of risk factors involved in kidney transplantation-associated cancers (Dr Didier Ducloux).
– Influence of immune genetic polymorphisms on breast cancer prognosis (Dr François Ghiringhelli).
– Study of Toll like Receptor polymorphisms in the setting of TLR ligand-based biotherapy (OM Pharma Company).
– Mesenchymal Stem Cells and the prevention of graft-versus-host disease (GvHD): this is a multicenter clinical study involving 21 clinical centres and 3 biological centres (Rennes, Grenoble, Besancon, *BioMonitorig platform*, coordination of the biological study: Pr K Tarte, Rennes).
– Allogeneic haematopoietic cell Transplantation: Biological follow-up of peripheral haematopoietic cell donors given G-CSF for stem cell mobilisation (Dr Eric Deconinck).
– Minimal residual disease (MRD) in haematological malignancies (Dr Francine Garnache Ottou).

Cap sur la recherche de transfert

CANCÉROPÔLE DU GRAND-EST



Etudes exploratoires et de validation

- Génomique
- Transcriptomique
- Protéomique
- Métabolomique

Bio-banques

Identification de candidats, études fonctionnelles

- Chimie médicinale
- Biochimie intégrative
- Puces à cellules transfectées

Evaluation pré-clinique : marqueurs & stratégies thérapeutiques

- Xénogreffes
- PharmImage

Evaluation clinique précoce

- Phases 0/I
- CIC cancer
- Evaluation des pratiques Qualité de Vie

Bio-informatique
O. Poch

Un Comité de Cliniciens (COCLIN)

pour renforcer la pratique, l'impact et la visibilité de la recherche translationnelle dans le CGE

Animateur
Pierre FUMOLEAU
CLCC GF-Leclerc, Dijon
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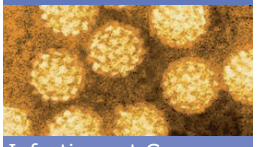
AXE 1

Epidémiologie : indicateurs de santé et évaluation des pratiques

AXE 2



Infections et Cancer

AXE 3

Contrôle local des cancers : imagerie, outils de diagnostic, sensibilité aux traitements, nouvelles thérapeutiques

AXE 4

Plasticité cellulaire, hétérogénéité tumorale et micro-environnement dans la progression tumorale

AXE 5

Maîtrise des échecs thérapeutiques

AXE 6

Thérapeutiques immunomoléculaires et cellulaires

CAISSE D'EPARGNE Réalisé avec le soutien financier des Caisses d'Epargne d'Alsace et de Lorraine Champagne-Ardenne

ERBITUX® 5 mg/ml
CETUXIMAB

agm magna 02/09

ERBITUX® 5 mg/ml solution pour perfusion. FORMES ET PRESENTATIONS : *solution pour perfusion à 5 mg/ml* (incolore) : flacons de 20 et de 100 ml, boîtes unitaires. **COMPOSITION* :** Cétuximab (DCI) 100 mg ou 500 mg par flacon. Le cétuximab est un anticorps monoclonal chimérique IgG1 produit par la technique de l'ADN recombinant. **INDICATIONS :** • Erbitux® est indiqué dans le traitement des patients présentant un cancer colorectal métastatique avec gène KRAS de type sauvage exprimant le récepteur du facteur de croissance épidermique (EGFR) : - en association avec une chimiothérapie, - en monothérapie après échec d'un traitement à base d'oxaliplatine et d'irinotécan et en cas d'intolérance à l'irinotécan. • Erbitux® est indiqué dans le traitement des patients présentant un carcinome épidermoïde de la tête et du cou : - en association avec la radiothérapie en cas de maladie localement avancée, - en association avec la chimiothérapie à base de platine en cas de maladie récidivante et/ou métastatique. **POSOLOGIE ET MODE D'ADMINISTRATION * :** Surveillance pendant et au moins 1 h après la fin de la perf. Disponibilité de matériel de réanimation. Avant la 1ère perf, prémédication par antihistaminique et corticostéroïde. 1ère dose : 400 mg/m². Doses hebdomadaires ultérieures : 250 mg/m². • CCRm : en association avec la chimiothérapie (médicaments administrés au moins 1 h après la fin de la perf) ou en monothérapie. • Carcinome épidermoïde de la tête et du cou : - localement avancé : débuter une semaine avant la radiothérapie ; - récidivant et/ou métastatique : associer à une chimiothérapie à base de platine (administrée au moins 1 h après la fin de la perf), suivie d'un traitement d'entretien par cétuximab. Administration : voie IV, par pompe à perfusion, goutte-à-goutte ou seringue électrique. Durée de perf : 120 mn (dose initiale), puis 60 mn (doses ultérieures) ; vitesse max de perfusion : 10 mg/mn. **CONTRE-INDICATIONS :** • Antécédents connus de réactions d'hypersensibilité sévères (grade 3 ou 4) au cétuximab. • Avant d'instaurer un traitement en association, il doit être tenu compte des contre-indications des médicaments chimiothérapeutiques utilisés simultanément ou de la radiothérapie. **MISES EN GARDE ET PRECAUTIONS D'EMPLOI* :** • Réactions liées à la perf : réduire sa vitesse si légères ou modérées, arrêter immédiatement le traitement si sévères. • Affections respiratoires : interrompre le traitement si diagnostic de maladie interstitielle pulmonaire. • Réaction cutanée sévère : interrompre le traitement jusqu'à régression au grade 2. • Déséquilibres électrolytiques (hypomagnésémie, hypokaliémie), risque accru d'apparition d'hypocalcémies sévères (particulièrement en cas de chimiothérapie à base de platine) : déterminer les concentrations sériques avant et pendant le traitement. • Neutropénie et complications infectieuses associées : chez les patients recevant le cétuximab en association avec une chimiothérapie à base de platine, le risque d'apparition d'une neutropénie sévère est accru, celle-ci pouvant entraîner ensuite des complications infectieuses, en particulier en cas de lésions cutanées, de mucite ou de diarrhée. **INTERACTIONS*. GROSSESSE ET ALLAITEMENT* :** Seulement si le bénéfice potentiel justifie le risque potentiel pour le fœtus. Allaitement déconseillé jusqu'à 2 mois après la dernière administration. **CONDUITE ET UTILISATION DE MACHINES*. EFFETS INDESIRABLES* :** Réactions cutanées, hypomagnésémie, mucite, élévation des enzymes hépatiques, maux de tête, conjonctivite, diarrhées, nausées, vomissements, déshydratation (en particulier secondaire à diarrhée ou mucite), hypocalcémie, anorexie, fatigue, réactions liées à la perfusion (fièvre, frissons, vertiges, dyspnées...) parfois sévères (bronchospasme, urticaire, hypotension, perte de conscience, état de choc). **SURDOSAGE*. PHARMACODYNAMIE* :** Agents antinéoplasiques, anticorps monoclonaux, code ATC : L01XC06. Anticorps monoclonal chimérique IgG1 spécifique de l'EGFR. **PHARMACOCINETIQUE*. SECURITE PRECLINIQUE*. INCOMPATIBILITES* :** Ne pas mélanger avec d'autres médicaments. Utiliser une ligne de perfusion séparée. **CONDITIONS DE CONSERVATION* :** A conserver au réfrigérateur (entre 2°C et 8°C). Ne pas congeler. *Après ouverture* : 48 h à 25°C. Utiliser immédiatement après ouverture. **MODALITES DE MANIPULATION*. RENSEIGNEMENTS ADMINISTRATIFS :** Titulaire de l'autorisation de mise sur le marché : Merck KGaA, Darmstadt, Allemagne. LISTE I. AMM EU/1/04/281/003 (2004, rév. 24.11.2008) ; CIP N° 570 750-8 (1 fl 20 ml). AMM EU/1/04/281/005 (2004, rév. 24.11.2008) ; CIP N° 570 752-0 (1 fl 100 ml). Médicament réservé à l'usage hospitalier. Prescription réservée aux médecins spécialistes ou compétents en oncologie ou en cancérologie. Inscrit sur la liste des spécialités prises en charge en sus de la T2A. Collect. **Merck Lipha Santé** : 37 rue Saint-Romain F 69379 Lyon cedex 08. Tél : 04 72 78 25 25. Information médicale/Pharmacovigilance : Tél (N° vert) : 0 800 888 024. Site web : www.merckserono.fr. Courriel : infoqualit@merck.fr. *Pour une information complète, consulter le RCP disponible sur le site internet de l'EMEA ou auprès de Merck Serono. MLC 2009-01-29. Merck Lipha Santé S.A.S. au capital de 16 398 285 € - 955 504 923 rcs Lyon.

Merck Serono Oncologie | *Cibler l'innovation*

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