Contribution of adipose stem cells from obese subjects to hepato-or breast-carcinoma tumorogenesis, through promotion of Th17 cells

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BOOK DES COMMUNICATIONS ORALES ET POSTERS

Table des matières

Progression & Tumor Resistance, Innovative Therapies

Poster # 1

Acute and Chronic Toxicity of the Aqueous Extract of Aristolochia Baetica

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Keywords: Aistolochia baetica L., acute toxicity, chronic toxicity, mouse, rat, Morocco

Aristolochia baetica belongs to the Aristolochiaceae family under the vernacular name of Bereztem. Although it is a plant known for its toxicity due to aristolochic acids (AA) which it contains, it is widely used in traditional medicine and treatment of numerous ailments, notably cancer, diabetes or digestive tract disorders (b). As the botanical identity and renal toxicity of used species remain unexplored, the safety of patients may be threatened.

This work focuses on toxicological evaluation of A. baetica aqueous extract of the roots. The study of the aqueous extract of the roots acute toxicity in male and female mice, administered orally, has verified its safety to a singles doses of 1g/kg to 10g/kg. Just as, the evaluation of the chronic toxicity of the aqueous extract in rat waster for 90 days at doses of 0.5 and 1.5g/kg body weight, showed a decrease in body weight and other signs morbidity and a disturbance of some parameters. We also noted a mortality rate that increases with doses. Biochemical parameters studied in this evaluation showed a significant increase in the concentration of plasma creatinine and in the concentration of sodium potassium. Histological examination showed alterations of the renal parenchyma and the liver which are greater in animals treated with high dose.

Poster # 2

Identification of Different Expression Patterns of FBL in Human Samples: Evidence of Ribosome Composition Heterogeneity in Breast Cancers

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Keywords: ribosome, fibrillarin, breast cancer, prognostic marker

Ribosomes are the machinery translating mRNAs into proteins. The ribosomal RNA (rRNA) undergoes chemical modifications, including 2'-O-ribose methylations (methylations). We showed for the first time that ribosomes can present different rRNA methylation profiles associated with different translational activities. In a mammary tumour initiation model, alterations of rRNA methylation resulting from an increased expression of the rRNA methyl-transferase, fibrillarin (FBL), favour the translation of a subset of mRNAs encoding oncoproteins. This regulatory mechanism was supported by our findings that high mRNA levels of FBL are independent markers of poor prognostic in breast cancer. Since then, we have reported in cancer cell lines that any change in FBL expression drastically affects rRNA methylation profile and translational control (see Abstract of Catez et al.). However, the different kind of FBL expression patterns occurring in breast tumours and their associated clinical characteristics remain unknown.

To identify all FBL expression patterns occurring in primary breast tumours, two independent test series were analysed. Series 1 is composed of 216 total RNA in which FBL mRNA levels were quantified by medium-throughput qPCR. Series 2 is composed of 440 tissues in which FBL was stained by immunohistochemistry (IHC). Novel alterations of FBL in breast tumours were identified. It appeared that low FBL mRNA levels and the lack of detection of FBL by IHC are associated with poor prognosis in breast cancer. Analyses of two other independent validation series are ongoing to strengthen these data.

Altogether, the identification of different FBL expression patterns in breast tumours and their association with overall survival supports our findings that alteration of FBL is deleterious for cancer patients. In addition, these data support the existence of ribosome composition heterogeneity that might be a useful novel biomarker and therapeutic target in breast cancers.

Poster # 3

Search of mRNAs Whose Translation is Directly Regulated by the BRCA1 Protein: Towards the Identification of New Therapeutic Tools for BRCA1-Deficient Breast Tumors

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Keywords: breast cancer, BRCA1, tumor suppressor, translation, biomarkers, mRNA

BRCA1 is one of the two major breast cancer susceptibility genes. The numerous binding partners of BRCA1 allow it to participate to several cellular pathways which globally contribute to its cell surveillance capacity. The team in which I performed my PhD identified a new binding partner of BRCA1, the Poly(A)-Binding Protein 1 and, consequently, a new function of this tumor suppressor, namely, the translation regulation. Moreover, recent studies suggest that under conditions dangerous for the cell and potentially oncogenic, such as a genotoxic stress, protein synthesis is strongly altered. My thesis work was aimed at demonstrating that this new function of BRCA1 contributes, like its nuclear functions, to its role of tumor suppressor. During my thesis, I identified the mRNAs "targets" of BRCA1 by the technique of immunoprecipitation of the ribonucleoprotein complexes (RIP), I validated that these mRNAs "targets" of BRCA1 are associated to it for their translational control by realizing a comparative analysis of the contents of the polysomes of MCF-7 mammary epithelial cells transiently expressing an interfering RNA directed against BRCA1 by the technique that I have set up in the laboratory, the polysomal profiles. Subsequently, I established the conditions of genotoxic stress inducing the cytoplasmic localization of BRCA1, and in collaboration with clinicians of the Center Léon Bérard Hospital, I allowed the acquisition of tumor samples. This will allow us to identify among these targets new diagnostic markers or new therapeutic targets for breast cancers deficient in BRCA1.

Poster # 4

Ciblage des cellules souches cancéreuses dans les cancers ORL : effet synergique de l'ABT-199 et du cetuximab en association à la radiothérapie photonique

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Mots clés : cancer ORL, cellules souches cancéreuses, Bcl2, sphéroïdes, invasion/migration, radiothérapie

Objet : Les cellules souches cancéreuses (CSCs) de cancer ORL, sous-population hautement migratoire, semblent être à l'origine de la résistance aux traitements. L'objectif de ce travail était d'étudier l'effet synergique d'un inhibiteur spécifique de Bcl-2, l'ABT-199, avec un anticorps monoclonal anti-EGFR, le cetuximab, en association à une irradiation photonique sur une lignée de cancer ORL et sa sous-population de CSCs.

Méthodes : La sous-population de CSCs de la lignée SQ20B a été isolée par double tri. Les cellules ont été exposées à de l'ABT-199 10µM et/ou du cetuximab 5nM +/- irradiation photonique à 4 Gy. La prolifération des SQ20B et SQ20B/CSCs a été mesurée par vidéomicroscopie. Les analyses de la migration et de l'invasion ont été réalisées par test de blessure avec et sans matrigel (IncuCyte). L'apoptose a été évaluée par le test des caspases 3/7 en microscopie à fluorescence. Les données ont été validées grâce à un modèle 3D de culture en sphéroïdes. L'activation des voies de signalisation intracellulaires (Phospho-AKT et Phospho-MEK1/2), et des voies anti-apoptotiques (Bcl-2 et Bcl-xl) a été étudiée en réponse aux traitements par western-blot (WES).

Résultat : Le cetuximab inhibe la prolifération, l'invasion et la migration des SQ20B mais n'a aucun effet sur la sous-population de SQ20B/CSCs. A l'inverse, l'ABT-199 inhibe significativement ces processus dans les SQ20B/CSCs. L'ABT-199 a peu d'effet sur les SQ20B, en revanche il potentialise l'effet du cetuximab dans les deux populations. De plus, l'irradiation photonique à 4 Gy majore l'effet de l'ABT-199 et renforce l'effet de l'association thérapeutique. Ces résultats s'expliquent par la surexpression de l'EGFR dans les SQ20B et de Bcl-2 et Bcl-xl dans les SQ20B/CSCs.

Conclusion : L'association ABT-199+Cetuximab combinée à l'irradiation photonique inhibe de façon synergique la prolifération, la migration et l'invasion des SQ20B et de leur sous-population de CSCs, et semble prometteuse en clinique.

Poster # 5

Regulation of Metabolic Enzymes by Lysine Deacetylase Inhibitors in A549 Non-Small Cell Lung Cancer Cells

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Keywords: Non-small cell lung cancer, metabolic reprogramming, inhibitors of lysine deacetylases, quantitative proteomics

Metabolic reprogramming is nowadays known as a hallmark of cancer. It enables cancer cells to adapt their cellular metabolism to new environmental conditions of limited nutrient and oxygen supply that are characteristic in the tumor microenvironment. Protein lysine acetylation has also emerged recently as a metabolism-coordinating mechanism and growing evidence has shown that acetylation regulation of metabolic enzymes plays an essential role in cancer. Consequently, inhibitors of lysine deacetylases (KDACis) have drawn attention as promising strategies for therapeutic intervention. According this statement, this study aimed to analyse the changes in the proteome of A549 non-small cell lung cancer (NSCLC) in response to hypoxia and KDACi treatments with a special focus on the proteins associated with metabolism. The quantitative proteomics approach was carried out by using the FASP (Filter Aided Sample Preparation) method combined with a dual protease digestion (Lys-C/ trypsin) before labelling the resulting peptides with iTRAQ 8-plex reagents. Then, the labelled peptides were fractionated in two dimensions (OFFGEL/RP nanoLC) prior MALDI-TOF/TOF mass spectrometry analysis. MS and MS/MS data were analysed by Protein Pilot software and quantitation was validated by the R package IsobarPTM. This proteomic approach led to the identification and quantitation of 834 proteins and evidenced the capacity of KDACis to reverse the tumor metabolic phenotype, an effect enhanced under hypoxia conditions, by changing metabolic enzyme expression profiles, especially in glycolysis and Krebs cycle. These enzyme expression changes were validated by Western Blot analysis. Moreover, glucose uptakes, production of lactate analysis and enzyme activity determination corroborated the implication of these enzymes in the reversion of the A549 NSCLC metabolic phenotype. Together, these results allow us to better understand how KDAC inhibitors control metabolic pathways under hypoxia in NSCLC.

Poster # 6

The 5'-Nucleotidases cN-II and CD73 Impact on the Metabolic Adaptability and Cell/Matrix Interactions of MDA-MB-231 Cells

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Keywords: nucleotides, metabolism, cell/matrix interactions

cN-II and CD73 are two 5'-nucleotidases that respectively dephosphorylate intracellular and extracellular AMP into adenosine. Considering nucleotide/nucleoside trafficking and their roles in cell biology, these enzymes could be involved together in other processes that increase cancer cells aggressiveness. In our project, we evaluate their impact on cell proliferation, metabolic adaptability and cell/matrix interaction. We modulated cN-II and/or CD73 expression in the human triple-negative breast cancer cell line MDA-MB-231 by shRNA.

These knockdowns did not affect MDA-MB-231 cells proliferation monitored by CFSE staining. Nevertheless, cN-II- cells displayed higher pAMPK activations than control cells in basal conditions. Surprisingly, these and CD73 low-expressing cells showed a better ability to survive under a stress that disables catabolic pathways, such as autophagy, ß-oxidation or glycolysis (30% less Annexin V positive cells, p

Poster # 7

Biomarqueurs protéiques associés la radiorésistance dans des modèles de gliomes de haut grade

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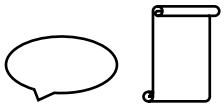
Keywords: gliome de haut grade, radiorésistance, RPPA, radiothérapie hypofractionnée

La radiothérapie joue un rôle majeur dans la prise en charge des gliomes de haut grade. Cependant, la radiorésistance de cellules de gliome limite son efficacité et entraine des récidives au sein du volume irradié à forte dose. Le but de cette étude, était d'identifier des biomarqueurs in vitro et in vivo prédictif de la radiorésistance des gliomes de haut grade. Nous avons testé la radiorésistance de 8 lignées cellulaire de gliome (survie clonogénique), de 6 modèles de xénogreffes dérivées de ces mêmes lignées et de 5 xénogreffes dérivées de patients (PDX) traitées par radiothérapie hypofractionnée. Une approche RPPA (Reverse Phase Protein Array) a été utilisée pour identifier des biomarqueurs protéiques prédictifs de la radiorésistance. Des protéines impliquées dans la réparation de l'ADN, l'apoptose, les voies de signalisation RTK, MAPK/ERK, SAPK/JNK, NFκB, HSP, PI3K/Akt, la régulation du cycle cellulaire et l'adhésion. In vitro, nous avons identifié FAK, HSP90, HSF1, HSPA2, la vimentine et l'intégrine ß4 comme biomarqueurs de la radiorésistance. EGFR/EGFR, Phospho-Chk1/Chk1 et VCP sont les marqueurs associés à la radiorésistance à une radiothérapie hypofractionnée in vivo. Nos données révèlent qu'aucun biomarqueur n'est conservé entre les modèles in vitro et in vivo soulignant la grande complexité des modèles étudiés. La radiorésistance des gliomes de haut grade est multiparamétrique et leur radiosensibilisation pourrait passer par une approche combinatoire.



Poster # 8

Translation Regulation of Selenoproteins, a Family of Selenium-Containing Enzymes Involved in Cancer Prevention

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Keywords: selenium, selenoproteins, translational control, mRNPs, cancer prevention, UGA recoding, SECIS

Selenium is an essential trace element in mammals, which deficiency leads to physiopathological disorders, increases cancer risks and reduces lifespan. Epidemiological studies provide evidences for the chemopreventive role of selenium in specific cancers, including prostate, lung, colorectal, stomach and multiple cancers. Several meta-analyses also confirm this finding. In addition, in several studies, long-term selenium supplementation of the diet reduces the risk of cancers incidence, and cancer death. However the cellular and molecular mechanism for selenium implication in cancer prevention remains poorly understood.

Selenium is incorporated as a rare aminoacid, selenocysteine (Sec), into an essential family of antioxidant enzymes, the selenoproteins, with contains 25 members in human. Selenocysteine is co-translationally inserted using a UGA codon, normally used to stop protein synthesis. Cells have evolved a mechanism of recoding UGA as Sec for the 25 selenoprotein mRNAs while maintaining its use as a stop codon in other cellular transcripts. Several cis- and trans-acting components of the selenocysteine insertion machinery have already been identified, including the stem-loop-stem-loop structure in the 3'UTR of selenoprotein mRNAs referred to as SECIS element. The SECIS is necessary and sufficient for faithful UGA/Sec recoding, and more importantly modulates the efficiency of Sec insertion in response to selenium variation, oxidative stress and cellular aging.

This UGA-selenocysteine translation elongation step, which is limiting for selenoprotein biosynthesis, is now evidenced to finely regulate the selective expression of the selenoproteome. In this work we present and discuss a model of translational control in which selenoprotein expression is mostly controlled at the level of selenocysteine insertion in response to various stimuli and/or pathophysiological conditions.

Poster # 9

Progesterone Signaling in Breast Cancer: Novel Insights

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Keywords: progesterone receptor, PRMT1, arginine methylation, breast cancer

Progesterone, a steroid hormone, through its receptors PRs regulates normal female reproductive development and functions. They have dual actions as a nuclear transcription factor and as a modulator of cytoplasmic signalling complexes that activates mitogenic protein kinases. Cellular response to progesterone is also regulated by receptor post-translational modifications that may affect receptor stability, subcellular localization and interactions with regulators. We have previously shown that PR is methylated by PRMT1 on several arginine residues and we identified three targets in its DNA-binding domain. Moreover, recent studies showed nuclear forms of PR when it is not activated by its hormone. These repressive actions are linked to interactions with proteins like the demethylase LDS1 or the Heterochromatin Protein 1 (HP1γ).

By in vitro and in vivo approaches, we are studying the impact of methylation on signalling pathways of PR. We also produced antibodies directed against the methylated forms of PRs; their characterization and their specificity are in progress. In T47D breast cancer cells, we demonstrated that PRs interact with PRMT1, mainly in the nucleus. Our results also indicate that PR methylation affects PR transcriptional activity and PRMT1 knockdown disrupts the rapid activation of protein kinase pathway after progestin stimulation. In nucleus, PR and PRMT1 interact without hormone stimulation and they seem to be part of a repressive complex on PR-target genes.

These data confirmed the importance of PRMT1-dependent methylation on PR signalling. In view of new light about PRs role in breast cancer, understanding its actions will be critical to develop new models that will advance our knowledge base and will reveal if these modified receptors can be therapeutically targeted.

Poster # 10

Characterization of NLRP7 Expression and Regulation in Normal and Tumor Human Placentation during the First Trimester of Pregnancy

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Keywords: choriocarcinoma, hydatidiform moles, metastasis, placenta, invasion, migration

Choriocarcinoma is a veritable placental cancer that develop upon abnormal pregnancies, such Hydatidiform moles (HMs). Choriocarcinoma can metastasis in multiple maternal organs, such as the lung, the vagina, the pelvic, the brain, and the liver. Recent studies established an association between recurrent HMs and mutations in a protein called NLRP7. NLRP7 is member of a new family of proteins involved in inflammatory processes. Nevertheless, the role and the biological functions of NLRP7 remain to be elucidated in normal and tumor human placentation. Using normal trophoblast cells (HTR), placental explants and choriocarcinoma cells (JEG3), we investigated NLRP7 expression, regulation and role in human placenta. Also, we compared its protein and mRNA levels in a cohort of HM patients' and gestational age matched controls.

We demonstrated that NLRP7 was more expressed during the hypoxic period of placental development compared to its oxygenated period. In HTR cells "normal trophoblast cells", hypoxia increased NLRP7 expression and its invalidation decreased their proliferation and increased their invasion. These results were confirmed in placental explant cultures. In JEG3 cells, NLRP7 expression was much higher compared to HTR cells and NLRP7 invalidation in JEG3 cells decreased their degree of invasion. More importantly, analyses of the normal and HM cohorts showed differential localization of NLRP7 within the placentas, along with a significant increase in its mRNA levels in HM patients.

Altogether our results demonstrate that NLRP7 is involved in normal placental development during the first trimester of pregnancy and that its deregulation might be associated to choriocarcinoma development. Its direct and differential control of trophoblast invasion in normal vs tumor trophoblast cells strongly suggest its potential involvement in choriocarcinoma progression. Further studies are ongoing to elucidate the role of this new protein in placental tumorogenesis.

Poster # 11

The sVEGFR1-i13 Splice Variant of VEGFR1 Regulates a BETA 1 Integrin/VEGFR Autocrine Loop Involved in the Response to Anti-Angiogenic Therapies of Squamous Cell Lung Carcinoma

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Keywords: squamous cell lung carcinoma, Bevacizumab, sVEGFR1, VEGFR-dependent autocrine loop

Vascular endothelial growth factors (VEGFs) and their receptors are regulators of physiological and pathological angiogenesis. Although targeting angiogenesis has become a key therapeutic strategy for cancer treatment, trials evaluating anti-angiogenic therapies (AAG) have failed to identify any benefits in patients with squamous cell lung carcinoma (SCC). Rather, these patients are at higher risk of bleeding complications when exposed to Bevacizumab (BVZ), a humanized monoclonal anti-VEGF-A antibody. The soluble VEGF receptor-1, namely sVEGFR1, is a truncated version of the cell membrane-spanning VEGFR1 that only retains the first six N-terminal Ig-like extracellular motifs of VEGFR1 owing to intron 13 retention and polyadenylation. Interestingly, some recent data highlighted sVEGFR1 as a potential biomarker of response to BVZ treatment. In this study, using various SCC cell lines, we showed that AAG increase the intra- and extra-cellular levels of sVEGFR1. Furthermore, in NCTU-induced SCC murine tumorgrafts models, we confirmed that the intra-tumoral level of sVEGFR1 significantly increases following VEGFR-TKI (sunitinib) or anti-VEGFR2 (DC101) treatment. Of note, this increase was never observed in the lung adenocarcinoma histological sub-type treated in the same conditions. At the molecular level, we unraveled an original signaling network by which sVEGFR1 regulates a ▫1 integrin/VEGFR1/VEGFR2 autocrine loop in an opposite manner, such an inverse regulation allowing to discriminate between AAG-sensitive or -resistant SCC cells. To conclude, our results provide the first evidence that AAG therapies upregulate sVEGFR1 expression in SCC cells. In addition, our data highlight sVEGFR1 as a dual factor that can trigger both anti-(expected) and pro-(unexpected) tumoral functions. These results might help: to define SCC patients eligible to anti-angiogenic therapies; to explain why SCC patients treated with Bevacizumab exhibit severe complications.

Poster # 12

Single Cell Genomics Analysis Pipeline Using DEParray Technology: An Application to Study Circulating Tumoral Cell Sub-Populations

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Keywords: single cell genomics, DEParray, Circulating tumoral cell

High-throughput Genomics analysis at single cell level resolution is a major revolution to understand molecular mechanisms occurring in heterogeneous tissues. These approaches allow us to decipher genetics portrait of cell subpopulations including rare cells present in physiological or pathological tissues (as stem cells or other underrepresented cells) or in blood. Different technologies coupling unique cell sorting (C1 single cell autoprep system from fluidigm, FACS, ICELL8 from Wafergen, chromium single cell from 10X genomics etc…) with DNA/RNA amplification followed by PCR or New Generation Sequencing analysis permit to reach genetics/transcriptomics and epigenetics knowledge at single cell level. However none of these technologies permit to associate cell phenotypic features such as size, shape, presence or absence of one or more target surface proteins and genetics features. The technology called DEPArray technology (Silicon Biosystems) allows purification of multiple different types of cells (by group of cells or at single cell level) from a single sample identified by combinations of multiple intracellular and extracellular markers, as well as with the use of morphological features such as circularity or size. This technology permits isolation of rare cells subpopulations coming from fresh culture, or Circulating Tumour Cells (CTC) (Molecular analysis of circulating tumor cells identifies distinct copy-number profiles in patients with chemosensitive and chemorefractory small-cell lung cancer. Carter L et al, Nat Med. 2016 Nov) but also from FFPE tissues (Digital Sorting of Pure Cell Populations Enables Unambiguous Genetic Analysis of Heterogeneous Formalin-Fixed Paraffin-Embedded Tumors by Next Generation Sequencing. Bolognesi C et al, Sci Rep. 2016 Feb).

ProfileXpert, the genomics and microgenomics platform of the Rhône-Alpes region has implemented recently this technology (fundings coming from the Gis-Ibisa and University Lyon1) and started in collaboration with the Hospices civils de Lyon a project on CTC analysis coming from patient with prostate cancer. We present here an example of workflow to analyse CTC including a preselection of CTC by the CellSearch followed by cell sorting with DEParray based on specific labelling (CD45-, EpCAM+, and cytokeratins 8, 18+, and/or 19+) and genotyping analysis by NGS of CTC population at single cell level.

Poster # 13

GNS561 a New Quinoline Derivative Inhibits the Growth of Hepatocellular Carcinoma in a Cirrhotic Rat Model

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Keywords: hepatocellular carcinoma (HCC), resistance, cancer, liver cirrhotic rat

Background: Quinoline derivatives are novel class of oral small molecules inducing inhibition of autophagy, apoptosis, and cell cycle modulation. Previously, anti-proliferative activity of GNS561 was demonstrated in vitro on HCC cell lines and the efficacy in vivo was evaluated in a PDX orthotopic BALB/c-nu mouse model. The aim of this study was to assess tolerance and efficacy of a new quinoline derivative GNS561 in a DEN-induced cirrhotic rat model with HCC.

Material and methods: Rats were diethylnitrosamine-injured during 14 weeks to obtain cirrhosis with fully developed HCC, then randomized into groups and treated for 6 weeks. Tumor progression was followed by MRI every 3 weeks. Pathological analysis and immunohistochemistry were blindly analysed.

Results: Mean number of tumors was significantly lower in GNS561 (n=46.4), in sorafanib (n=65.1) and in combination group of GNS561+sorafenib (n=40.6), when compared to control (n=100.4; p=0.0018, p=0.0295 and p=0.0003). Tumor decrease measured by MRI was associated with a significantly reduced proliferation of tumor cells, measured by Ki67 and Cyclin D1, particularly in GNS561 group (70%) and combination (84%) compared to control, whereas the effect of sorafenib alone on proliferation was modest (30%). Moreover, fibrosis area was reduced in GNS561 group compared to control (p=0.04) and in combination group compared to control (p=0.001) and compared to sorafenib group (p=0.015).

Conclusions: GNS561 was more efficient than sorafenib to control tumor growth in preclinical cirrhotic rat model of HCC and GNS561 combination with sorafenib exerted additive effect in controlling tumor progression. Based on its potent activity, GNS561 is now aimed to further reach clinical development in patients with HCC in 2017.

Poster # 14

Hétérogénéité tumorale et échappement métastatique des carcinomes mammaires triples négatifs

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Mots clés : cancer du sein triple négatif, métastase, hétérogénéité tumorale

Avec une incidence cumulée supérieure à 12%, le cancer du sein est, de loin, le cancer le plus fréquent chez la femme. Les tumeurs mammaires « Triple Négatif » (TNBC) restent un véritable challenge car leur statut invalide de fait les approches thérapeutiques ciblées (aucune expression des récepteurs aux œstrogènes, à la progestérone et expression nulle à faible de HER2). En effet, elles sont habituellement sensibles à la chimiothérapie pendant la phase initiale du traitement, mais près de la moitié met en place un mécanisme d'échappement, qui conduit à une récidive rapide, souvent dans les 2 ans. Cette forte adaptabilité témoigne de la présence de clones cellulaires aux propriétés très variées au sein d'une même tumeur. Comprendre l'échappement vers la production de métastases passe donc par l'analyse des tumeurs à l'échelle de la cellule unique et de leur interaction avec leur microenvironnement. Pour chaque couple tumeur primaire/métastase, la méthodologie retenue consiste à commencer par l'analyse fine du transcriptome des métastases afin d'en tirer une signature. Grâce à la technologie C1 de Fluidigm, cette signature sera alors recherchée dans un échantillonnage de cellules uniques issues de la tumeur primaire, afin de déterminer si certaines de ses cellules présentaient déjà le profil de la future métastase, et dans quelle proportion.
Les variations d'expressions observées seront confirmées au niveau protéique par TMA.
Une approche in vivo sur souris permettra également de rechercher et de caractériser les cellules tumorales circulantes avant qu'elles ne se fixent en métastases.
La corrélation des signatures "métastase" / "tumeur primaire" donnera des éléments qui permettront, à terme, de prédire le pouvoir métastatique d'une tumeur mammaire triple négative. Le traitement pourrait alors être adaptée.

Ce projet est financé par le CPER Epicure 2016 et intégrera les résultats générés sur les 3 modèles de cancer (prostate, colon et sein).

Poster # 15

Characterization of Alternative Splicing Signatures of Glioblastoma Stem Cells Deriving from Human Xenografts

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Keywords: glioablastom, cancer stem cells, alternative splicing,

Glioblastomas (GBM) are the most aggressive tumors in the adult brain. They contain a small number of treatment-resistant cells, the Glioma Stem Cells (GSCs), which are responsible for tumor initiation, growth, and relapse after standard treatments. Understanding the mechanisms underlying the survival and tumorigenic capacities of GSCs is needed to develop innovative and efficient therapies that are lacking to date. Alternative splicing is a major driver of transcriptome diversity and splicing alterations are known to promote tumorigenesis and invasion in solid cancers. As it remains unclear to what extent alternative splicing regulation favors GBM initiation and progression, we propose to investigate the alterations of splicing programs in 5 GSC in vitro models.

Using an RNA-Seq approach, we identified two alternative splicing factors enriched in GSC compared to differentiated samples. We are currently investigating their implication in the GSC phenotype using a loss-of-function strategy. In addition, using bio-informatics algorithms, we are analyzing the 5 GSC models transcriptome in order to identify specific splicing variants that might be regulated by our two candidates.

The perspective of this study aims at identifying splicing programs (qualitative changes) and gene signatures (quantitative changes) enriched in GSC comparing the transcriptomes of GSC, neural stem cells, and in vitro differentiated samples. This analysis will further establish a molecular signature of these aggressive cells, and potential new targets emerging from this work will be considered to improve the clinical outcome of patients with personalized treatments.

Poster # 16

Implication of Neuropilin-2 in the Progression of Small Intestinal Neuroendocrine Tumors: Towards a Promising Therapeutic Target?

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Keywords: gastroenteropancreatic neuroendocrine tumors, progression, axon guidance molecules, neuropilins

Object : Small intestinal neuroendocrine tumors (siNETs) are rare tumors that raise several clinical and therapeutic challenges. Current treatments are not efficient, in part because of a poor understanding of their mechanisms of progression. We recently highlighted some axon guidance molecules (AGMs) that might be implicated in the progression of siNETs. Among them, we are currently focusing our efforts on the semaphorin 3F receptor neuropilin-2 (NRP2). The objective of this study is to validate NRP2 as a potential therapeutic target.

Method : A tissue microarray gathering 34 cases of siNETs has been utilized to assess NRP2 expression. NRP2 serum level has been dosed in patients. In vitro, NRP2 expression has been silenced in the STC-1 cell line. An in vivo mouse model of tumor cell dissemination has also been used.

Results : We demonstrate that NRP2 is highly expressed in human siNETs. In addition, NRP2 serum level are increased in patients compared to controls. While NRP2 silencing does not seem to impact cell viability, proliferation or apoptosis, the use of a NRP2 blocking antibody decreases the viability of wild-type STC-1 cells. These clones also show a decrease in VEGFR2 expression. When grafted in nude mice, NRP2 silencing induces a strong antitumoral effect, associated with a diminution of cell proliferation and a decreased mTOR activation.

Conclusion : Our results suggest NRP2 as a potential promising therapeutic target for siNETs. Ongoing mechanistic studies aim at deciphering the underlying molecular mechanisms.

Poster # 17

Modulation of Androgen Sensitivity by Liver X Receptors in Prostate Adenocarcinomas

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Keywords: castration-resistant prostate cancer, androgen, LXR, cholesterol

Therapeutic treatment of metastatic and advanced prostate cancer is based on androgen deprivation, inducing tumor regression. However, most of the patients become resistant to hormone treatment leading to the progression of the pre-existing disease and/or the appearance of new metastases. This tumor growth corresponds to the emergence of the castration-resistant stage. Deciphering the molecular mechanisms underlying castration resistance development is needed. Among metabolic alterations associated with prostate cancer progression, cholesterol accumulation is now considered as a hallmark of cancer aggressiveness. This supports tumor growth and metastatic dissemination of tumor cells and is particularly deregulated in castration-resistant state.

Nuclear Receptors LXRs (Liver X Receptors) are major sensors of intracellular cholesterol. They exert anti-proliferative and pro-apoptotic effects in prostate cancer cells. Previous works from our team demonstrated that LXRs reduce tumor invasiveness and limit metastatic dissemination of prostate tumor cells in vivo. Moreover, our lab and others showed that LXRs can modulate transcriptional activity of androgen receptor in normal mouse prostate.

In this context, we analyze the response to the castration of our mouse model of metastatic prostate cancer invalidated for Pten and Lxrs (Ptenpc-/-;Lxrαß-/-) in comparison with the common model of prostate adenocarcinoma invalidated for Pten alone (Ptenpc-/-).

Lxr knockout leads to an increase in cell proliferation and expression of prostate cancer aggressiveness markers in absence of androgens. This correlates with an in vivo and in vitro altered expression of androgen regulated genes. Moreover, Ptenpc-/-;Lxrαß-/- mice show a dramatic increase in metastatic dissemination in response to castration. Finally, Lxr knock-out leads to the establishment of a very aggressive castration-resistant prostate cancer in Ptenpc-/- mouse model, with an enhanced metastatic dissemination.

Poster # 18

Characterization of a Specialized Ribosome Biogenesis Factor in Embryonic Stem Cells : Towards the Concept of Specialized Ribosomes

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Keywords: stem cells, ribosome biogenesis factor

Embryonic stem cells (ESC) and cancer stem cells (CSC) share properties like self-renewal (the ability to proliferate in a same state) and acute cell plasticity (pluripotency and multipotency, respectively). Several teams have thus identified shared gene expression signatures between ESC and CSC. Understanding the mechanisms governing ESCs, and more generally stem cell maintenance present thus a great potential for identifying innovative therapeutic strategies in oncology. ESC identity (self-renewal and pluripotency) and regulation of gene expression programs are controlled at different level: epigenetic, transcriptional and post-transcriptional level. More recently, the role of the translational machinery, ribosomes, has emerged as implicated in the hometostasis of adult and embryonic stem cell in several model organisms. Starting our study from transcriptomic data, my team has identified different ribosome-associated proteins (RaPs) significantly enriched in mouse embryonic stem cells compared to differentiated murine cell lines. Among these candidates, we have focused on one particular RaP which expression profile suggests specific role during differentiation: it demonstrates a strong enrichment at the transcriptomicl and proteomic level in naïve ESCs while its expression is significantly decreased upon differentiation. We are currently evaluating the contribution of this ribosome biogenesis factor in embryonic stem cell homeostasis and uncovering whether this factor may participate in generating stem cell specialized ribosomes. Interestingly, we established that this RaP is also expressed in human ESCs and in glioblastoma cancer stem cell models. Therefore, pursuing the molecular characterization of this RaP in ESC will pave the road towards defining its function in this pathological context and establishing whether targeting its activity is a reliable strategy to manipulate cancer stem cell fate decisions.

Poster # 19

Role of a Ribosome Biogenesis Protein in Embryonic Stem Cells Maintenance

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Keywords: embryonic stem cells, ribosome biogenesis, self-renewal, pluripotency

Control of gene expression programs orchestrating embryogenesis and early development is an active field of research. Progression in the understanding of these mechanisms could contribute to progress in regenerative medicine, human pathologies modeling and oncology. Indeed, embryonic stem cells (ESC) and cancer stem cells (CSC) share properties like self-renewal (the ability to proliferate in a same state) and acute cell plasticity (pluripotency and multipotency, respectively). Some teams have thus demonstrated shared gene expression signatures between ESC and CSC in different models. Understanding the mechanisms governing ESC maintenance present thus a great potential for identifying innovative therapeutic strategies in oncology. ESC identity (self-renewal and pluripotency) and regulation of gene expression programs is controlled at different level: epigenetic, transcriptionnal (by transcription factors like Oct4, Nanog, Sox2, Myc and Klf4) and post-transcriptionnal level (alternative splicing and non-coding RNAs). More recently, the role of the translationnal machinery, ribosomes, has emerged as implicated in stem cell homeostasis in different species. Starting the analysis from transcriptomic data (RNA-seq, GEO database), my team has identified different ribosome-associated proteins (RaPs) significantly enriched in mouse embryonic stem cells compared to differentiated murine cell lines. Among these candidates, we have focused on one particular RaPs which expression profile suggests specific role during differentiation: enrichment at the transcriptionnal and protein level in ESC and important decrease during differentiation. We are currently defining the function of our candidate protein in mouse ESC maintenance (high cell proliferation potential, self-renewal and pluripotency).

Poster # 20

The rRNA Epigenetic Hypothesis: Role of Ribosome Heterogeneity in Tumorigenesis

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Keywords: ribosome, rRNA, 2'-O-Methylation, translation, breast cancer

Ribosomes are molecular machines responsible for all cellular protein synthesis. Ribosomes are composed of 80 proteins and 4 RNAs. Ribosomal RNAs (rRNA) are key components of the translation machinery, carrying-out the decoding of mRNAs and catalyzing the peptide-bond formation. Human rRNAs carry more than 200 chemical modifications, including pseudouridylation and 2'-O-ribose methylation (2'-O-Me).

Alteration of ribosome biogenesis is a landmark of cancer cells. Recently, our group demonstrated that cancer cells not only produce more ribosomes, but ribosomes that are qualitatively different compared to non-transformed cells. Specifically, in breast tumor cells, 2'-O-Me of rRNA is increased as a consequence of up-regulation of Fibrillarin (FBL) the unique methyl-transferase of rRNAs (Marcel. V, et al. Cancer Cell 2013).

We are exploring the clinical relevance of rRNA modifying factors and rRNA 2'-O-methylation, and their impact on tumorigenesis. In several breast cancer cohorts, FBL expression correlated with disease outcome. In cellular models of breast and colorectal cancer, FBL over-expression favored tumorigenesis.

Using RiboMethSeq, a new RNA-Seq based method to exhaustively and quantitatively map rRNA 2'-O-methylation, we showed that 2'-O-Me can be modulated at critical position within the ribosome structure and provides functional plasticity to the ribosome. Using in cellulo and in vitro approaches, we show that modifying 2'-O-Me of rRNAs altered translation fidelity, and translation control of internal ribosome entry site containing (IRES) mRNA that are involved in tumorigenesis.

In conclusion, our studies demonstrate that tumor cells produce ribosomes with an altered rRNA composition, which impacts translational control. 2'-O-Me plasticity expands the repertoire of ribosome composition and contributes to the diversity of ribosomes population. These data highlight rRNAs as target of RNA epitranscriptomic regulation of gene expression during tumorigenesis.

Poster # 21

Crosstalk Between IGF1 & Estrogen Receptor Non-Genomic Signaling Pathway in Breast Cancer

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Keywords: breast cancer, estrogen non-genomic signaling, IGF-1R, methylation, phosphorylation

Breast cancer is a major health problem currently affecting 1 out of 5 women. Seventy percent of breast cancers are hormone-dependent, and are treated by hormonal therapies targeting estrogen receptor and consequently the inhibition of its pro-tumorigenic effects. In parallel to the genomic estrogen signaling, non-genomic signaling has been described, where ERα recruits Src kinase and PI3K at the plasma membrane and thus activates downstream phosphorylation cascades like AKT, which in turn leads to survival and proliferation of cancer cells. Our team has found that estrogen-induced methylation of arginine 260 of ERα is a prerequisite for the formation of this non-genomic complex, regulating cell proliferation (Le Romancer et al. Mol Cell, 2008).

In 2012, we have shown that this pathway is activated in aggressive breast tumors representing a new potential target for breast cancer therapy (Poulard et al. Embo Mol Med, 2012).

Crosstalk between estrogen and growth factors signaling involving phosphorylation has been largely described. Hence, we investigated if ERα R260 methylation could be involved in this crosstalk. Among several growth factors, we found that IGF-1 only was able to induce methylation of ERα in an estrogen-independent manner. Like estrogen, IGF-1 induces a rapid and transient methylation of ERα by the Protein Arginine Methyltransferase (PRMT1) concomitant with the formation of ERα/Src/PI3K complex. Moreover, we found that upon IGF-1 stimulation, IGF-1R and ERα showed increased interaction using In vitro and PLA assays. In line with these results, we found an interaction between those receptors in patient's positive tumors and we're validating these data on TMAs of a cohort of 440 breast tumors from Centre Leon Berard. Interestingly, we've recently found also that IGF1R phosphorylates the DNA binding domain of ERα that could modulate the latter downstream signaling. These results open new perspectives of combining therapies targeting the two pathways.

Poster # 22

Unveiling the Mechanisms of the RNA-Binding Protein Musashi1 in Stemness and Drug Resistance of Intestinal Epithelial Cells

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Keywords: intestinal stem cells, intestinal cancer, drug resistance

Background. The RNA-binding protein Musashi1 (Msi1) plays a role in stem cell biology of several tissues including the intestinal epithelium. In addition a link between Msi1 expression levels and intestinal tumor stage or with drug resistance has also been observed. With the aim to investigate the mechanisms of Msi1 function on intestinal stem cells and drug resistance, we took advantage of the v-Msi1 mice recently developed in our laboratory, harboring an ectopic Msi1 expression in the whole intestinal epithelium.

Results. We used an approach of 3D-organoid cultures from WT and v-Msi1 crypts treated or not with 1.5 ug/mL 5-FU, a drug commonly used to treat intestinal cancers. We first assessed the number of viable organoids and their growth characteristics at different time-points after treatment. Interestingly, 5-FU treatment strongly reduced the growth and viability of WT but was inefficient on v-Msi1 organoids. In order to link the effects on organoid's growth with drug resistance and stemness, we analysed the expression of enzymes involved in 5-FU metabolism and action and the role on Lgr5-positive stem cells. Contrariwise to the WT condition, the v-Msi1 organoids presented a drug resistant phenotype, as indicated by the expression pattern of 5-FU metabolizing enzymes, and that stem cells were not affected by 5-FU treatment.

In conclusions. These results suggest that Msi1 is a key regulator of both stemness and resistance to 5-FU. Our next objectives will be an in depth investigation of this process in animals in vivo in both physiological conditions and in tumors.

Poster # 23

The Pyrrolopyrimidine Derivative PP-13 is a Novel Tubulin-Binding Agent with Promising Anticancer and Antimetastatic Properties

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Keywords: pyrrolo-pyrimidin structure, resistance, spindle poison

Despite the emergence of targeted therapies and immunotherapy, chemotherapy remains a gold-standard for the treatment of numerous cancers. Spindle poisons that interfere with microtubule dynamics are commonly used in chemotherapy drug combinations. However, their troublesome side effects and the emergence of resistance make essential the development of alternative agents. Thanks to a high throughput cell-based assay, we screened agents able to restore apoptosis in apoptosis-resistant cancer cells. We selected a new microtubule-targeting agent (PP-13) with pyrrolo-pyrimidine structure and investigated its anti-cancer effects in vitro and in vivo.

PP-13 effects on cell cycle, cytotoxicity, and apoptosis were determined in representative cancer cell lines by flow cytometry, MTT, and western blot. The molecular target of PP-13 was identified using in vitro tubulin polymerization test, competition fluorescence assay and electron microscopy. Subcellular effects of PP-13 were followed by immunofluorescence assays and time-lapse videomicroscopy. Anticancer effects of PP-13 were evaluated by wound healing and angiogenesis assays in vitro, and in vivo tumor growth of xenografts on chicken chorioallantoic membrane.

PP-13 exerted cytotoxic effects on a large panel of cancer cell lines, including those resistant to targeted therapies and chemotherapy with IC50 in the nanomolar range. By interfering with mitotic spindles organization and dynamics, PP-13 impaired the congression of the chromosomes, promoted mitotic cell cycle arrest and finally led to aneuploidy, mitotic slippage or direct apoptotic death. PP-13 targeted tubulin and competed with colchicine for the binding to the same site. Interestingly, in vivo experiments showed that PP-13 inhibited tumor and metastases growth without any noticeable toxicity.

Our results suggested PP-13 to be a promising and potent alternative to conventional spindle poisons for cancer treatment, even for chemoresistant cancers.

Poster # 24

Analyse de l'ADN circulant dans le plasma ou sérum de patients porteurs de neuroblastome

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Mots clés : biopsie liquide, ADN circulant, neuroblastome

Le neuroblastome (NB), prolifération maligne d'origine neuroectodermique est la tumeur solide la plus fréquente chez l'enfant de moins de 5 ans. Cette tumeur présente une grande hétérogénéité clinique. Différents marqueurs génomiques ont été identifiés tels que : l'amplification de l'oncogène MYCN, les mutations du gène ALK et la présence d'altérations segmentaires au niveau des chromosomes (présence de gain ou perte de fragment de chromosomes). Ces marqueurs sont actuellement utilisés pour orienter la décision thérapeutique.
Comme la tumeur n'est pas toujours disponible pour permettre l'analyse de ces marqueurs génomiques, notre laboratoire s'intéresse depuis plus de 10 ans à l'analyse de l'ADN libre circulant (cfDNA) issus du plasma ou sérum de patients porteurs d'un NB. Utilisant la qPCR, la ddPCR (droplet digital PCR) ou encore les puces Oncoscan, nous avons montré que:
-La quantité de cfDNA augmente avec le stade de la maladie.
-Les séquences d'ADN MYCN circulantes peuvent être détectées par qPCR ou ddPCR au diagnostic dans le plasma ou le sérum. La spécificité du test est de 100% et la sensibilité est stade dépendante (10% (stage I-II), 75% (stage III) and 85% (stage IV or IVS).
-La détection des séquences MYCN circulantes représente un bon marqueur pour évaluer la réponse à la thérapie et identifier la progression tumorale chez les patients porteurs d'une tumeur montrant une amplification de MYCN.
-Les mutations d'ALK peuvent être identifiées par ddPCR au diagnostic dans le plasma ou le sérum. Il existe une excellente concordance entre les résultats obtenus sur cfDNA et l'ADN issu de la tumeur primaire.
-Le cfDNA peut refléter l'hétérogénéité tumorale.
L'ensemble de ces résultats montre que, chez les enfants porteurs d'un NB de stade avancé, le cfDNA issus de biopsies liquides (comme le plasma ou le sérum) contient de l'ADN tumoral circulant qui peut être analysé afin d'identifier des altérations génomiques et ainsi permettre d'orienter la thérapie.

Poster # 25

Expression of Δ40p53 and Δ133p53, Two Isoforms of p53 Lacking the N-Terminus, in Cutaneous Melanoma

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Keywords: melanoma, metastasis, p53, isoforms, p53 inactivation

Object: Cutaneous melanomas are highly malignant tumours that retain wild-type TP53 in the majority of cases (90%), suggesting that p53 suppressive activity is abrogated through alternative mechanisms. The TP53 gene encodes 12 isoforms that differ in their N- and C-terminus due to alternative splicing, promoter or codon initiation usage. So far, there is growing evidence that some of the isoforms can have an important role to play as dominant inhibitors of p53 suppressive activity.

Method: We developed a strategy for identifying p53 isoforms at both mRNA and protein levels and analysed their expression in several cell lines derived from malignant melanoma. We have also used mRNA extracted from stage I-IV human cutaneous melanoma samples, with well defined metastatic status to analyse the expression of FSp53 (Fully-Spliced) mRNA, p53I2 and p53I4 mRNA, encoding Δ40p53 and Δ133p53, respectively.

Results: In our study, p53 and Δ40p53 appear to be the predominant isoforms expressed in melanoma cell lines. Compared to primary stage I-II melanoma, enhanced expression of either p53I2 or p53I4 was detected in a substantial proportion of metastatic cases (70%; 21/30). When sub-grouped according to metastatic status, enhanced expression of either p53I2 or p53I4 was more frequent in nodal (9/10) and distant (8/10) than in regional dermal (4/10) lesions (p=0.044, Fisher-Exact). Whereas enhanced expression of FSp53 mRNA was detected only in cases with enhanced expression **of p53I2, p53I4 alone was increased in 6/30 lesions (20%).**

Conclusion: Overall, these results demonstrate that expression of mRNA encoding N-terminal p53 isoforms Δ40p53 and Δ133p53 increased during metastatic progression of cutaneous melanoma, suggesting a role for p53 isoforms in inactivating p53 suppressor functions.

Poster # 26

Effects of Spiro-bisheterocycles on Proliferation and Apoptosis in Human Breast Cancer Cell Lines

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Keywords: spiro-bisheterocycles, oxygen and nitrogen heterocycles, breast cancer, cell proliferation, apoptosis, p53, MDM2.

Breast cancer is the first cause of cancer death in women worldwide and a major concern to public health. The main axes of this work consist in studying the antiproliferative and pro-apoptotic effects of spiro-bisheterocycles on human breast cancer cell lines MCF-7 and MDA-MB-231. On the structural point of view, the compounds feature a hydantoin moiety attached to either diazole, isoxazole, diazepine, oxazepine or benzodiazepine via the privileged tetrahedral spiro-linkage. The treatment with compounds 3 and 6, corresponding to spiro [hydantoin-isoxazole] and spiro [hydantoin-oxazepine] respectively, resulted in a significant decrease of cell proliferation and the induction of the apoptosis in both breast cancer cell lines. However, the compound 4 (spiro [hydantoin-diazepine]) demonstrated an antiproliferative activity only against MDA-MB-231. The qRT-PCR revealed an up-regulation of MDM2, strictly p53-dependent, and an increase of the expression of pro-apoptotic genes such as caspase 3 and BAX in MCF-7 wild-type p53 and MDA-MB-231 mutant p53 breast cancer cells. In summary, the results suggested that our compounds promoted the apoptosis of breast cancer cell lines through p53-dependent and independent pathways.

Poster # 27

L'épissage alternatif des transcrits : un rôle dans l'échappement des tumeurs pulmonaires aux thérapies anti-EGFR ?

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Keywords: cancer du poumon, EGFR-TKI, résistance, épissage alternatif, protéines SR

Les thérapies ciblant l'activité tyrosine kinase de l'EGFR (EGFR-TKI, Géfitinib) sont particulièrement intéressantes dans les adénocarcinomes pulmonaires présentant une mutation activatrice de l'EGFR et sont utilisées en première ligne de traitement dans cette indication. Cependant après 15 à 20 mois de traitement, la tumeur progresse à nouveau. La caractérisation des mécanismes de résistance acquis en réponse aux EGFR-TKI est donc un enjeu crucial. Les protéines sérine/arginine rich (SR) sont impliquées dans le recrutement du splicéosome et la sélection des sites d'épissage de l'ARN pré-messager. Il a été montré que des anomalies de l'épissage alternatif pouvaient être à l'origine de mécanismes de résistance aux thérapies ciblées dans certains cancers. Nous avons généré des modèles cellulaires de tumeur pulmonaire avec une résistance acquise au gefitinib, qui ne présentent aucun mécanisme de résistance connu. En comparaison à la lignée parentale sensible, nous avons observé une augmentation de l'expression et/ou de la phosphorylation des protéines SR SRSF1/2. De façon importante, la neutralisation par siARN de ces facteurs d'épissage re-sensibilise les clones résistants à l'apoptose induite par le gefitinib. Ceci suggère qu'une signalisation impliquant certaines protéines SR pourrait contribuer à l'échappement des cellules tumorales aux thérapies ciblées anti-EGFR dans les cancers du poumon. Grâce à une analyse de séquençage de l'ARN à large échelle, nous avons identifié et validé des variations d'épissage alternatif affectant plusieurs dizaines de gènes dans les clones résistants. Parmi ces gènes certains voient leur expression régulée par SRSF1/2 en réponse au géfitinib. Nous avons identifié un gène de l'autophagie, ATG16L1, dont l'épissage est redirigé vers un profil « sensible-like » lors de la resensibilisation des clones au gefitinib via la neutralisation de SRSF1/2.

Poster # 28

Développement et caractérisation d'un modèle *in vitro* pour l'étude du microenvironnement du cancer de la prostate

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Keywords: cancer de la prostate, sphéroïdes, microenvironnement, culture en trois dimensions, LNCaP-Luc, hypoxie, résistance aux médicaments

Dans le cancer de la prostate, de plus en plus d'études tendent à démontrer le rôle prépondérant de l'interaction des cellules tumorales avec leur microenvironnement et soulignent la pertinence des approches thérapeutiques ciblant ces deux composantes. Ainsi, l'émergence de ces stratégies nécessite le développement de modèles se rapprochant de l'environnement cellulaire, prenant en compte l'architecture tissulaire et les régulations entre cellules et matrices telles que les nouvelles techniques de culture en 3D (sphéroïdes). L'objectif de ce projet est de créer et de caractériser un modèle de sphéroïdes de cellules de cancer de la prostate humaine, exprimant le PSMA, pour étudier l'efficacité de molécules thérapeutiques in vitro. Pour cela, 1000 cellules LNCaP-Luc ont été ensemencées avec 0,5% de méthylcellulose dans des plaques 96 puits non adhérentes. Les études d'immunofluorescence, montre une prolifération cellulaire (Ki-67) essentiellement en périphérie du sphéroïde associée à la formation d'un cœur apoptotique (TUNEL), dès le stade J7. La microscopie électronique à balayage et les imageries bioluminescentes et fluorescentes (sonde Lox-1), révèlent l'installation d'un gradient d'oxygène, entrainant la formation d'une zone hypoxique au cours de la croissance. Cette hypoxie a pu être corrélée à une augmentation du VEGF secrété. La sensibilité vis-à-vis de chimiothérapies a été évaluée sur culture 2D et 3D. Les sphéroïdes montrent une résistance plus importante au docétaxel et au TH-302, activable en hypoxie, comparativement aux cultures 2D. Pour le docétaxel, cette résistance augmente avec le stade des sphéroïdes alors que l'activité du TH-302 est potentialisée par l'environnement hypoxique. Pour conclure, le développement de sphéroïdes de cellules LNCaP-Luc propose un modèle simple, se rapprochant d'une micro-tumeur, qui semble particulièrement pertinent pour la validation de nouvelles approches thérapeutiques ciblant prolifération et microenvironnement.

Poster # 29

The Anticancer Drug Transporter, ABCG2, and its Involvement in Chemoresistance: Structural and Mechanical Study

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Keywords: BCRP/ABCG2, ABC transporter, multidrug resistance, chemotherapy, structure, inhibitors

The Breast Cancer Resistance Protein (BCRP) or ABCG2 is a member of the ATP-Binding Cassette (ABC) transporters superfamily. It was first discovered in 1998 by its involvement in resistance to breast cancer treatments. It is an efflux pump which exports a wide range of chemically unrelated drug molecules out of the cells. Hence, it is responsible for the multidrug resistance phenotype encountered in chemotherapy. Many efforts are made to develop inhibitors of the transporter that would restore the sensitivity of cancer cells. However, the high resolution structure of ABCG2 is yet unknown, which limits the understanding of the transport mechanism and thus the rational design of these inhibitors.

The aim of this project is the resolution of the ABCG2 three dimensional structure by X-ray crystallography. The human ABCG2 is overexpressed in the bacterial system E.coli, and purified by Nickel affinity chromatography followed by size exclusion chromatography, yielding an homogeneous pure population. We showed that ABCG2 binds its ligand Hoechst 33342 in a cooperative binding manner. The binding affinity is 3 µM (KD = 3 µM) and it is accompanied by a blue shift of tryptophan fluorescence indicating an environmental change to a more hydrophobic region. This value is comparable to the KM of Hoechst 33342 transport by ABCG2 through the membrane in HEK293 cells, a native environment, therefore revealing that the protein is correctly folded in our system.
These results pave the way to future studies of this transporter to better understand its mechanism of action, based on structure-function relationships and consequently facilitate inhibitors conception.

Poster # 30

Effect of Novel AKT Inhibitor ARQ 751 as Single Agent and its Combination with Sorafenib on Hepatocellular Carcinoma in a Cirrhotic Rat Model

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Keywords: hepatocellular carcinoma , cirrhotic rat model, fibrosis

Background and aim: Hepatocellular carcinoma (HCC) is the 2nd leading cause of cancer-related mortality worldwide. AKT pathway is activated in almost 50% of HCC cases and longer exposure to Sorafenib, classical treatment of advanced HCC over-activates AKT pathway, leading to HCC resistance to Sorafenib treatment. Here, we studied the efficacy of a new 2nd generation AKT inhibitor ARQ 751 alone or in combination with Sorafenib. To identify specific adverse effects related to the background of cirrhosis, we used diethylnitrosamine (DEN)-induced cirrhotic rat model which faithfully reproduce human scenario of advanced HCC.

Method: Rats were treated weekly with intra-peritoneal injections of DEN during 14 weeks to obtain cirrhosis with fully developed HCC. After that, rats were randomized into 4 groups (n=7/group): control, sorafenib, ARQ 751 and combination of sorafenib+ARQ 751 and treated for 6 weeks. Tumor progression was followed by 3 MRI. Pathological analysis and immunohistochemistry was analysed in a double blind manner.

Results: Tumor progression in rat liver was significantly reduced with treatment of ARQ 751 as single agent (91.5%) compared to the control group (158.8%), but the greatest effect was observed in combination group (49.4%) compared to the control, ARQ 751 or sorafenib group (105.7%). Tumor size was significantly reduced in both ARQ 751 group and combination group compared to the control group or sorafenib group. Similarly, mean number of tumors was significantly lower in ARQ 751 group and combination group when compared to the control group or sorafenib group. Tumor cell proliferation was decreased and fibrosis was improved by ARQ 751 and combination treatment.

Conclusion: ARQ 751 as single agent and its combination with sorafenib exerted strong suppression in tumor progression and improved liver fibrosis. Thus, results provide a strong rationale for testing ARQ 751 in clinical settings and also confirm the importance of targeting AKT in HCC.

Poster # 31

The Splicing Factor SRSF2 is an Early Component of the DNA Damage Response in Lung Cancer Cells

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Keywords: lung cancer, DNA damage response, cisplatin, SRSF2

The splicing factor SRSF2 belongs to the family of SR (Ser-Rich Arginine) proteins. These proteins play a crucial role in both constitutive and alternative splicing of pre-mRNA. As compared to normal lung tissues, we previously described the upregulation of SRSF2 protein in Non Small Cell Lung Carcinoma as well as in neuroendocrine lung carcinoma (Gout et al., 2012, Edmond et al., 2013). These results indicated that a deregulated expression of SRSF2 likely contributes to lung tumorigenesis. In addition, we previously demonstrated that SRSF2 accumulates in various NSCLC cell lines in response to cisplatin and is required for cisplatin-induced apoptosis (Edmond et al., 2011). These data indicated that SRSF2 contributes to the signaling pathways that are activated following DNA Damage, known as the DNA Damage Response (DDR). The goal of this study was to deepen these results. We first demonstrated that the knock-down of SRSF2 prevents the activation of early markers of DDR such as phosphorylation of ATM(Ser1981), H2AX(Ser139) or NBS1(Ser143), as well as acetylation of H2AX(K5) in cells treated with cisplatin. In addition, using several technics (co-immunoprecipitation, Proximity Ligation Assay, GST Pull-Down), we provided the first evidence that SRSF2 interacts with the MRN complex, a sensor of DNA double strand breaks including MRE11, RAD50 and NBS1 proteins, and promotes its accumulation in chromatin-enriched fractions. This interaction was stimulated by cisplatin treatment. By using siRNA, we further demonstrated that MRN integrity is required for SRSF2-induced DDR activation. Importantly, a mutant of SRSF2 that less interacts with MRN was less efficient in triggering DDR activation in response to cisplatin. Finally, we showed that SRSF2 interferes with DNA repair pathways such as homologous recombination and Non Homologous End Joining. As a whole, these data support a role of SRSF2 as an early component of the DNA Damage Response in lung cancer cells.

Poster # 32

Crosstalk Between LKB1 and the Arginine Methyltransferase PRMT5 in Breast Cancer

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Keywords: breast cancer, non-genomic pathway, LKB1, PRMT5, phosphorylation.

Breast cancer is the most occurring cancer in women. In addition to the estrogen genomic pathway, the non-genomic pathway also exists where ERα forms a cytoplasmic complex with Src and PI3K. Our team has demonstrated that the methylation of ERα on R260 is a prerequisite for the complex formation and that this complex is highly expressed in aggressive breast tumors. Amongst the partners of metERα in the cytoplasmic complex, the serine/threonine kinase LKB1 was identified. It is known for its onco-suppressive properties through negative regulation of the mTOR pathway.

Our team identified LKB1 to be a specific protein partner of PRMT5, a major type II arginine methyltransferase implicated in oncogenic processes. PRMT5 is found to be overexpressed in many cancer types including breast cancer where its expression is associated with poor patient outcome.

To understand the functional role between LKB1 and the PRMT5 in breast cancer, we used in vitro and in cellulo approaches allowing us to demonstrate the existence of a direct interaction between LKB1 and PRMT5 in various breast cancer cell lines. Interestingly, using in vitro phosphorylation approaches, we demonstrated that LKB1 phosphorylates PRMT5 in its N-terminal domain and consequently, the phosphorylated residues were identified.

Our team is currently investigating the downstream consequences of PRMT5 phosphorylation by LKB1 to decipher specific signaling pathways initiated by this modification and the consequent cellular functions.

Poster # 33

Biomarqueurs prédictifs de la réponse au traitement par radiothérapie et chimiothérapie dans les cancers des VADS : résultats préliminaires de l'étude ancillaire CHEMRAD

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Mots clés : cancer des VADS, radiothérapie, facteurs predictifs, biomarqueurs

Introduction : Malgré la combinaison de stratégies thérapeutiques comme la chirurgie, la chimio- et radio-thérapie, la survie à 5ans des patients atteints de cancer des VADS reste inférieure à 30% en raison de l'apparition de récidives locorégionales et/ou de métastases. L'étude ancillaire ChemRad a pour but d'identifier des marqueurs biologiques de la réponse tumorale à la radio-chimiothérapie afin d'orienter précocement les patients vers la meilleure stratégie thérapeutique.

Méthodes : Trois prélèvement biopsiques tumoraux et deux sanguins sont réalisés, avant traitement, pendant (chimiothérapie d'induction +/- radiothérapie ou RT seule), et en cas de récidive, sur une cohorte de 60 patients. Un des échantillons biopsiques est maintenu en culture ex vivo afin de tester la réponse à la RT, un autre est destiné à l'établissement d'une lignée primaire et le dernier permet le typage HPV et différentes analyses moléculaires. Les prélèvements sanguins sont destinés à l'isolement et l'analyse des cellules tumorales circulantes (CTCs), marqueur d'agressivité et de progression tumorale.

Résultats : Les différentes étapes permettant le maintien en survie des biopsies ex-vivo ont été mises au point ainsi que le protocole d'irradiation et de suivi de la réparation des cassures double-brin de l'ADN. Le protocole d'isolement et de comptage des CTCs a été développé en parallèle ainsi que leur caractérisation immuno-histochimique, en particulier par des marqueurs de cellules souches cancéreuses et de la transition épithélio-mésenchymateuse. Une corrélation sera établie entre les résultats obtenus à partir de la biopsie, de la lignée primaire et des CTCs et la réponse au traitement des patients.

Conclusion : Ce travail permettra à terme une meilleure compréhension du mécanisme d'échappement tumoral et l'identification de marqueurs prédictifs de la résistance aux traitements.
Soutenu par Cancéropôle CLARA, Grenoble-Alpes Métropole, Conseil Régional Rhône-Alpes et Conseil Général.

Poster # 34

AKT Inhibitor ARQ 092 and Sorafenib Additively Inhibit Progression of Hepatocellular Carcinoma and Improve Liver Microenvironment in Cirrhotic Rat Model

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Keywords: liver, HCC, cirrhosis

Hepatocellular carcinoma (HCC) is the second most common cause of cancer-related mortality worldwide. Longer exposure to classical treatment of advanced HCC, sorafenib, often over-activates AKT pathway, leading to HCC resistance. Moreover, AKT pathway itself is activated in almost half of HCC cases. Therefore, we investigated the efficacy of combination of Sorafenib with allosteric Akt inhibitor ARQ 092 in a diethylnitrosamine (DEN)-induced cirrhotic rat model with HCC.

Fisher 344 rats were DEN-injured during 14 weeks to obtain cirrhosis with fully developed HCC, then randomized into 4 groups: control, sorafenib, ARQ 092 or combination of ARQ 092+sorafenib and treated for 6 weeks.

MRI showed significantly reduced tumor progression by combination treatment (53 %) compared to the control (158 %; $p<0.0001$), sorafenib (106 %; $p=0.006$) or ARQ 092 group (105 %; $p=0.010$). Mean number of tumors and tumor size were lower in combination group when compared to the control or sorafenib group. Tumor decrease was associated with a significant reduction in tumor cell proliferation and an increased apoptosis. CD34 staining showed reduced angiogenesis in combination group compared to the control or sorafenib group. The results from Sirius red staining showed a decrease in fibrosis of animals treated with ARQ 092 alone or with combination of ARQ 092. Granulocyte/T-cells ratio in blood was decreased in all treated groups and the accumulation of neutrophils and macrophages in liver tissue was reduced compared to the control group. Western blot analysis of liver tissues showed a significant reduction of phosphorylation of AKT and its downstream signalling actors in both ARQ 092 and combination groups.

In conclusion, combination of ARQ 092 and sorafenib exerted additive effect in controlling tumor progression and improved immune response in blood and liver. Our results confirm the importance of targeting AKT in HCC.

Poster # 35

Understanding and Controlling Specific Dynamic Equilibrium in Adhesion Structures

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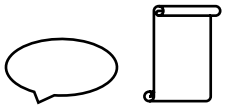
Keywords: optogenetic, oncogene, invasion, cell signaling

Migration and invasion processes are dependent of specific adhesive structures. Strikingly, these different structures are sharing numerous identical components. Thus, it was proposed that the characteristic of these adhesion structures is not only dependent of a specific signaling node but could also be under the control of specific dynamic equilibrium. It appears that manipulating in space and/or in time these dynamic equilibria could be a good strategy to understand and control them. We can manipulate these equilibria through the use of multiple methods of optogenetic allows to target specific pathways implicated in adhesion at the min- and micron-scale.



Poster # 36

Technical Insights into Highly Sensitive Isolation and Molecular Characterization of Circulating Tumor Cells for Early Detection of Tumor Invasion

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Keywords: ISET®, Circulating Tumor Cell (CTC), Circulating Cancer Cell (CCC), Circulating Rare Cells (CRC), cellfree DNA (cfDNA), theranostic, tumor invasion, NextGeneration Sequencing (NGS), liquid biopsy

Introduction: An unsolved technical issue in the Circulating Tumor Cells (CTC) field is how to obtain highly sensitive and unbiased collection of these fragile and heterogeneous cells, in both live and fixed form, for their molecular study when they are extremely rare, thus at the beginning of the invasion process.

Material and Methods: We have explored the potential of ISET® (Isolation by SizE of Tumor Cells), an open platform developed for marker-independent isolation of tumor cells from blood, to collect intact fixed and live cancer cells by using spiking analyses with extremely low numbers of fluorescent cultured cells. Then we have assessed the feasibility of Next Generation Sequencing (NGS) analyses targeted to single cells enriched by ISET using the hotspot cancer panel v2 on the Ion Torrent platform. This panel can detect 6893 possible COSMIC mutations over 50 oncogenes and tumor suppressor genes.

Results and Discussion: Our results consistently show the feasibility of isolating fixed and live tumor cells with a sensitivity close to one cancer cell per 10 mL of blood. We also demonstrate the feasibility of NGS analysis of single live cells enriched by ISET® with Ion Torrent Plateform. Our data provide evidence that the analysis of three single cells enriched by ISET® is sufficient to reliably identify the mutated allele frequency in the target cell population.

Conclusions: These technical improvements should help the study of circulating cancer cells at the early steps of tumor invasion.

Poster # 37

Evolution of Barrett's Esophagus through Space and Time at Single-Crypt and Whole-Biopsy Levels

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Keywords: Barrett's Oesophagus, evolution, intra-tumour heterogeneity, genomics

Barrett's Oesophagus (BO) is a metaplastic lesion in which individual glands replace the squamous lining of the oesophagus. BO is a precursor of oesophageal adenocarcinoma (OAC) but patients without dysplasia at diagnosis rarely progress to OAC, there is thus a need for improved biomarkers. They may be found by assaying the evolutionary process underlying BO development. Although previous work has focused on BO evolution in whole biopsies, it fundamentally acts at the gland level. Here we performed copy-number alteration (CNA) analysis of individual glands to characterise clonal evolution over space and time at unprecedented resolution. In 4 non-progressors, 3 biopsies from two time-points were analysed. In 4 progressors, 3 biopsies from an initial endoscopy and 8 biopsies from the later resected oesophagus were analysed. 8 individual glands were microdissected from each biopsy. Each gland and remaining epithelium from the biopsy were assayed for CNAs using SNP-arrays (612 samples total), which allowed us to characterize genetic diversity within and across biopsies. In 6 out of 8 patients, CNAs could be detected in all glands and biopsies, suggesting that the lesion derived from a single ancestor. Whole biopsies frequently failed to detect alterations that were present only in individual glands. However, we find that multiple biopsies efficiently characterise genetic diversity within a lesion, the 'macro-diversity' between biopsies reflecting the 'micro-diversity' between glands of a biopsy (R^2 = 0.72, p=0.007). Genetic distances between glands were unrelated to physical distances in the lesion (all p>0.05) and rare clonal expansions indicated that the BO lesion is mostly evolving neutrally. Finally, mutation rates calculated at crypt and whole-biopsy levels were not significantly different. These results draw on genetic analyses performed at an unprecedented spatial resolution and shed new light on the evolutionary dynamics and origins of BO.

Poster # 38

Search of the Mechanism of Action of a Novel Pharmacological Agent that Sensitizes Cells to Paclitaxel

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Keywords: microtubules, cancer, drugs, treatment

Paclitaxel (Taxol®), a pharmacological agent that targets microtubules has been utilized successfully in the treatment of solid tumors for several decades. However, severe side effects are often associated with this treatment, due to the microtubules numerous functions in normal cells. Moreover, many cancers are de novo resistant or acquired resistance to such chemotherapy.

A strategy to circumvent these limitations is to identify drugs that target microtubule regulators rather than compounds interacting directly with the microtubule network. Such drugs can be selected using phenotypic screens, in which a compound collection is directly assayed on cells.

We have used a cell toxicity assay and screened a library of 8,000 compounds, to select compounds able to sensitize cells to a non-toxic low dose of paclitaxel.

We have isolated a carbazole derivative, "T4", that - at a concentration that is non-toxic to cells when administrated alone - is able to induce cell death in conjugation to a non-toxic dose of paclitaxel.

This compound impacts microtubule dynamics in cells, induces abnormal mitotic spindles and blocks cells in mitosis. Moreover, T4 target is conserved among species as similar results were observed when applied on the yeast S. Cerevisiae.

Thus T4 appears to behave as a mitotic drug. Our aims are now to identify its target, mainly by using approaches based on yeast genetics and by characterizing more precisely its effects in cells.

We have thus used yeast to screen a DNA bank. The aim was to overexpress the 6000 genes of S. Cerevisiae and to select the clones that survive in presence of lethal dose of T4. These clones have been analyzed by sequencing. Among the genes that permit S.c to survive, we found several genes involved in the regulation of microtubule and actin dynamics, as well as genes involved in the regulation of the cell cycle and of the metabolism. We are currently testing the relevance of these candidates.

Poster # 39

Role of Rho Proteins in the Migratory and Invasive Properties of Basal-Like Breast Tumors

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Keywords: rho proteins, basal-like breast cancer, BRCA1

Basal-like breast cancers are among the most aggressive cancers and effective targeted therapies are still missing. In order to identify new therapeutic targets, we performed RNA-Seq of 10 breast cancer cell lines with different phenotypes. Several genes involved in epithelial cell migration have been identified overexpressed in basal-like breast cancer cells.

Among them, we have focused on the RhoA and RhoB genes that encode small GTPases known to have a role on the actin cytoskeleton allowing the cells to migrate. We confirmed by qRT-PCR and Western blot that strong RhoA expression and low RhoB expression was associated with basal-like subtype. An RNA interference protocol showed that a decrease of RhoA reduced the migratory and invasive capacities of the basal-like MDA-MB231 and HCC1937 cell lines, whereas a decrease of RhoB increased them. Rhosin, a RhoA inhibitor, could also reduce migration of basal-like cell lines.

By fluorescent actin labeling, we could show that the inhibition of RhoA expression also induced a decrease in the formation of stress fibers, which are a conformation of the actin cytoskeleton of migrating cells and a known mechanism of Rho proteins. Inhibition of RhoB does not seem to have an effect on this parameter.

BRCA1, a gene frequently inactivated in basal-like tumors, seemed to have a role in the differential expression of RhoA and RhoB in the basal-like tumors, since the restoration of BRCA1 expression in the basal-like mutated SUM1315 cell line decreased RhoA expression and increased that of RhoB, leading to a reduction of its migratory capacities.

Our study suggested the Rho proteins as potential therapeutic targets for basal-like and BRCA1 mutated breast cancers. It seems that migration and mesenchymal properties acquisition of basal-like breast cancer cells are key functional pathways in these tumors with a high metastatic potential.

Poster # 40

Sequential Activation of RAS/MAPK and PI3K/AKT/TOR pathways via an EGFR-Autocrine Loop Resumes Prostate Cancer Hallmarks in the Drosophila Accessory Gland

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Keywords: prostate, tumorigenesis, drosophila, AKT/mTOR, Ras/MAPK, EGFR signaling

Background: Many factors play a role in prostate cancer etiology and progression. Particularly, analyses of human prostate cancer specimens have revealed the importance of Ras/MAPK and PI3K/AKT/mTOR signaling pathways for the late stages of this disease. However, human and biological data lack to understand their implication in earlier stages of tumorigenesis.

Methods: To study the underlying molecular mechanisms by which these signaling pathways operate during initiation and progression of the disease, we propose an alternative in vivo model of prostate tumorigenesis in the accessory gland of Drosophila, which is a functional homolog of the human prostate.

Results: Hyperactivation of AKT/TOR pathway induces cell overgrowth but not tumorigenesis. Conversely, hyperactivation of Ras/MAPK pathway by expression of RasV12 induces formation of cells masses that recapitulate many cancer cells hallmarks such as uncontrolled cell growth and proliferation, loss of cell identity, tissue disruption, enhanced matrix metalloproteinase expression, neovascularisation-like tracheogenesis.
In this model, we furthermore demonstrate that Ras/MAPK pathway may induce tumor development by establishment of an autocrine feedback loop (EGFR dependent signaling). More, this tumorigenesis required AKT/TOR pathway recruitment to allow tumor growth and extravasation.

Conclusions: We have developed a Drosophila model which seems to be efficient to study phenomena that are describe in human prostate tumorigenesis. As our results suggest that Ras/MAPK pathway deregulation may be a promoter of prostate tumorigenesis, the next step is to observe if this really happens in human samples with a Tissu MicroArray analysis.
We also plan to use our model to search for the molecular mechanisms that could be implicated in this tumorigenesis and, in particular, implication of metalloproteinases, which seem to be expressed on a short time window during the tumorigenic process.

Poster # 41

Physiologic and Pathologic SMYD2 and SMYD3 Lysine Methylation Signalings

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Keywords: lysine methyltransferases, SMYD2, SMYD3, pancreatic cancer

Protein lysine methylation is a highly dynamic and regulated set of post translational modifications (PTMs) that has been firstly described as a fundamental component of the “histone code”. However, a growing number of non-histone proteins have been shown to undergo lysine methylation and might affects various cellular processes with a finely tuned regulation. Their deregulation is implicated in multiple pathologies such as cancers.

My PhD project aims to characterize new significant signalings for SMYD2 and SMYD3 (SET and MYND domain protein 2 and 3), 2 lysine methyltransferases (KMTs) implicated in pancreatic ductal adenocarcinoma (PDAC) progression (Mazur, Reynoird et al. Nature 2014; Reynoird et al. Genes and Development 2016). These 2 KMTs are overexpressed in other contexts and might participate in yet unidentified pathways involved in PDAC or other cancers.

In order to decipher their implication in such signalings, we identified 28 substrates for SMYD2 and 17 for SMYD3 in HeLa cells by using a proteomic approach of SILAC and mass spectrometry combined with methylated proteins enrichment by 3xMBT. We validated a number of substrates by in vitro methylation assays and we are identifying the site and the state of each confirmed methylation event by point mutation analysis and mass spectrometry. We will focus our research in human digestive cancers and I will characterize the most relevant substrates in this context. I will decipher the molecular mechanisms involved in SMYD2 and SMYD3 signalings, by observing the consequences of the presence or absence of each pair of KMT/substrate in cell lines and understand the importance of each methylation event in the regulation of cell homeostasis.

This project proposes a better characterization of SMYD2 and SMYD3 signaling pathways involved in various cancers. This study should lead to the identification of new prognostic markers and new therapeutic targets in order to improve patients’ treatment.

Poster # 42

Identification du mode d'action et des cibles de EZH2 dans le contexte du cancer cortico-surrénalien

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Mots clés : carcinome cortico-surrénalien, EZH2, E2F1, activateur transcriptionnel, RRM2, PRC1, PTTG1, bio-informatique.

Les carcinomes cortico-surrénaliens (CCS) sont des tumeurs agressives de mauvais pronostic, dans lesquelles la surexpression de la méthyl-transférase EZH2 participe à la progression maligne. Ce facteur possède un mode d'action canonique associé à la répression de la transcription via le complexe PRC2 et la mise en place de la marque histone H3K27me3. Cependant EZH2 est également capable d'activer l'expression génique via son interaction avec des facteurs de transcription.

Afin d'identifier le mode d'action de EZH2 dans le CCS, nous disposions de données d'expression génique de trois cohortes de patients. Les analyses bio-informatiques effectuées ont montré que EZH2 agit préférentiellement comme un activateur transcriptionnel. Ce mode d'action semble reposer sur son interaction avec le facteur de transcription E2F1, afin de stimuler l'expression de gènes associés à la progression tumorale. Afin de déterminer les cibles les plus pertinentes pour ce mode d'action, nous avons croisé les données des patients avec le transcriptome de souris invalidées pour Ezh2 dans la cortico-surrénale (Ezh2-KO). Nous avons identifié RRM2, PRC1 et PTTG1 comme trois cibles de l'activité positive de Ezh2, dont la surexpression est associée au mauvais pronostic dans le CCS. Ces trois gènes pourraient être des cibles thérapeutiques intéressantes. En effet l'inhibition du gène RRM2 par l'inhibiteur pharmacologique GW8510 montre une réduction importante de la prolifération des cellules de lignée de corticosurrénalome humaine H295R.

Les analyses réalisées jusqu'à présent suggèrent donc que EZH2 pourrait interagir avec E2F1 afin de stimuler l'expression de gènes cibles qui sont associés au mauvais pronostic dans le CCS.

Poster # 43

CovIsoLink™: New Bacterial Transglutaminase Q-tag Substrate for the Development of Site Specifique Antibody Drug Conjugates

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Keywords: Antibody-Drug-Conjugates, Q-tag, bacterial transglutaminase

CovIsoLink™ (Covalently Isopeptide crosslinking) relates to methods for enzymatic covalently coupling drugs and other compounds through transglutaminase site specific generated in the targeted proteins including, polypeptides, proteins and immunoglobulins (patent pending 1).
Transglutaminases (TGases) catalyze covalent cross-linking of specific glutamine residues to the primary amines. Using an in house peptide library, we identified several amino acid sequences that were recognized as glutamine donor substrates. The optimum peptide sequences were selected and we further confirmed that these sequences have improved affinity compared with the conventional small peptides Z-QG. In different experiments we engineered Fc-containing polypeptide at the C-terminal domain and showed that mTG incorporates with high efficiency several types of amine donors to the engineered antibodies.
CovIsoLink™ is now used to develop new antibody drug conjugates (ADCs) since the major advantage of this method is to obtain a homogenous immunoconjugate with uniform stoichiometry.
We developed and characterized different recombinant anti Her2 IgG1 Mab carrying optimized enzymatic substrates (tag) by genetic insertion in the coding sequence of MAb. We then evaluated the best linkers and conformation to incorporate different compounds through mTG enzymatic reaction. We set up experimental conditions, production, purification, HPLC/MS analysis and control of the immunoreactivity of CovIsoLink™ Her2-ADC. Using mTG, we obtained site specific conjugation of different modified drugs with optimized linker on the anti Her2 IgG1 antibody. By HIC analysis, we validated a specific and reproducible DAR reaching DAR2 depending on drugs and experimental conditions. In vitro and In vivo characterization and dose response studies of CovIsolink-ADC specificity and reactivity are currently performed in Her2 positive models by comparison with Kadcyla (T-DM1).

1) PCT/EP2014/0792278

Poster # 44

Nov/CCN3 is a Negative Target of the Methyltranserase EZH2 in Adrenocortical Carcinoma

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Keywords: adrenocortical carcinoma, EZH2, apoptosis, Nov. nuclear receptor SF1

Adrenocortical carcinoma (ACC) is a rare tumour with an incidence of 0.5 to 2 new cases per million per year. However it is associated with poor prognosis. Although surgical resection remains the treatment of choice in localized ACC, it is still inefficient to treat patients with metastases. It is thus essential to identify the actors of malignant progression in order to develop more effective therapeutic strategies.

We have recently shown that the histone methyltransferase EZH2 is the most deregulated epigenetic histone modifier in ACC. EZH2 is overexpressed downstream of TP53/RB1/E2F1 and is associated with tumour proliferation and poor prognosis. Pharmacological inhibition of EZH2 inhibits H295R cells growth and aggressive behaviour and induces apoptosis. This suggests the involvement of EZH2 in malignant progression. Thus, EZH2 could represent an interesting therapeutic target.

Expression of the pro-apoptotic factor NOV/CCN3 is decreased in ACC, which is correlated with poor prognosis. Our molecular analyses show that EZH2 inhibition by siRNA or pharmacological treatment with DZNep increases expression of NOV/CCN3, suggesting that EZH2 overexpression may favour malignant progression in ACC by inhibition of apoptosis stimulators. NOV has previously been identified as a negative target of the nuclear receptor SF1 in ACC cells, although the molecular mechanisms underlying this inhibition were unidentified. Our preliminary results showed that the knockdown of EZH2 prevented Nov inhibition by the nuclear receptor SF1 in H295R cells. In addition, the combined knockdown of SF1 and EZH2 induced a higher increase in Nov expression. By ChIP assays, we further observed SF1-dependent enrichment of EZH2 on NOV promoter and enhancer. Altogether, these data suggest that SF1 and EZH2 interact functionally to inhibit expression of NOV in ACC.

Poster # 45

INOVOTION Tests for Drug Discovery: Early Identification of Low / Value Leads Keywords

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Keywords: oncology, drug discovery, in vivo assay, preclinical tests, efficacy & toxicity evaluation, target validation, multi cancer screening, chick embryo model

Since its introduction, the chick embryo model involving the technique of chorioallantoic grafting has proved extremely valuable for the in vivo studies of tumor development, angiogenesis and malignant cell dissemination. The ability of the chick embryo's chorioallantoic membrane (CAM) to efficiently support the growth of inoculated xenogenic tumor cells greatly facilitates the analysis of human tumor cell metastasis. We have developed highly sensitive and reproducible assays for monitoring the growth and the metastatic dissemination of human tumor cells in the chick embryo. These tests are validated for 13 human tumors cell lines including various carcinomas, gliomas and melanomas as well as reference drugs currently marketed. Using these assays we can investigate the efficacy and the toxicity of new drug lead candidates in oncology. These assays can also be used to study genetically modified cell lines. The data obtained with this model are much faster, more reliable, less expensive and need only minute amounts of drug (>1,000 times less) compared to the mouse model. Our tests are applicable to any preclinical anti-cancer drug discovery program. Moreover, they could be used for the early evaluation of the toxicity of any new drug candidates, i.e. not just in oncology. Altogether, our tests make the chick embryo CAM system an attractive model to reduce animal experimentation for drug discovery.

Poster # 46

Role of HIF-1α in the Resistance of Cancer Stem Cells to Photon and Carbon Ion Irradiations

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Keywords: hypoxia, cancer stem cells, carbon ions, HIF-1a

Purpose: The resistance of HNSCC to radiotherapy is partly explained by the presence of Cancer Stem Cells (CSCs) located in hypoxic niches. The protein HIF-1α (Hypoxia-Inducible Factor 1α) is considered as the major transcriptional regulator of the cellular response to oxygen homeostasis. Compared to X-rays, carbon ion efficiency relies on better ballistic properties and a higher relative biological effectiveness independent of the oxygen concentration. This work aims at clarifying the role of HIF-1α in the response of CSCs to photon and carbon ion irradiations.

Methods: HNSCC-CSCs were transfected with a non-relevant or a siRNA targeting HIF-1α. Oxygen Enhancement Ratio (OER) was calculated at the ratio of dose inducing 10% survival after photon or carbon ion irradiation in hypoxic (1% O_2) versus normoxic conditions. DNA Double Strand Breaks (DSBs) were quantified by the γ-H_2Ax assay and ROS with a CM-H_2DCFDA dye.

Results: For two HNSCC cell lines and their CSC subpopulation, in response to photons under hypoxia, an OER>1.2 was associated with HIF-1α expression. This stabilization appears earlier in CSCs than in non CSCs and is correlated with the variation of ROS levels, confirming the adaptive properties of CSCs to hypoxia. Compared with photons, the oxygen effect is canceled after carbon ion exposure (OER=1) and no stabilization of HIF-1α was observed in normoxia. Inhibition of HIF-1α with a siRNA leads to the decrease of CSC survival after both radiations in hypoxic conditions (OER<1). Furthermore, radiosensitization is associated with a significant increase of residual DSBs.

Conclusion: These results demonstrate that HIF-1α plays a key role in the response of CSCs to photon and carbon ion irradiation. It participates to radioresistance by increasing cell survival and DNA repair and contributes to tumor escape. This makes the HIF-1α targeting an attractive therapeutic challenge.

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Bioinformatics, Modeling

Poster # 47

Network-Based Systems Analysis of microRNAs to Infer Cancer Target microRNAs

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Keywords: bioinformatics, microRNA, network, cancer, systems biology

Bioinformatics, microRNA, network, cancer, systems biology

MicroRNAs post-transcriptionally regulate protein coding genes through specific binding to their mRNA targets. MicroRNAs are predicted to target multiple protein coding genes while at the same time, most protein coding genes are predicted to be the target of multiple microRNAs. As a result, we have inferred microRNA networks in which a microRNA is a node, and two nodes are linked together if they share a given number of common targets. We evaluated the robustness of the approach by comparing our results with different microRNA target prediction algorithms used.

The microRNA hubs, the nodes with the most neighbors, form two distinct and dense subnetworks / clubs. As the functions of microRNAs are directly associated with the regulation of their targets, we performed gene ontology enrichment for the shared targets of each club to predict the associated biological pathways. The first club was found to be associated with the regulation of the transcription / translation machinery, whereas the second club, made up of three microRNAs, was found to be associated to the regulation of signaling through small GTPases. The three microRNAs has been experimentally validated on cell lines to modulate cytoskeleton organization, proliferation and invasion potential. We showed that miR-940 was consistently deregulated in breast cancer tissues from public data of three independent studies.

Interestingly, the microRNAs in the neighborhood of each club shared their ontology enrichments. It further confirms that the topology of the network conveys biological information.

Finally, we developed miRViz, a web interface to visualize microRNA data onto the associated network. The interest of this approach lies in the fact that neighbor microRNAs tend to have similar expression. I will present various publicly available cancer data overlaid on the microRNA networks. A focus will be given to the prognostic potential of microRNAs in adrenocortical carcinomas.

Poster # 48

Prediction of the Physicochemical and ADMET Properties of Combretastatin A-4 Derivatives

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Keywords: ADMET predictions, physicochemical properties, anti-proliferative agents, tubuline

Combretastatin A-4, commonly named as CA-4, is a (Z)-stilbenoid derivative, first isolated in the 90s by Georges Petit from Combretum caffrum, a South African willow tree.1
Based on the structure of this natural product, we have previously designed, synthesized and evaluated some (Z)- and isomethylene heterocyclic trimethoxyaryl analogues.

These synthetic compounds proved to have interesting tubulin polymerization inhibition properties and anti-proliferative effects against some cancer cell lines, blocking the cell cycle at the G2/M phase.2

Using in silico approaches on a selection of these molecules to predict physicochemical, ADME and Toxicity properties, the aim of our project is to select the best one(s) for further studies.

To this purpose, we used two softwares:
- Molinspiration for the calculation of molecular properties (e.g. LogP, TPSA),
- ACD Labs / Percepta Predictors (e.g. cytochrome P450 substrates, health effects).

The main results will be presented, which give valuable indications for the lead optimization phase.

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Poster # 49

Probing Interstand CrossLinks Impact on DNA Structure by Classical Molecular Dynamics Simulations

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Keywords: complex DNA damage, molecular modeling, interstrand crosslinks

Interstrands cross-links are highly cytotoxic oxidatively-generated DNA damages. They result from abasic sites (Ap) reaction with a purine on the facing strand of the double-helix. The aldehyde group of the abasic site condenses with the exocyclic amino group of the purine, leading to a covalent adduct, bonding the two strands.

This type of lesions is very deleterious for cells since it avoids the strands separation during DNA replication, leading to apoptosis [1]. The feasability of such lesions has been prooved [2,3], with high yields (15-70%) reported for the formation of Ap-Adenine cross linking [4]. These observations suggest that B-helix is flexible enough to allow the two reactants to approach and react, despite the mechanical constraints that it could induce.

We investigated the structural impact of these interstrands cross-links in 21 base pair oligonucleotides, generating 100ns molecular dynamics simulations. Structural analysis on these conformationnal sampling allowed us to probe the effect of such lesions on the double-helix. This study showed strong structural rearrangements in the double-helix, presenting two stable conformations, both with π-stacking modifications of the surrounding nucleobases and C33 exclusion, the difference between the two relying on the T12 exclusion.

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Poster # 50

Integrative Analysis Reveals Novel Subtypes of Medulloblastoma Subgroups

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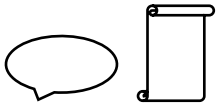
Keywords: brain tumor, medulloblastoma, subgroups, integrative analysis methylation, gene expression, copy-number

Medulloblastoma is the most common malignant brain tumor in children. It is is now accepted to be comprised of four distinct molecular variants, and current clinical trials are stratifying patients using a combined biological and clinical risk stratification. However inside each subgroup, we observe tremendous clinical heterogeneity suggesting additional substructure. What remains unclear is the degree of biological substructure within subgroups.

We performed an integrative analysis of 763 primary medulloblastoma samples with gene expression and genome-wide DNA methylation data with the Similarity Network Fusion method (SNF) to uncover this structure. The integrative clustering faithfully recapitulated the core subgroups with a clear boundary between Group 3 and 4, that is not readily apparent with a single data type. A subsequent analysis within each subgroup revealed varying degrees of biological heterogeneity. After integration of somatic chromosomal arm-level alterations, focal somatic copy number alterations, and clinical features, we identified twelve medulloblastoma subtypes with clear clinical and molecular characteristics; two WNT, four SHH, three Group 3 and three Group 4 subtypes. We discovered two clear infants SHH subtypes with disparate outcome and distinct copy number profiles, a childhood group with a poor prognosis and an adult group. One Group 3 subtype with the worst prognosis had MYC amplicons and isochromosome 17q without other focal aberrations. Pathway analysis results support the subtype specificity of biological processes. Notably, we observe an enrichment of developmental pathways in one of the two SHH infant subtypes.

As current therapies result in significant long-term neurocognitive and neuroendocrine sequelae, the identification of distinct biological processes within each subgroup allows for more refinement in biological risk stratification as well as the possible identification of novel agents for future rationale targeted therapies.



Poster # 51

Detecting Very Low Abundance Mutations from Multi-Sample NGS Data with Needlestack

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Keywords: next generation sequencing, circulating tumor DNA, bioinformatics, somatic variant calling

The comprehensive characterization of somatic mutations by screening cancer genomes can help to understand cancer appearance and progression but also to identify accurately predictive biomarkers such as circulating tumor DNA (ctDNA). Nevertheless detecting somatic mutations remains an unsolved problem, exacerbated when trying to identify mutations in ctDNA due to reduced variant allelic fractions (AF). Indeed, Next Generation Sequencing (NGS) error level can reach this low proportion, and somatic variant calling from ctDNA is like finding a needle in a needlestack.

Here we present needlestack, an highly sensitive variant caller which estimates the distribution of sequencing errors directly from the data. Needlestack is based on the idea that analyzing large number of samples together can help estimate the distribution of sequencing errors to accurately identify variants present in very low proportion. At each position and for each candidate alteration, both single nucleotide substitutions and short insertions or deletions, we model the sequencing error distribution using a robust negative binomial regression and detect variants as being outliers from this error model.

We show using both in silico trials and real NGS data that needlestack is able to detect as low as 0.1% AF mutations. We demonstrate that detectable AF are highly error-rate dependent, and that the error rate varies between positions along the DNA sequence, supporting the need to estimate this parameter independently across positions. Finally we applied needlestack on TP53 circulating-free DNA deep sequencing in a case-control study for the early detection of small-cell lung cancer.

Poster # 52

Modélisation par dynamique moléculaire de l'endommagement complexe de l'ADN

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Mots clés : dynamique moléculaire, lésions de l'ADN, nanoparticules d'or

L'endommagement et la réparation de l'ADN mettent en jeu des réactions et interactions chimiques complexes, qui nécessitent d'être clairement comprises pour développer des traitements innovants. C'est notamment le cas de l'hadronthérapie. L'utilisation des nanoparticules d'or pour un traitement du cancer plus efficace est également en phase de recherche. Ces nanoparticules amplifient les dommages faits par les radiations grâce à une réaction en chaîne. Il s'agit alors de comprendre ce phénomène d'amplification et l'effet de taille associé.

Nous présentons de premiers résultats obtenus par dynamique moléculaire pour deux systèmes:
(1) un brin d'ADN interagissant avec des nanoparticules d'or (Au38), de manière à avoir un point de départ pour l'étude de réactivité, et fournir des paramètres pour des simulations Monte-Carlo.

(2) un brin d'ADN présentant 2 lésions oxoguanines en interaction avec l'enzyme de réparation MutM [2], un cas de réparation connu expérimentalement pour être difficile [1]. Nos dynamiques moléculaires montrent sans ambiguïté que la présence d'une deuxième oxoguanine "gèle" l'enzyme au niveau de la triade d'intercalation (M75/R109/F111) et de l'asparagine (B149).

La simulation nous permet ainsi de mettre en évidence, avec une résolution atomique, les interactions ADN-protéine qui bloquent la réparation pour des lésions sévères de l'ADN, et l'interaction entre des clusters métalliques et des biomolécules [3].

Ces études prennent place dans le cadre du Labex PRIMES (financement de la bourse doctorale de Chen Hui CHAN).

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Poster # 53

Méthode statistique pour la comparaison de pipelines utilisés dans le séquençage à haut débit

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Mots clés : méthodes statistiques, séquençage haut débit, comparaison de pipelines, sensibilité, spécificité

Today, DNA sequencing is performed using Massive Parallel sequencing (MPS) which cuts drastically the time and expenses of genome sequencing. Nevertheless, Sanger sequencing is still largely used for variants validation. The analysis of MPS data involves many steps for which several bioinformatic tools, academic or commercial, have been developed. These tools may be combined into MPS data analysis pipelines. In this work, a statistical method to compare MPS pipelines is presented. This method was applied to the comparison of an academic pipeline (BWA-GATK) with a commercial pipeline (TMAP-NextGENe®) in two conditions (with and without reference to a gold standard; here, Sanger sequencing) on a panel of 41 genes sequenced in 43 epileptic patients.

To determine whether the margins and the ORs for agreement were heterogeneous, four log-linear models were used: a full model, a homogeneous-margin model, and a model with single odds ratio (OR) for pipeline agreements shared by all patients, and a model with single intercept shared by all patients. Then a log-linear mixed model was fitted taking the biological variability into account through a random effect.

Briefly, the results showed that, among the 390,339 base-pairs sequenced, TMAP-NextGENe® found, on average, 2253.48 variants (substitutions and indels) vs. 1857.13 variants for BWA-GATK. Using Sanger sequencing as gold standard, we found that the specificities of BWA-GATK and TMAP-NextGENe® were relatively close but significantly different (99.57% vs. 99.65%; $p < 0.001$); and the sensitivities between the two pipelines were similar (63.47% vs. 63.42%). Same-trend results were obtained when only substitutions were considered as variants (99.98% specificity and 76.81% sensitivity for the two pipelines).

Poster # 54

Prioritizing Chemicals for Risk Assessment Using Chemoinformatics: Examples from the IARC Monographs on Pesticides

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Keywords: pesticides, chemoinformatics, cancer, hazard identification, text mining

Identifying cancer hazards is the first step towards cancer prevention.The IARC Monographs Programme, which has evaluated nearly 1000 agents for carcinogenic potential since 1971, typically selects agents for hazard identification on the basis of public nominations, expert advice, published data on carcinogenicity, and public health importance. PURPOSE. Here we present a novel and complementary strategy for identifying agents for hazard evaluation using chemoinformatics, database integration and automated text mining. METHODS. To inform selection among a broad range of pesticides nominated for evaluation, we identified and screened nearly 6000 relevant chemical structures. RESULTS. We systematically compiled information on 980 pesticides, creating chemical similarity network maps that allowed cluster visualization by chemical similarity, class, and the number of publications concerning epidemiology, cancer bioassays, and carcinogenic mechanisms. For the IARC Monograph meetings that took place in March and June 2015, this approach supported high priority evaluation of glyphosate, malathion, parathion, tetrachlorvinphos, diazinon, DDT, lindane, and 2,4-D. CONCLUSIONS. This systematic approach, accounting for chemical similarity and overlaying multiple data sources, can be used by risk assessors as well as researchers to systematize, inform and increase efficiency in selecting and prioritizing agents for hazard identification, risk assessment, regulation or further investigation. This approach could be extended to an array of outcomes and agents, including occupational carcinogens, drugs, and foods.

Poster # 55

Mise au point d'un test moléculaire améliorant le diagnostic pre-opératoire des nodules thyroïdiens.

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Keywords: thyroid nodule, fine needle aspiration cytology, thyroid carcinoma

Introduction : L'évaluation pre-opératoire du risque de malignité des nodules thyroïdiens repose sur l'échographie et la cytoponction, au terme desquels 30% sont classés indéterminés.

Objectif : Construire un test moléculaire dont les performances seront évaluées en association avec le résultat cytologique et adaptées au contexte clinique dans le but d'affiner le diagnostic pré-opératoire des nodules thyroïdiens.

Matériel et Méthodes : Dans cette étude prospective, le matériel de cytoponction des nodules possédant un diagnostic histologique était analysé par une puce transcriptomique de 20 gènes construite lors d'un travail antérieur. L'ajustement d'un modèle de régression logistique a permis de sélectionner les 7 gènes les plus pertinents. Ce prédicteur moléculaire a été intégré, avec le résultat cytologique Bethesda, dans un modèle de régression logistique. Les performances de ce prédicteur combiné ont été optimisées pour différentes prévalences de malignité ainsi qu'en pondérant les conséquences d'éventuelles erreurs diagnostiques (ratios bénéfice-risque). Ce modèle a été comparé à la classification Bethesda seule en évaluant l'aire sous la courbe ROC (ASC).

Résultats : 722 cytoponctions ont été incluses, 128 ont bénéficié du test moléculaire dont 46 nodules malins. Le prédicteur combiné présentait dans notre cohorte (prévalence de 36%) une sensibilité de 76% et une spécificité de 95%. L'ASC du test combiné était significativement supérieure à celle de la classification Bethesda seule (p=0.004). Pour une prévalence de 7%, nous obtenions une spécificité maximale de 100% et une sensibilité plus basse de 47.8%.

Conclusion : Ce test moléculaire, évalué en association avec la cytologie et optimisé selon le contexte clinique, présente une excellente spécificité et pourrait contribuer à une amélioration de l'évaluation pre-opératoire des nodules thyroïdiens.

Poster # 56

Network-Based Systems Approach for Disease Biomarkers and Relevant Signaling Pathways Contextualisation

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Keywords: protein-protein interaction network, systems biology, gene expression, signalling pathways, cancer deregulation

Background: To survive, organisms must adapt to evolving environment by detecting, processing and responding adequately to changing extracellular cues. This adaptation relies on intracellular signaling cross talks built over highly dynamic protein-protein interactions networks (PPINs) and allows the emergence of fundamental properties of any living systems such as signal integration, anticipation, shielding towards cues, processes separation, memorization and cellular proliferation or differentiation. Recent studies demonstrated that robustness of these networks buffers genetic mutations and that multiple alterations of PPINs are compulsory to cancer development, suggesting that disease diagnosis and therapy must integrate a systemic dimension.

Methods: Starting from a set of molecular entities selected from public databases and literature on non small cell lung cancer or breast cancer, we developed a PPIN based method to extend and contextualize the relevant information into signaling pathways. Datasets of interest were analyzed using differential gene expression assessment with a supervised approach on R/Bioconductor. PPIs are retrieved from BIOGRID database using Cytoscape environment, manually curated and analyzed with shortest path algorithm. The contextualized network was reconstructed with SBGN standards under VANTED environment.

Results and perspectives: Our methodology results in a comprehensive understanding of biomarkers behavior in a given context. More broadly, our pipeline paves the way for network-based investigation of cellular processes, biomarkers, and their dynamical behavior within their respective signaling pathway.

Poster # 57

Prise en compte de la technologie dans la quantification des biomarqueurs

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Mots clés : protéomique, quantification, biomarqueurs, variabilité, analyse statistique, plan expérimental

Introduction : Les techniques de spectrométrie de masse sont très séduisantes pour détecter des biomarqueurs protéiques de manière sensible et rapide. Cependant, contrôler la variabilité technologique sur ces chaînes d'analyse reste un enjeu majeur pour la recherche et la validation de biomarqueurs. Ceci nécessite de développer des techniques de traitement de l'information intégrant la variabilité technologique. Des algorithmes de traitement dédiés respectivement aux chaînes d'acquisition MALDI en mode découverte de biomarqueurs et aux chaînes d'acquisition SRM en mode validation ont été développés dans le cadre du projet BHI-Pro. Des plans expérimentaux et des plans d'analyses spécifiques sont proposés pour les deux modes afin de contrôler la variabilité technologique en regard de la variabilité biologique dans la quantification des protéines.

Méthodes : Des données expérimentales sont obtenues à partir d'échantillons biologiques en contrôlant la variabilité biologique par dilution. Une gamme de concentrations relatives est obtenue en fonction des facteurs de dilution. Ces rampes de dilution sont ensuite traitées selon les étapes biochimiques propres à chaque chaine d'analyse. Les plans expérimentaux comprennent des réplicats pour chaque étape de la chaine d'analyse afin de répéter les mesures pour chacun de ces facteurs de variation. Le plan d'analyse prend en compte chacun de ces facteurs dans des modèles hiérarchiques en modélisant la relation entre la valeur des mesures et la quantité relative de protéine. Une décomposition de la variance des mesures permet de quantifier les parts de variabilité biologique, de variabilité technologique et d'erreur due au modèle. Ces critères sont utilisés pour comparer les algorithmes de traitement développés par rapport au traitement classique pour chaque chaine analytique (MALDI et SRM).

Résultats : En mode MALDI, on retrouve la relation entre l'intensité du pic correspondant à la protéine et sa concentration relative pour 4 protéines sur 11. L'algorithme de traitement des spectres développé dans BHI-Pro permet de diminuer la part de variance technologique de 2 à 3 et celle de l'erreur due au modèle d'environ autant.
En mode SRM, on retrouve la relation entre la quantité estimée de la protéine et sa concentration relative pour 6 protéines sur 12. L'algorithme de quantification développé dans BHI-Pro permet de diminuer la part de variance technologique sous certaines conditions.

Conclusions : La prise en compte des sources de variabilité de la mesure due à la technologie utilisée permet de contrôler la part technologique de la variance de la mesure. La quantification des biomarqueurs en mode découverte comme en mode de validation peut être ainsi améliorée.

Poster # 58

NanOx^{TM}, a New Multiscale Model to Predict Ion RBE in Hadrontherapy

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Keywords: RBE, multiscale modeling, hadrontherapy, oxidative stress

Object: Hadrontherapy is becoming an increasingly attractive modality for cancer treatment due to the favourable depth-dose profile of ions and high relative biological effectiveness (RBE) in the tumour region. Since RBE depends on multiple parameters related both to the irradiation beam and the cell properties, biophysical models are essential to comply with the demands of a clinical environment. NanOxTM addresses some of the flaws in the models currently implemented in the treatment planning systems, and shows many innovative features.

Method: The model takes into account the fully stochastic nature of ionizing radiation by considering dose fluctuations both at nanometric and micrometric scales, and introduces the concept of chemical dose. The latter represents the induction of cell death by “non-local” events as the accumulation of cellular oxidative stress or sub-lethal lesions induced by radical species. Such “non-local” events are complementary to the “local” events, which take place at a very localized scale and are considered as lethal since can singly cause cell death.

Results: NanOxTM predictions for V79 and HSG cell lines irradiated by photons, protons and carbon ions are in good agreement with the experimental data. The model is able to describe the effectiveness of ions, including the overkill effect at high LET values. Moreover, the typical shoulder in cell survival curves is reproduced owing to the introduction of the chemical dose which varies with LET.

Conclusion: The promising results obtained with NanOxTM stress its potential in the context of hadrontherapy, and may lead in the future to apply it to neutron beam therapy or photoactivation of nanoparticles. Despite a rigorous mathematical approach, its implementation remains simple and compatible with the constraints of clinical application. The model relies in fact on the fit of a reduced set of parameters, and its pragmatic architecture facilitates improvements and optimizations.

Poster # 59

Modélisation biophysique des effets radiosensibilisants des nanoparticules

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Mots clés : modélisation, nanoparticules, radiosensibilisation, échelle nanométrique

L'enjeu majeur de la radiothérapie est de concentrer la dose d'irradiation dans les cellules cancéreuses tout en épargnant au mieux les cellules saines. Parmi les stratégies envisagées, l'utilisation de radiosensibilisants vise à amplifier les effets destructeurs de dose dans la tumeur. Les nanoparticules de métaux lourds tels que l'or, le gadolinium, sont des radiosensibilisants particulièrement prometteurs. Si leur effets radiosensibilisants sont étudiés depuis quelques dizaines d'années, l'origine de ce phénomène est encore mal connue et peu quantifiée.

La littérature suggère que les radiations généreraient un effet physique appelé cascade Auger. Cet effet aurait pour conséquence de déposer davantage de dose, amplifiant les dommages cellulaires critiques par cassure directe de molécules sensibles (ADN) ou par boost de radicaux libres. Ces effets sont produits à des échelles nanométriques et dans des temps extrêmement courts (10-15 à 10-12 seconde) mais ont des conséquences à échelle du patient.

Parce que ces processus physico-chimiques ne sont pas observables, l'outil de simulation est indispensable pour mieux comprendre les processus initiaux. Notre objectif est dans un premier temps de développer une simulation permettant de calculer les distributions spatiales de dose et de radicaux libres autour des nanoparticules et de quantifier le boost induit. Dans un second temps, nous injecterons ces résultats dans le modèle Nanox, développé dans le cadre de l'optimisation de soin par hadronthérapie, pour traduire ces effets en termes d'augmentation de dose biologique et de mort cellulaire.

Ces deux étapes feront l'objet d'une confrontation avec des données expérimentales pour évaluer la qualité des modèles et de la pertinence des scénarios proposés dans la littérature. L'objectif final serait de guider le développement des nanoparticules et si possible d'aider à la planification clinique de traitements radiothérapeutiques basés sur les nanoparticules.

Poster # 60

Identification of Systematic Sources of Variation in DNA Methylation Measurements

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***Keywords**: Big Data, epigenetic, technological challenges, normalization, biostatistics*

Background: Methylation levels are characterized by environmental variability, but also by systematic variation due to laboratory treatment, including batch, the chip position within the batch, the position of the sample within the chip, etc. In this work the contribution of laboratory variables explaining overall variability of methylation levels was quantified. The performance of methods to correct this variation was investigated. Data from a nested case-control study on breast cancer in the European Prospective Investigation into Cancer and nutrition study were used.

Methods: Illumina Infinium HumanMethylation450K was used to acquire methylation levels in over 421.000 CpGs sites for 902 study participants. Variables expressing laboratory treatment were initially inspected via box-plots. Two normalizing techniques called COMBAT and Surrogate Variables Analysis (SVA) and a method based on the computation of residuals in a linear regression model were applied. A Principal Component Partial R2 (PC-PR2) analysis was used to quantify the contribution of laboratory factors to total variability using methylation levels before and after the correction step.

Results: Both box-plots and the PC-PR2 methods picked out the same laboratory variables: the row sample position and the batch explained the greatest proportion of variability, i.e. 11.4% and 9.5% of total variation, respectively. The chip position explained 6.5 % of the variability. When COMBAT or the residual methods were used to correct for these factors, their contribution was reduced to 0.2% and 1.3%, respectively. On top of that, SVA also allowed reducing the variability due to other sources such as the chip position.

Conclusion: The row sample position and the batch were identified as the most important systematic sources of variability in the DNA methylation measurements. The three interrogated methods succeeded in reducing their influence to marginal levels. SVA also reduced other sources of variability.

Poster # 61

Impact of Global DNA Hypomethylation on the Transcriptional Deregulation Linked to L1 Chimeric Transcripts Initiated by LINE-1 Transposable Elements in Gliomas

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Keywords: Transposable elements, LINE-1, Gliomas, LCT

L1 repeats represent 17% of the human genome. The 5' region of the youngest L1 sub-families (L1PA1 to 6) contains a bidirectional promoter consisting, in addition to the internal sense promoter, of an antisense promoter (ASP). A hallmark of cancer genomes consists in a global DNA hypomethylation which affects specially L1 promoters. In tumors, evidences suggest that this hypomethylation can result in transcription from ASP, leading to the expression of aberrant L1-chimeric transcripts (LCT) composed of L1 5' end and its adjacent sequence. To investigate the pangenomic extent of this transcriptional deregulation and its impact in tumor process, a dedicated bioinformatic tool, CLIFinder, was designed to select putative LCT among RNA-seq reads.

Analysis of 13 gliomas and 3 control brains identify 3000 chimeras. Most of them are detected in tumors and 82% implicate young L1. Analyses of 63 chimeras with young L1 demonstrate that 88% of them correspond to LCT initiated at ASP. Their expression study in a larger cohort of gliomas shows a basal transcription from ASP in normal tissues and a frequent overexpression in tumors for LCT detected only in tumors by CLIFinder. This overexpression seems to result from either L1 hypomethylation or transcriptional locus deregulation.

Among 22 LCT overexpressed, 12 appear as biomarker predictive of patients' survival and 4 of them are supposed to play a role in tumor process. Further experiments are required to validate mechanisms implied in overexpression and supply proof of concept of a functional role for the 4 candidates.

This study has validated CLIFinder as a bioinformatic tool useful for the identification of LCTs . Its use for analyzing other RNA-seq data will allow to assess the extent of this deregulation in other tumors types.

Poster # 62

Identification of Epigenetic Hotspots in Lung Cancer

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Keywords: epigenetics, lung cancer, methylome, differential expression, (de)regulation

Epigenetic deregulation of tumor-suppressor genes and oncogenes is one of the key step of tumorigenesis. In particular, many genome-scale studies have revealed extensive changes in methylation profiles that contribute to oncogenesis. However, the crucial role of epigenetic alterations in cancer remains poorly understood. The considerable amount of large-scale data offers now novel opportunities to systematically study epigenomic information in the context of cancer. In this work, we aim to investigate the role of epigenetic alterations in the regulation of differentially expressed genes.

We addressed this question by applying a systematic statistical analysis on transcriptomic and DNA copy number variation (CNV) data issued from publicly available studies on healthy and cancerous lung tissues (collected by the The Cancer Genome Atlas). We identified genes or genomic regions whose variations in activity observed in lung cancer could not be explained by a change in copy number (amplification or deletion). We are comparing and classifying methylation profiles of tumoral and non tumoral lung tissue to identify typical pattern of epigenetic marks.

This should allow the identification of epigenetic hotspots of cancer (de)regulation. We will evaluate their impact on gene expression, and we will try to propose models that interpret or predict functionally meaningful cancer epigenetic “hot” domains. The results obtained will be correlated with clinical data (prognosis, cancer predisposition, treatment response, etc).
These results implicate that epigenetic regulation before and during cancer development mediates tumorigenesis. We expect to discover new oncogenic mechanisms and epigenetic biomarkers that could be used to develop novel therapeutic strategies.

Poster # 63

DeCovA : un logiciel d'analyse de couverture et de détection de CNV sur des panels de gènes

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Mots clés : couverture, profondeur, CNV, panels de gènes, diagnostic, logiciel

Le séquençage de panels de gènes par des technologies à haut débit est désormais couramment utilisé pour le diagnostic génétique en routine. Cette stratégie s'impose notamment pour la détection de variants somatiques qui nécessite des profondeurs de séquençage importantes, ne pouvant pas être obtenues par séquençage de génomes ou d'exomes complets dans un cadre diagnostic.
L'étude de la profondeur et de la couverture du séquençage est capitale pour l'analyse des panels. En effet, ils s'enrichissent régulièrement de nouveaux gènes ce qui impose une validation bioinformatique dont profondeur et couverture sont des paramètres clefs. En routine diagnostique, l'analyse de ces variables permet d'identifier les régions d'intérêt mal couvertes chez le patient. Enfin, l'analyse de la profondeur est le point de départ de nombreuses méthodes de détection de variations du nombre de copie de l'ADN (CNV). Les outils classiques d'analyse de données NGS n'apportent pas de réponse pertinente à ces problématiques : trop généraux (profondeur moyenne, couverture à une profondeur donnée) ou trop précis (information base par base), ils ne permettent pas d'identifier sans traitement additionnel les régions trop peu couvertes et offrent rarement une sortie visuelle intuitive.

Pour permettre aux cliniciens et biologistes d'analyser facilement la couverture des gènes d'intérêt, nous avons développé le logiciel DeCovA. Il détermine les régions exoniques à analyser à partir d'un fichier d'intervalle ou d'une liste de gène/transcrits, et utilise BedTools ou GATK pour en réaliser l'analyse de profondeur base par base, après filtrage éventuel des reads. Il produit ensuite différentes sorties dont les principales sont : (1) un schéma de chaque gène/transcrit, par patient, avec la profondeur et les régions insuffisamment couvertes, (2) un schéma similaire de chaque gène/transcrit permettant d'apprécier le nombre de patients étudiés atteignant au moins une profondeur de séquençage donnée, (3) un graphe montrant la fraction de la région d'intérêt couverte à différents seuils de profondeur, (4) un fichier bed listant les intervalles dont la couverture est insuffisante. L'analyse de profondeur peut être aussi utilisée pour détecter des CNV sur un groupe de patients : pour chaque intervalle, après une normalisation sur la profondeur totale pour chaque patient, la profondeur de chaque patient est comparée à la profondeur médiane, les régions et patients trop variables étant mis à l'écart de façon itérative. Le sexe des patients est pris en compte dans l'analyse et les résultats sont complétés par des graphes par chromosome et par gène.

En conclusion, DeCovA permet de réaliser une analyse de couverture et une recherche de CNV de façon simple et efficace. Il fournit des sorties graphiques faciles à interpréter pour le diagnostic. Il peut être utilisé indépendamment ou intégré à un pipeline d'analyse. DeCovA est un logiciel libre, disponible sur demande à thomas.simonet@chu-lyon.fr.

Poster # 64

Optimism Bias Correction in Omic Studies: Assessment of Penalized Methods on Simulated Data

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Keywords: stratified/personalized medicine, biomarker discovery

With the development of omic technology, thousands of biological variables can be analysed at the same time. This leads to complex model selection and parameter estimation which are subject to an optimism bias. Our work intents to study the correction of optimism bias by penalty regression methods in case-control studies involving both clinical and omic variables.

Datasets with 2000 observations were simulated, from which we derived training sets with different sizes. The evaluation of multivariate methods based on LASSO ("ordinary" LASSO, adaptive LASSO and regularized LASSO for selection followed by a ridge regression) was achieved by calculation of the power, the False positive rate (FPR), the False discovery rate (FDR) with each method and by direct comparison of the estimated parameters with the theoretical ones.

The "ordinary" LASSO corrects too much the optimism bias: parameters of the omic variables are underestimated. The adaptive LASSO based on the LASSO estimation of the weights corrects too much the underestimation of the ordinary LASSO: parameters are overestimated. The adaptive LASSO based on ridge estimation showed the best parameter estimation among all LASSO-based methods used for selection and estimation. The regularized LASSO selection showed a slight optimism bias which is reduced with the increase of the training set size.

The optimism bias is a direct consequence of variable selection. The optimism reduces with the increase of the number of variables selected among truly differentially-expressed variables. However, the power is highly correlated with the FPR and the FDR. Thus, it is important to find a compromise between appropriate-model selection and estimation accuracy. LASSO-based methods are adapted for variable selection, whereas they provide biased estimates in non-asymptotic situations. Adaptive LASSO based on ridge estimation of the weights provides the best estimates but it presents the highest value of FDR whatever the size of the training sets.

Poster # 65

mIDEA, an Innovative Adaptative Trial Algorithm for covariate-Adjusted Dose-Finding : Design and First Implementation

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Keywords: bayesian, clinical trial, adaptive, individualized, dose escalation, dose finding

Context: For many drugs in cancer therapy, identification of the optimal non-toxic and effective dose is difficult. Dose adaptation is frequently performed empirically based on personal clinician experience, in a way that is not easily shared between teams, and may be affected by covariates that are not addressed in prescription guidelines. An illustration of such a situation is everolimus, known to be effective at doses frequently associated with a high risk of oral mucositis, thereby requiring empirical dose reductions.

Methods: We built the Multivariate Intrapatient Dose Escalation Algorithm (mIDEA) to optimize dose finding based on patient covariates (body surface area, age and serum albumin) along with toxicity. Using a Bayesian hierarchical pharmacometric population model, at each cycle the mIDEA program proposes an individual dose that is continually reassessed based on covariates and tolerance observed so far both in the individual and in the cohort. The suggested dose is associated with a preset risk of toxicity. Given that clinicians keep the right to choose dosage and ignore mIDEA's recommendation, and that mIDEA's Bayesian prior captures their initial beliefs, mIDEA can safely be assessed in normal clinical practice.

Application: mIDEA is being implemented for everolimus at Centre Hospitalier Lyon-Sud (CITOHL) to examine whether and how dosage should be adapted to body surface area, age and serum albumin, and observed mucositis. It initially proposes dosages deviating by 10% [extrema 0-25%] from those a priori proposed by blinded clinicians. These will then be continually re-adjusted based on clinical observations. A total of 30 to 40 patients are necessary.

Perspectives: mIDEA could be used to optimize the search for treatment doses associated with an acceptable toxicity, based on patients covariates, in many settings. It could be a time- and cost-saving method to identify the best individualized dosage during drug development.

Poster # 66

Comparison of Performances of Three Technologies for Detection of RAS Mutations in cfDNA (NGS strategy, BEAMing Assay and ddPCR BioRAD Detection Assays)

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Keywords: lung cancer, cfDNA, colorectal cancer, digital PCR, BEAMing, NGS

A number of RAS mutations confer resistance to anti-EGFR treatment used in management of colon cancer and lung cancer. The objective of this study was to evaluate the analyze of cell-free plasma DNA (cfDNA) as a reflect of tumor mutation status in order to use liquid biopsy instead of invasive and painful tumor biopsy during the tumor progression. A cohort of 24 lung and colon cancers was constituted in Hospices Civils of Lyon. The molecular profile from biopsies were assessed with PGM NGS technology in the routine use. In this study, plasma samples were sampling at diagnosis (colon cancer) and during progression (lung cancer). KRAS and NRAS somatic alterations were quantified using three different technologies: picoliter-droplet digital polymerase chain reaction (ddPCR) from BioRad and Sysmex Innostics manufacturers and new generation sequencing (NGS, NextSEQ500 by Illumina) of library preparing of 56G oncology panel Kit from Swift Biosciences.

Poster # 67

International Cancer Genome Consortium (ICGC)

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Keywords: oncogene , genomics , mutagenesis , transcription

The International Cancer Genome Consortium (ICGC) was established to bring together researchers from around the globe to comprehensively analyze the genomic, transcriptomic, and epigenomic changes in 50 different tumour types or subtypes that are of clinical and societal importance across the globe (International network of cancer genome projects. *Nature* 464, 993-998 (15 April 2010)).

As of February 2017, the ICGC has received commitments from researchers and funding organizations in Asia, Australia, Europe, North America and South America for 89 project teams in 17 jurisdictions to study more than 25,000 tumour genomes.

Processed data is available via the Data Coordination Centre (http://dcc.icgc.org) based at the Ontario Institute for Cancer Research and is updated semi-annually. The latest data release (Version 23) in December 2016 includes datasets spanning 70 ICGC projects. In total, ICGC data release 23 comprises molecular data from 16,246 cancer genomes.

Raw datasets exist for > 18,000 tumours, including 4,754 whole genome sequences, 7,489 exomes, 21,920 copy number alteration profiles, 20,951 transcriptomes (RNASeq, miRNA-Seq and array-based expression) and 9,317 epigenomes submitted to ICGC.

In Boston, ICGC will report on progress on a number of fronts, including the PanCancer Analysis of Whole Genomes (PCAWG) project and ICGCmed.

In the next two years, ICGC will deliver on its initial and new objectives, including 1) sequencing a cumulative number of >25,000 tumour genomes from 50 or more tumour types; 2) improving data quality of ICGC datasets; 3) developing a scalable software infrastructure to support data management and cancer genome research; 4) streamlining data access mechanisms to accelerate usage and downstream discoveries without compromising the need to protect patient confidentiality; 5) coordinating Pan Cancer and other cross-tumour analyses; 6) and training basic and clinician scientists to use ICGC datasets and tools. More information can be found on www.icgc.org.

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Poster # 68

Identification de nouveaux gènes de prédisposition à l'adénocarcinome ovarien séreux de haut grade, par une approche exome

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Mots clés : prédisposition héréditaire, cancer de l'ovaire, exome

Contexte : l'adénocarcinome séreux ovarien de haut grade (ASOHG) est un cancer rare de mauvais pronostic en raison d'un diagnostic tardif fréquent. Les mesures de dépistages n'étant pas efficaces, l'identification de patientes à haut risque génétique permettrait de leur proposer une prise en charge adaptée et d'explorer d'autres voies thérapeutiques. Au moins 24 % de ces cancers ont une origine génétique, mais aucune mutation n'est identifiée dans 50 à 85 % des cas familiaux, après analyse de panels de gènes. Le séquençage de l'exome apparaît donc nécessaire. Des études basées sur une approche "série de cas non apparentés" n'ayant pu identifier de nouveaux gènes de prédisposition, nous proposons donc une approche familiale. Nos données préliminaires ont trouvé 62 gènes candidats (GC), qui nécessitent des explorations complémentaires.

Méthodologie : Sélection prospective de familles présentant au moins 2 cas d'ASOHG sans mutation BRCA 1 et 2 (monocentrique, multicentrique France / Russie). Un pré-screening par panel sélectionnera les familles sans mutation d'un gène prédisposant connu. Le séquençage de l'exome sera ensuite effectué, le rôle des GC identifiés dans la prédisposition à l'ASOHG sera évalué par :

- Comparaison des analyses constitutionnelles/somatiques sur pièce tumorale
- Analyse d'expression génique/protéique sur pièce tumorale/saine. Analyse à l'échelle de la cellule unique des populations clonales tumorales (C1, Fluidigm)
- Analyse in silico (TCGA, IGCG), avec recrutement d'une population contrôle indépendante
- Analyse de ségrégation
- Analyses fonctionnelles sur culture cellulaire. Seront mesurées différents paramètres décrits dans la cancérogenèse : prolifération, migration, invasion, réparation…

Résultats attendus : Les analyses fonctionnelles proposées confirmeront l'implication des GC. Ils pourront alors être ajoutés aux tests diagnostiques de prédisposition et ouvriront de nouvelles perspectives de thérapies ciblées.

Clinical Research

Poster # 69

Recueil de données en vie réelle concernant l'efficacité des traitements anticancéreux chez les patientes présentant un cancer du sein avancé ou métastatique

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Mots clés : cancer du sein métastatique, données en vie réelle, traitements anticancéreux

En France, on estime à 48763 nouveaux cas de cancers du sein par an (INCa, 2012), dont environ 5% sont métastatiques d'emblée. Le cancer du sein métastatique reste une maladie incurable avec une médiane de survie comprise entre 16 et 29 mois. A ce jour, il existe peu d'informations sur le bénéfice clinique apporté par les protocoles de chimiothérapies actuels sur la population globale de patientes concernées. L'étude CASCADE a montré la diminution rapide des taux de réponses objectives des patientes traitées entre la 1ère et 3ème ligne. Les différents sous-types tumoraux influencent également les réponses tumorales observées (de Paz et al. ESMO, 2016). Une étude observationnelle a été menée à l'hôpital privé Drôme Ardèche pour estimer la survie des patientes chez lesquelles un cancer du sein avancé ou métastatique a été diagnostiqué entre le 01/08/2007 et le 31/12/2013, et traité par au moins une ligne de chimiothérapie. Les données ont été collectées à partir des dossiers médicaux et des logiciels de prescription entre le 01/08/2007 et le 31/12/2016. L'âge médian des 54 patientes était de 63 ans. Parmi les cancers métastatiques diagnostiqués durant la période de l'étude, 35% étaient métastatiques d'emblée. 65% des patientes avaient des métastases osseuses et 26% des métastases ganglionnaires et/ou pulmonaires. En termes de survie, les résultats préliminaires montrent que les patientes ayant présenté une maladie stable ou une progression en 1ère ou 2ème ligne ont une survie médiane globale estimée à 39,4 mois. Parmi les 15 patientes en réponse partielle ou complète en 1ère et/ou en 2ème ligne, 7 patientes sont toujours en rémission partielle ou complète au 31/12/2016. Les faibles effectifs de cette cohorte monocentrique ne permettent pas d'évaluer la survie globale en fonction des sous-types tumoraux, quelle que soit la réponse aux traitements. Toutefois, cette étude montre la faisabilité du recueil de données en vie réelle avec une bonne disponibilité des données.

Poster # 70

Cancer non-médullaire de la thyroide non syndromique et osteosarcome : à propos d'une famille

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Keywords: prédisposition héréditaire, osteosarcome, cancer thyroïde

Contexte : Les cancers non médullaires de la thyroïde familiaux représentent entre 3 et 9 % des cancers thyroïdiens. Environ 5 % de ceux-ci s'intègrent dans un syndrome de prédisposition héréditaire (syndrome de Cowden, de Gardner, de Werner, de DICER1, polypose adénomateuse familiale, complexe de Carney). Dans les formes non syndromiques, plusieurs gènes candidats à pénétrance faible à modérée ont été identifiés (HABP2, FOXE1, SRGAP1).
Nous présentons une famille comportant une association de cancer thyroïdien et d'ostéosarcome, évoquant une prédisposition héréditaire au cancer à haute pénétrance non décrit à ce jour.

Présentation clinique : Parmi une fratrie de 4 individus, nous décrivons deux adénocarcinomes papillaires thyroïdiens précoces (un homme à 21 ans, une femme à 22 ans) et un ostéosarcome huméral (une femme, à 33 ans).
Dans la branche paternelle : nous décrivons un cas de sarcome thyroïdien chez le grand-père à 62 ans, une leucémie chez la grand-mère à 25 ans. Le père présente de multiples nodules thyroïdiens.
Dans la branche maternelle : nous retrouvons un goitre thyroïdien multi-nodulaire traité par thyroïdectomie chez la mère à 49 ans, et la notion d'une thyroïdectomie chez la grand-mère à 75 ans pour une cause indéterminée.
Dans la descendance, on retrouve deux cas de polyarthrite juvénile dont un cas chez le fils de l'individu indemne de la fratrie.

Discussion : La présentation familiale évoque une prédisposition héréditaire au cancer, à transmission autosomique dominante ou récessive, à haute pénétrance, dans un syndrome associant un risque de cancer thyroïdien et d'ostéosarcome.
La présence d'une polyarthrite juvénile familiale peut également s'intégrer dans un syndrome associant une prédisposition au cancer et à la polyarthrite juvénile, à transmission autosomique dominante.
Des explorations moléculaires, notamment par séquençage de l'exome, seront nécessaires afin d'en déterminer la cause.

Poster # 71

Establishing a Regional Network for the Evaluation of the Clinical Utility Of Liquid Biopsies in Lung Cancer

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Keywords: liquid biopsies, ctDNA, lung cancer

The presence of a targetable driver mutation in nearly 50% non-squamous non-small cell lung cancer (NSCLC) patients has enabled tailoring therapy regimens to improve survival.
Serial repeat biopsies can offer an instrumental y indication into the longitudinal evolution of cancer. Mutation detection in plasma tumor DNA as a “liquid biopsy” has been suggested as non-invasive approach to monitor tumor dynamics over time.
We have established a protocol (LIBIL, NCT01511288) for the collection of liquid biopsies from stage IIIB/IV NSCLC patients either untreated, on treatment or progressive to targeted therapy. LIBIL has included 134 NSCLC patients for whom clinical and molecular information is collected prospectively.
Targeted sequencing of plasma samples, using the eTAm-Seq™ assay (Inivata), has allowed the detection of driver mutations with a concordance rate (CR) of 93,3% in matched plasma and tissue samples obtained at diagnosis (n=12/20; unavailable tissue for 8/20 of the evaluated patients). In plasma samples from patients that relapsed under 1st generation EGFR inhibitors (EGFRi) we evidenced the EGFR T790M mutation in 57% of patients (CR=90,9%).
Analysis of serial samples collected from 3 patients under EGFRi showed the emergence of an EGFR T790M mutation 11 weeks before the radiographic confirmation of progression (P1); differential dynamics in the fractions of mutated clones that reflected the pattern of dissociated tumor response (P2) and the presence of concomitant resistance mutations in a patient treated with osimertinib (P3).
We recently established a regional LIBIL network, including 8 Cancer Centers, General Hospitals and University Hospitals.
Our preliminary results provide further evidence on the use of liquid biopsies for monitoring response and resistance to treatment and tumor heterogeneity. The consolidation of the LIBIL network is a great opportunity to foster regional interactions to further evaluate the clinical utility of liquid biopsies.

Poster # 72

Evaluation du profil oxydatif d'une population de l'Ouest algérien atteinte de Cancer Colorectal

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Mots clés : cancer colorectal, stress oxydatif, FORT, FORD, MDA, carbonyles, catalase, superoxyde dismutase, ouest algérien

Objectif : Le stress oxydatif est impliqué dans l'étiologie de plusieurs pathologies telles que le cancer. Notre étude cas-témoin vise à évaluer le profil oxydatif d'une population de l'Ouest algérien(n=32) atteinte d'un cancer colorectal comparé à celui d'une population saine de la même région(n=32).

Méthodes : Dans ce contexte, nous avons étudié au niveau plasmatique de ses populations l'attaque radicalaire par le test FORT et la capacité antioxydante globale par le test FORD en utilisant un photomètre FORMplus (FORM®, CR3000) ainsi que quelques marqueurs de la production radicalaire à savoir le taux de malondialdéhyde selon la technique de quintanilha et al, 1982 et des carbonyles selon la technique de Levine et al., 1990 produits de la peroxydation lipidique et protéique respectivement. En parallèle, nous avons évalué l'activité antioxydante de la catalase (CAT EC 1.11.1.6) selon la technique de Gòth,1991 et de la superoxyde dismutase(SOD EC 1.15.1.1) selon la technique de Maklund et al.,1974. Une analyse statistique a été réalisée pour comparer les moyennes obtenues des deux populations en utilisant le test t de student à l'aide du logiciel SPSS version 20.0.0, une différence est considérée significative si $p<0.05$.

Résultats : Nous avons noté une élévation fortement significative ($p<0.001$) de tous les marqueurs de l'attaque radicalaire ; FORT(mmol H_2O_2/l) 2.69 ± 1.08 vs 1.76 ± 0.20; MDA(µmol/l) 5.98 ± 2.85 vs 1.87 ± 0.44; carbonyles (nmol/mg de protéines) 4.94 ± 2.55 vs 2.13 ± 0.25 chez les cancéreux par rapport aux sains. Une réduction non significative de l'activité de la catalase(kU/l) 11.91 ± 3.30 vs 12.08 ± 1.13 ($p>0.05$) contre une réduction fortement significative de celle de la superoxyde dismutase(U/l) 134.29 ± 34.54 vs 182.51 ± 63.03 ($p=0.001$) et de la capacité antioxydante FORD(mmol/l Trolox) 1.08 ± 0.36 vs 1.33 ± 0.12($p<0.001$).

Conclusion : Notre population cancéreuse présente un stress oxydatif bien marqué par rapport à la population saine.

Poster # 73

Impact of Chemotherapy-Induced Menopause in Young Women with Non-Metastatic Breast Cancer

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Keywords: breast cancer, young women, chemothery-induced menopause, quality of life, hormonal variations

Background: Breast cancer mostly affects women over 50, but 10 to 15% of patients are still in childbearing age and are mostly treated by chemotherapy. Young women could face a chemotherapy-induced menopause (CIM), so it seems important to study its impact on quality of life (QOL). In this context, the Jean Perrin Comprehensive Cancer Center has promoted MENOCOR study. The main objective of this trial is to assess the CIM's impact on QOL 2 years after chemotherapy.

Methods: Menocor is a prospective, multicenter study, in women aged 18 to 45 years and diagnosed with a non-metastatic breast cancer. The main objective is assessed using the functional score of the QLQ-C30. The other aspects of QOL are studied using self-questionnaires (incl. QLQ-BR23) and blood samples are collected to evaluate the hormonal variations (AMH, FSH and estradiol). In our interim analysis, 2 groups of patients have been defined: amenorrhea ≤ 9 months and amenorrhea > 9 months that characterises the CIM. Data to compare both patients groups concern outcomes at 6 months post-chemotherapy.

Results: 59 patients are included in this analysis: 25 women (42%) had an amenorrhea >9 months. No significant difference between the 2 groups was found for the functional scores of the QLQ-C30 and QLQ-BR23 (p=0.09 and p=0.67, resp.). However other parameters showed that the CIM group differs from the other one: in particular amenorrhea appears faster in CIM patients [lower number of chemotherapy cycles until amenorrhea (p=0.005; 2.8±0.9 vs 4.0±1.6)] and they are 3 years older (p=0.03; 40.8±4.1 vs 38.0±5.9). Regarding hormonal variations, CIM patients saw their AMH levels decrease stronger between inclusion and end of chemotherapy (p=0.03).

Conclusion: We expect that with the inclusion of the next 180 patients, QOL differences will reach significance. This interim analysis underlines that amenorrhea >9 months depends on particular clinical parameters which could enable to anticipate CIM in the future.

Poster # 74

Febrile Neutropenia Induced by Chemotherapy in Solid Cancers: Exceptional Complications but Rapidly Fatal

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Keywords: neutropenia-chemotherapy-solid cancers

Introduction : Febrile neutropenia occurred in 13% of patients treated for solid tumors. The purpose of this study is to describe the clinical and therapeutic profile of febrile neutropenia.

Patients and methods : This is prospective study in oncology and radiotherapy center of University Hospital of Casablanca during one year (2013). All patients who presented fever ≥ 38 ° C and neutropenia after chemotherapy were included. Database was analyzed by SPSS 20 with ANOVA one factor.

Results : 25 patients were included. Median age was 45 years [11-73 years] and sex ratio (M / F) was 0.4. The cancer sites were: breast (10 cases) , cervix (3) , nasopharynx (3), germ cell tumors (2), osteosarcoma (2), rectum (2), bladder (1), oral cavity (2). Tumors were metastatic (17 cases) or locally advanced (5 cases) or localized (3 cases). The chemotherapy regimens incrimined were:

- PF (3 cases) and docetaxel (3 cases)
- FOLFOX4, docetaxel / carboplatin, AC60, 3AC60 +3 T, docetaxel / capecitabine, API / AI with 2cases for each.
- TAC, gemcitabin, BEP, 3FEC +3 T, Gemzar / carboplatin, EP, TPF: one case for each.

The median time between chemotherapy and aplasia was 12 days.
According to the WHO grading, neutropenia was grade 4 in 20 patients. Anemia, thrombocytopenia and mucositis were associated with neutropenia in respectively 17, 12 and 16 patients. Bi-antibiotic (gentamycin / Triaxon) was administered in 15 cases, 22 patients received growth factors (ESA), 19 transfusions (red blood cells or platelets). The evolution was favorable in 21 patients. Unfortunately we recorded 4 patients (16%) in our study. Difference in median platelet (patients died versus favorable group) was close to significance (p = 0.063). Median duration of hospitalisation was 6.4 days.

Conclusion: Febrile neutropenia is very deadly in our context despite taking standardized load. The combination of mucositis, anemia, thrombocytopenia or mucositis explains this high mortality.

Poster # 75

Prise en charge des métastases choroïdiennes en radiothérapie externe : étude rétrospective et synthèse de la littérature

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Keywords: métastases-choroidiennes-cancers-radiothérapie

Objectif : Les métastases choroïdiennes sont rares au cours de l'évolution des cancers solides et constituent des sites métastatiques exceptionnels mettant en jeu le pronostic fonctionnel visuel. Nous avons effectué une étude rétrospective afin de déterminer l'intérêt de la radiothérapie externe pour le traitement des métastases choroïdiennes.

Patients et méthodes : Nous avons revu les dossiers de 28 patients avec des métastases choroïdiennes qui présentaient un cancer du sein (n=15), du poumon (n=9), ovaire (n=1), rein (n=1), prostate (n=1), sans primitif retrouvé (n=1). L'âge médian était 58 ans (Extrêmes : 34 à 71 ans). Le stade tumoral avant la découverte des métastases choroïdiennes était métastatique pour 50% des patients. L'atteinte oculaire était unilatérale (n=22) ou bilatérale (n=6). Les doses délivrées variaient de 20 à 50 Gy avec un fractionnement de 3 à 5 Gy en technique 2D (n=5), conformationnelle 3D (n=21), modulation d'intensité (n=2). Le schéma de prescription le plus utilisé délivrait 30 Gy en 10 fractions (64%) par l'intermédiaire de 2 faisceaux de photons d'énergie 6 MV.

Résultats : En fin d'irradiation, 13 patients (46%) ont présenté une amélioration des symptômes ophtalmologiques. Pour les autres, une stabilisation des symptômes a été notée (n= 15). Aucun patient n'a présenté de dégradation visuelle. Aucune toxicité aigüe ou tardive de grade 2-3 n'a été objectivée. Le type histologique n'influençait pas la réponse (p=0,5). Il n'existait aucune relation dose –réponse dans notre série.

Conclusion : La radiothérapie externe est une technique utile dans le traitement palliatif des métastases choroïdiennes. Les toxicités aigues et tardives restent acceptables.

Poster # 76

Cancer du foie en milieu congolais: Profil épidémiologique, clinique, para clinique et évolutif à propos de 35 cas

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Mots clés : cancers du foie, epidémiologie, CUK, HGRM, Kinshasa, RDC

Contexte : Les tumeurs du foie sont diagnostiquées au stade de cancer dans les pays en voie de développement où le niveau socioéconomique ne favorise pas la prise en charge des pathologies chroniques.

Objectif : L'objectif général de cette étude est d'évaluer l'ampleur des tumeurs du foie dans les hôpitaux de la ville de Kinshasa.

Méthodologie : Étude transversale descriptive de 5 ans, réalisée dans les services de médecine interne des Cliniques Universitaires de Kinshasa et de l'Hôpital Général de Référence de Makala, portant sur 35 patients ayant un cancer du foie. Certains des patients ont bénéficié d'une exploration échographique abdominale et d'autres d'un dosage du taux d'alpha-foetoprotéine, aucun patient n'avait réalisé un examen histologique hépatique.

Résultats : La fréquence des cancers du foie dans la population d'étude était de 0,3%, les hommes représentaient 71,4%, l'âge moyen était de 57,2±16,9 ans. L'alcool était l'antécédent le plus fréquent (62,9%). L'ascite, l'hépatomégalie, la douleur abdominale étaient les signes cliniques les plus fréquents, l'examen du foie est plus marqué par un foie augmenté de volume, de bord inférieur tranchant dans 60%, dur dans 45,7%, sensible (51,4%) ; l'ascite (68,6%) et l'ictère (60,0%) étaient les complications retrouvées ; le VHC et le VHB étaient retrouvés dans 60,0% et 58,3% respectivement. 57,9 % des patients étaient au stade C de Child Pugh et 45,7% étaient décédés.

Conclusion : Dans nos cliniques, le profil des cancers du foie est semblable à celui observé dans la littérature, le contrôle des facteurs de risque permet de réduire son incidence en Afrique.

Poster # 77

Profil immunohistochimique du cancer du sein de la femme dans le département d'Annaba

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Mots clés : cancer du sein, cancer du sein , Afrique du Nord , immunohistochimie , incidence

Objectif : L'objectif de notre travail est de déterminer le nombre de nouveaux cas (incidence) du cancer du sein selon le type IHC.

Matériel et Méthode : Les données ont été extraites du registre du cancer d'Annaba et ont concerné tous les cas de cancer du sein (C50 selon CIM10), survenus durant l'année 2016.
Les numérateurs des taux d'incidence ont été calculés en rapportant les % des types IHC retrouvés parmi les cas où l'information était disponible au total des nouveaux cas de cancers . Ces taux ont été exprimés en nombre de cas pour 100 000 femmes.

Résultats : Le nombre de nouveaux cas du cancer du sein survenus chez la femme est de 215 cas soit une incidence annuelle de l'ordre de 60,6 cas/ 100 000 Femmes (F).
Le nombre de cas avec information disponible sur les récepteurs hormonaux était de 148 cas. Parmi lesquels, la fréquence des tumeurs à récepteurs ostrogéniques positifs (RE+) était de 73,0% (IC 95% = 65,1 – 79,9) soit une incidence de 29,9 cas /100 000 F, la fréquence des tumeurs à récepteurs de progestérone positifs (RP+) était de 68,9% (IC 95% = 60,8 – 73,3) soit une incidence de 28,2 cas /100 000 F et de 26, 0% (IC 95% = 19,1 – 33,9) pour les tumeurs avec Her2 positifs avec une incidence de 10,7 cas /100 000 F Les tumeurs triple négatif représentent 16.9% (IC 95% = 11,2 – 23,9) de l'ensemble des cas avec une incidence de l'ordre de 7,04 cas /100 000 F

Conclusion : Les proportions des types IHC dans notre travail ne différent pas des proportions retrouvées sur les séries de cas. L'incidence de la forme triple négative est faible comparativement aux incidences de cette forme qui sont plus élevées dans les pays occidentaux.

Poster # 78

Enquête épidémiologique et analyse biochimique du stress oxydatif dans le cancer du sein à l'ouest algérien

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Mots clés : cancer du sein, contraception, stress oxydatif, TBARS, FORD

Introduction : Le cancer du sein reste le premier cancer féminin en termes de fréquence et de mortalité. L'étiologie de ce cancer est plurifactorielle ; elle repose sur la définition des indicateurs individuels de risque, entre autres les facteurs environnementaux et le mode de vie.

Objectif : Le présent travail a pour objectif de réaliser une étude des facteurs de risque, qui semblent être liés au cancer du sein, et d'évaluer l'état du stress oxydatif chez des patientes issues d'une population ouest-algérienne.

Matériels et méthodes : Nous avons procédé à une étude cas témoins couvrant la période de 2010 à 2012. Les informations ont été colligées par le biais d'un questionnaire bien établi. Par ailleurs, l'analyse de l'état du stress oxydatif au niveau sanguin a inclus 15 patientes n'ayant subi aucune thérapie anticancéreuse et 15 témoins. Le test FORD est réalisé par l'analyseur FORM plus tandis que les TBARS ont été mesurés par spectrophotométrie.

Résultats : Dans notre série de malades, la fréquence du cancer du sein est faible avant 35 ans puis augmente régulièrement avec l'âge jusqu'à atteindre un pic entre 45 et 54 ans. La régularité des cycles menstruels a été observée chez 75,60% des cas. Le sein gauche s'avère le plus atteint avec une fréquence de 53,23%. Une très faible pratique du sport est observée chez les malades avec 11,91% environ 1 fois/mois.
A l'issue de l'analyse du sang, il ressort que la différence est hautement significative entre les taux moyens des TBARS au niveau du plasma des malades comparativement aux témoins. Les taux moyens du FORD sont significativement plus faibles chez les patientes atteintes de cancer du sein (1,11 ± 0,22 mmol/l Trolox) en comparaison avec les témoins (1,31 ± 0,12 mmol/l Trolox) avec p < 0,0001.

Conclusion : La surveillance clinique des patientes atteintes de cancer du sein par le dosage des marqueurs du stress oxydatif pourrait avoir un intérêt très important dans le pronostic et le suivi thérapeutique.

Poster # 79

Tobacco and Precocity of Colorectal Cancer and Diverticulitis

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Keywords: tobacco, colorectal cancer, onset age, risk-factor

Tobacco impact on carcinogenesis is well documented, even in colorectal cancers (CCR) where it plays an indirect role. But literature about an earlier onset of CCR due to smoking is scarce. This study focuses on this topic.

Methods: From June to September 2002, a prospective cross-sectional survey was conducted to gather all cases of colorectal surgery in 81 French hospitals. 1,426 resections were collected of which 997 were performed for a non-hereditary CRC and 424 for sigmoid diverticulitis (SDV). Present smoking and number of cigarette packs-years (PY) were noted. This information was reported in 1,226 patients: 170 were smokers (CRC = 112 and SDV = 57). Other factors were available: gender, BMI, alcohol.

Results: Among smokers, 56 smoked ≤ 10 PY and 114 > 10 PY. Age at CCR onset was 69.6 ± 12.6 years (y) for non-smokers and 64.1 ± 11.6 (Δ = 5.5 y, p=2.4 10^{-6}) for smokers, and for SDV resp. 62.9 ± 13.8 and 52.4 ± 11.6 (Δ = 10.5 y, p<10^{-7}). Multivariate analysis showed that smoking was the most significant factor (p<10^{-7}) for early onset of both CRC and SDV, followed by gender (p=0.0000014) and obesity (BMI > 30, p=0.023). CRC precocity due to tobacco varied according to cancer location: smoking shortened CRC onset by 14 y in transversal colon (p=0.0021), 8 y in rectum (p=0.0007) and 6 y in sigmoid colon (p=0.0007).

Conclusion: Tobacco is not only a risk factor for CRC and SDV, but it favors their early onset. Influence of tobacco on CRC onset depending on location suggests that a mechanistic cause modulates the biological patterns of CRC carcinogenesis: smokers' saliva carries tobacco carcinogens all through the digestive track. Slow transit segments of the bowel may be longer exposed to these deleterious molecules and develop faster local defects possibly evolving in CRC or SDV. This study demonstrates by the way that wide studies regarding age at cancer onset could provide valuable information about mechanism of carcinogenesis and cancer prognosis.

Environment, Nutrition & Epidemiology

Poster # 80

Study of Body Composition During Adjuvant Antihormonal Therapy in Postmenopausal Breast Cancer Patients

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Keywords: breast cancer, antihormonal therapy, weight variations, body composition, fat mass

Context: Breast cancer (BC) treatments can lead to weight variations which may turn out to be pejorative if higher than 5% of initial weight (iW). A prospective study conducted in Jean Perrin Comprehensive Cancer Center studied weight variations and body composition in 50 BC patients treated by adjuvant chemotherapy. Results showed that after 6 cycles (T1), 20% of patients lost weight, 20% gained weight. Six months post-chemotherapy (T2), these variations were maintained. In patients who gained weight, a gain in fat mass was observed, but only 6 months after the end of chemotherapy (T2).

Objective: To assess the evolution of body composition of the same group of patients currently treated by antihormonal therapy (AHT) (n=33).
Methods: Dual-Energy X-ray Absorptiometry was used to measure fat and lean body mass. Body water was assessed by impedancemetry.

Results: After an average of three years of AHT (T3), patients gained 2kg ±5.4 (2.9% ± 7.9) (p=0.031) compared to their weight prior to chemotherapy (T0). This weight gain mainly occurred after the initiation of AHT (+2kg ±5.5; p=0.04). After three years of AHT following chemotherapy, 9% (n=3) lost more than 5% of their iW and 30% (n=10) gained more than 5% of their iW. The average lost was 5kg ±0.6 (8.3% ±1.7) and the average gain was 7.9kg ±5.9 (11.6% ±8.4). This weight gain was characterized by a fat mass gain (4kg ±5; p=0.034) especially in trunk (2.3kg ±2.8; p=0.03) and a lean body mass gain (4.6 ±3.5; p=0.003) mainly reflecting a water gain (2.5 ±2.3; p=0.018). At T3, 86% (n=6) of patients who had lost weight and 50% (n=4) of patients who had gained weight at T2 returned to their iW. For patients with a stable weight at T2, 33% (n=6) lost more than 5% of their iW and 11% (n=2) gained more than 5% at T3.

Conclusion: This study shows the impact of adjuvant treatments on weight and body composition of BC patients. Results suggest that weight gain, especially fat mass gain, mainly occurs after AHT initiation.

Poster # 81

Lifetime and Baseline Alcohol Use and Risk of Pancreatic Cancer in the European Prospective Investigation on Cancer and Nutrition Study

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Keywords: pancreatic cancer, EPIC, lifetime alcohol, alcohol subtypes, dose-response analysis

Background: Recent evidence from a large meta-analysis of 18 prospective studies suggested a weak relationship between average alcohol consumption and pancreatic cancer (PC) risk. In the present study the association between lifetime and baseline alcohol intakes and the risk of PC was examined within the European Prospective Investigation into Cancer and Nutrition (EPIC) study.

Methods: From 476,106 cancer-free participants enrolled between 1992 and 2000, 1,283 incident PC cases (57% women) were developed over a median follow-up of 14 years. Amounts of lifetime and baseline alcohol - differentiating wine, beer, and spirits intakes - were collected at recruitment by lifestyle and dietary questionnaires, respectively. Cox proportional hazard models with age as primary time variable were performed to estimate PC hazard ratios (HR), after adjustment for confounding factors and dose-response analysis was performed.

Results: Lifetime alcohol use was positively associated with PC risk in men, with HR equal to 1.06 (95%CI: 1.02, 1.10, ptrend60g/day) for both lifetime (HR=1.69; 95%CI: 1.04, 2.75) and baseline (HR=1.72; 95%CI: 1.21, 2.43) alcohol intakes. Lifetime and baseline alcohol use were not related to PC risk in women. Baseline alcohol results were homogeneous with respect to smoking status.

Conclusions: Findings from a large European prospective study suggest that the risk of developing PC is positively associated to extreme levels of alcohol intake in men.

Poster # 82

Tumor patterns, penetrance and excess cancer risk in germline TP53 mutation carriers.

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Keywords: P53 mutation, tumor, temporal patterns, penetrance, excess risk

Purpose: Germline TP53 mutation predisposes to multiple cancers but age-dependent risk is still poorly understood. We have used data from IARC TP53 database (p53.iarc.fr) to reevaluate age-dependent cancer patterns and excess risk as compared to non-carriers.

Method: A total of 2,079 carriers from 775 families were analyzed (excluding carriers of the Brazilian founder mutation p.R337H).

Results: Overall 2,550 tumors were reported, with a median age at cancer diagnosis of 28 and 31 years in males and in females, respectively. Penetrance was estimated to 80% at 70 years in both genders, with higher penetrance in males than in females during childhood and adolescence (P

Poster # 83

Determination of a Geographic Information System (GIS) Based Indicator to Assess Environmental Dioxins Exposure in Lyon and Through Comparisons with an Atmospheric Dispersion Model Results

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Keywords: dioxins, GIS, dispersion modelling, exposure assessment, SIRANE

Purpose: To classify the subjects of the E3N cohort placed in the Lyon urban area according to past dioxin exposure level by means of GIS based indicators. The accuracy of these indicators was evaluate by a systematic comparison with the dioxin concentrations computed by an atmospheric dispersion model.

Methods: Industrials sources were the main contributors for dioxin exposure over the study period (CITEPA, 2015). They were selected and characterized with the Standardized Toolkit for Identification and Quantification of Dioxin and Furan Releases of the United Nation Environment Program. Atmospheric dioxin dispersion was modeled with SIRANE, an urban Gaussian model, for four years: 1996, 2002, 2007 and 2008. In order to validate the SIRANE model performances, its results were compared to measures of weekly average concentrations collected within the Lyon urban area and available for 2007 and 2008.
Furthermore, a sensitivity analysis of the SIRANE results allowed us to identify the parameters that mostly affect the dioxin concentration levels at each of the 300 subject. Those parameters were subsequently integrated in the GIS based indicator to assess dioxin exposure. In order to compare the exposure levels provided by SIRANE and the GIS based indicator, subjects were classified in quintiles. Cohen's kappa coefficients were calculated to estimate the agreement at each year between the 2 methods before and after the additions of the impacting parameters.

Results: Kappa coefficients that were only based on proximity to dioxin sources were all below 0.55. Adding impacting parameters raised Kappa coefficients to values between 0.71 and 0.84. In absence of nationwide dioxin emission data, to assess dioxin exposure in the GEO3N nested case-control study, we showed the capability to use a GIS based indicator of dioxin exposure with substantial agreement with dispersion modelling across different settings.

Funding sources:
GEO3N : ADEME, CLARA, UCBL

Poster # 84

Circulating Inflammatory Markers and Risk of Differentiated Thyroid Carcinoma in Epic

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Keywords: differentiated thyroid carcinoma, inflammation, obesity

Purpose: This work is part of an ongoing project to study the aetiology of differentiated thyroid carcinomas (TC) within the European Prospective Investigation into Cancer and nutrition (EPIC) cohort. Previous results showed direct associations of TC risk with obesity, total energy intake, low consumption of polyunsaturated fatty acids, low alcohol intake. For biomarkers, we found positive associations with circulating concentrations of thyroglobulin and insulin-like growth factor and a negative association with thyroid stimulating hormone. To further understand the association between overweight and obesity (a state of chronic inflammation) with TC risk, we have initiated a study to investigate the relationship between the risk of differentiated TC and the concentrations of leptin, adiponectin, C-reactive protein, interleukin(IL)-6, IL-10 and TNF-α.

Methods: A case-control study has been nested within EPIC (475 incident TC cases and 1017 controls). Biomarkers have been measured using previously validated, highly sensitive commercially available immunoassays. Relative risk of TC by levels of each biomarker has been estimated using conditional logistic regression.

Results: Preliminary analyses indicate a significant increase in TC risk with higher levels of IL-10, overall and in analyses restricted to women and a significant decrease in risk with higher adiponectin levels among women only. Both these associations remained statistically significant after adjustment for BMI. A borderline significant positive association was observed with IL-6 overall but was strongly attenuated after BMI adjustment. No significant associations were observed with other markers. Further statistical analyses are ongoing to refine these results.

Conclusions: The proposed study is the first prospective study conducted on inflammation, cytokines and differentiated TC risk, and will provide insights on mechanisms relating overweight and obesity to TC risk.

Funding: INCa SHS-E-SP

Poster # 85

Epigenetic Precursors of Childhood Cancer and Associated Early-Life Exposure

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Keywords: childhood cancer, in utero exposure, environmental factors, DNA methylation, birth cohorts, I4C

Object: Childhood cancer remains the first cause of disease-related death in children, with increasing incidence worldwide. Its risk factors are largely unidentified and mostly based on retrospective evidence. During embryogenesis, a global redistribution of DNA methylation occurs to enable tissue differentiation. Hence, we hypothesize that DNA methylation during *in utero* development may act as a sensor of environmental exposures and mediate risk to childhood cancer later in life.

Method: We profiled the genome-wide methylation levels in cord blood samples from the International Childhood Cancer Cohort Consortium (I4C), the largest prospective investigation into childhood cancer based on mother-child birth cohorts. Starting with a major I4C cohort, DNA methylation levels of more than 450,000 cytosines (CpGs) were compared (using HM450-BeadChip) between nested cases (n=80, representing similar proportions of leukemias, central nervous system tumors and other tumors) and controls (n=160, matched to cases by birth year).

Results: We identified two differentially methylated 200-bp regions (DMRs) in leukemias relative to controls (FDR<0.05). A mean difference of 5-10% methylation was consistently found across several CpG sites in each DMR and was validated using bisulfite pyrosequencing. The observed associations were not influenced by covariates such as blood cell subtype distribution and age. These potential epigenetic signatures of childhood leukemia are currently being replicated and analyzed in relevance to early-life exposure factors in additional I4C cohorts. Preliminary findings suggest a role for early-life infection, maternal smoking during pregnancy and maternal use of hormone contraception preceding pregnancy.

Conclusion: These findings may place DNA methylation in the causal pathways linking early-life exposures and childhood leukemia and may contribute to a 'leap forward' in deciphering mechanistic precursors of childhood cancer.

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Poster # 86

Impact du régime hyperlipidique sur la migration des cellules immunitaires dans un modèle murin de carcinogenèse mammaire

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Mots clés : cancer du sein, immunité, obésité

Objet : Les modifications des défenses immunitaires systémiques et tissulaires induites par l'obésité sont des facteurs de risque de cancer mammaire en post-ménopause. Notre objectif est d'évaluer l'impact d'un régime hyperlipidique sur la réponse cellulaire immunitaire au niveau systémique et tumoral.

Méthode : Des souris femelles C57/bl6 âgées (33 semaines) ovariectomisées sont nourries avec un régime standard (SD : 3,4 kcal/g, lipides 10 % AET) ou un régime hyperlipidique (HL : 4,3 kcal/g, lipides 45% AET). Après 4 semaines, un groupe de chaque régime reçoit une implantation de cellules tumorales (EO771). La croissance tumorale est mesurée tout au long de l'expérimentation. Au sacrifice, un phénotypage des cellules immunocompétentes est conduit par cytométrie en flux à partir des tumeurs et des principaux tissus immunitaires (thymus, rate, ganglions…).

Résultats : Le régime HL induit une augmentation du volume tumoral (1114 ± 793 vs 384 ± 339 mm3 à 16 jours, p=0,04). Les organes lymphoïdes périphériques (rate et ganglions inguinaux) présentent une hypertrophie corrélée à la taille de la tumeur (r=0,4652, p=0,0004 pour la rate). Le phénotypage des cellules immunitaires infiltrées dans la tumeur révèle, sous régime HL vs SD, une augmentation des populations immunosuppressives, associée à une modification du ratio lymphocytes T cytotoxiques / T régulateurs (LTc/LTreg : 1,4 ± 0,1 vs 12,8 ± 6,8, p=0,05). Des altérations similaires (rapport LTc/LTreg) sont retrouvées dans les organes lymphoïdes périphériques.

Conclusions : Le régime HL induit une croissance tumorale accélérée et favorise la migration des cellules immunitaires depuis les organes lymphoïdes secondaires vers la tumeur. Le régime HL induit ainsi un recrutement intratumoral de cellules immunosuppressives favorisant la carcinogenèse, associé à une repolarisation de la réponse immunitaire au niveau systémique et tumoral.

Poster # 87

Highlights from Recent IARC Monographs on Pesticides: A Meta-Analysis of 2,4-D and NHL by the IARC Working Group

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Keywords: pesticide, cancer, 2,4-D, non-Hodgkin lymphoma

2,4-dichlorophenoxyacetic acid (2,4-D), an herbicide used widely to control weeds, was recently classified as "possibly carcinogenic to humans" (Group 2B) by IARC in June 2015. The evaluation was based on limited evidence for carcinogenicity in experimental animals, strong mechanistic evidence that 2,4-D induces oxidative stress in humans as well as moderate evidence of immunosuppression, and inadequate evidence for carcinogenicity in humans. The IARC Working Group conducted a meta-analysis to assess the association between exposure to 2,4-D and non-Hodgkin's lymphoid neoplasms (including CLL, HCL, and MM) to aid its evaluation of the epidemiological evidence. Various sensitivity analyses were conducted to assess the robustness of the results. A primary analysis with the best adjusted estimates from 13 studies (10 case-control, 3 cohort) yielded a meta-relative risk (meta-RR) of 1.04 (95%CI, 0.88-1.22; I2=6%) but which was slightly higher when using the least adjusted estimates from the studies (meta-RR, 1.31; 95%CI, 1.10-1.56; I2=37.6%). Since pesticide exposures usually occur as mixtures, use of other pesticides was an important consideration: the meta-RRs for whether or not studies adjusted for other pesticides were 1.10 (95%CI, 0.86-1.42; I2=9.7%) and 0.98(95%CI, 0.78-1.24; I2=9%), respectively. The meta-RR restricted to the 10 case-control studies was 0.97 (95%CI, 0.82-1.15; I2=0%). The IARC Working Group concluded that at present there was inadequate evidence for an association between exposure to 2,4-D and the risk of non-Hodgkin's lymphoid neoplasms in humans.

Poster # 88

Modeling Cancer Driver Events *In Vitro* Using Carcinogen Exposure and Immortalization Assays in Combination with Massively Parallel Sequencing

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Keywords: cancer driver, mutation, carcinogen, primary cell immortalization, massively parallel sequencing

Objective: Cancer genomes harbor mutation spectra reflecting exposures to external factors and endogenous events underlying tumor development. Information on candidate cancer driver alterations is accessible from public compendia of somatic mutations, yet much of this knowledge is descriptive and of limited mechanistic insight. Well-controlled experimental systems are thus needed to study the functional impact of carcinogenic exposures on the genome and on cancer cell growth.

Methods: We devised barrier bypass-clonal expansion (BBCE) assays based on cultured primary mouse embryonic fibroblasts, which upon exposure to mutagenic carcinogens circumvent senescence, a selective pressure barrier, and immortalize. Whole exomes of 25 independently arising cell lines were sequenced to decipher both the mutational signatures and the putative functional driver events selected and enriched for during the outgrowth phase.

Results: Almost 200 COSMIC Cancer Gene Census genes were found mutated, many of them recurrently, in the set of analyzed cell lines. The recurrent alterations affected pathways regulating DNA damage response and repair, transcription and chromatin structure, cell cycle, and cell death, as well as developmental pathways. The functional impact of the mutations manifested through a number of known cancer gene hotspots. When investigating the contribution of particular, putative cancer driver mutations to the immortalized phenotype, we identified a novel Ras-mediated dependence of non-synonymous Smarcd2 and Smarcc1 mutations on PRC2 histone methyltransferase activity. This interplay is in keeping with the roles of mutations in other BAF complex subunits in cancer cells.

Conclusion: We propose that the mutation catalogue generated by deep sequencing of the BBCE cell lines constitutes a unique resource that can yield mechanistic insights into individual or combinatorial driver events relevant to human cancer development.

Acknowledgements
INCa-Inserm Plan

Poster # 89

Mutational Signatures of Aflatoxin in Cells, Mice and Human Tumours: Implications for Molecular Cancer Epidemiology

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Keywords: aflatoxin, hepatocellular carcinoma, mutational signatures, whole-genome sequencing, mouse model

Objective: Aflatoxin B1 (AFB1) is IARC Group 1 carcinogen that causes hepatocellular carcinoma (HCC). The genome-wide mutational signature of AFB1 has been proposed but not validated experimentally. Here we report a genome-scale analysis of experimentally induced mutational signature of AFB1 in human cell lines and mouse tumours, and link these results to mutational signatures observed in human HCCs.

Methods: We performed whole-genome sequencing of two human cell-lines exposed to AFB1 and of tumours induced by AFB1 exposure in mice, with one mouse model harbouring the hepatitis B surface antigen transgene to mimic HBV infection which synergizes with aflatoxin in human HCC. Whole genome and exome sequencing of human tumours from populations likely exposed to AFB1 was also performed, and mutational signatures obtained in all systems were compared.

Results: AFB1 mutational signatures from all experimental systems were remarkably similar, with dominating G>T mutations accumulating on the non-transcribed strand. Comparison of the experimental data with data from newly-sequenced HCCs from Qidong County, China, a region of well-studied aflatoxin exposure, showed that COSMIC mutational signature 24, previously hypothesized to stem from aflatoxin exposure, indeed likely reflects AFB1 exposure, possibly combined with other exposures. Among published somatic mutation data from 347 HCC genomes, we found evidence of AFB1 exposure in 16% of HCCs from Hong Kong, but only 1% of HCCs from Japan.

Conclusion: Aflatoxin exposure apparently remains a substantial public health issue in some areas. This aspect of our study exemplifies the promise of future widespread resequencing of tumour genomes in providing new insights into the contribution of mutagenic exposures to cancer incidence.

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Poster # 90

The Dynamics and Topology of the Epigenomic Landscape During Carcinogen-Induced Cell Immortalization: An *In Vitro* Approach

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Keywords: human mammary epithelial cells, carcinogen exposure, genomics, epigenetics, integrated analysis

Objective: Detailed identification of somatic alterations in human tumours can reveal information on past carcinogenic exposures and cancer etiology. However, analyses of human cancer data are challenging due to the complexity of exposures and the interactions with endogenous factors underlying the clonal selection of genetic as well as epigenetic driver events. To better understand cancer etiology, we employ human mammary epithelial cells (HMEC) as an in vitro carcinogen exposure system to introduce genetic alterations and epigenetic changes, in order to study their functional interplay during cell immortalization.

Methods & results: HMECs are a unique human primary cell type shown to immortalize due to carcinogen exposure, by first bypassing the initial stress-related cell cycle arrest followed by evasion of replicative senescence. We immortalized HMEC by treatment with potent mutagenic carcinogens aristolochic acid, methylnitronitrosoguanidine and benzo(a)pyrene. Cytotoxicity and genotoxicity assays were employed to establish exposure doses and times. Morphological and growth characteristics were used to assess cell senescence and its bypass. A temporal, integrated genomic and epigenomic analysis is applied to the immortalized cell lines, involving whole genome sequencing alongside genomic location/topology analysis (ChIP-seq, ATAC-seq) and transcriptomic profiling (RNA-seq).

Conclusion: Well-controlled experimental exposure/immortalization models can yield important insights into the dynamics and functional relationships between carcinogen effects and genetic/epigenetic driver events usually challenging to disentangle from the end-point complex human cancer data. We propose that deep integrated analysis of this suitable model system can reveal novel (epi)genetic signatures of carcinogen-induced cellular immortalization, thus proving beneficial for understanding the causes of cancer at the molecular level.

Acknowledgements
INCa-Inserm Plan Cancer 2015 to JZ

Poster # 91

The IARC Mutspec Project: Innovative Genome-Wide Modeling of Mutation Spectra of Human Carcinogens

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Keywords: carcinogens, primary cell immortalization, deep sequencing, mutational signature

Objective: Interactions of carcinogenic compounds with DNA can produce characteristic mutation patterns (computationally discernible 'mutational signatures') which can provide insights into cancer etiology. While primary cancer genome data accumulate due to large-scale sequencing efforts, there is a profound lack of simple and robust experimental strategies elucidating the impact of particular carcinogens on the genome. To fill this gap, we devised a multi-system in vitro and in vivo screening approach to identify carcinogen-specific mutational signatures.

Methods: We first assessed the cytotoxic and genotoxic effects of selected chemicals (acrylamide, glycidamide, ochratoxin A) on primary mouse embryonic fibroblasts, followed by carrying the exposed, senescing cells to clonal outgrowth and expansion. The TP53 gene was screened for mutations to select clones for subsequent whole-exome sequencing (WES). Mutational signatures were extracted from WES data by non-negative matrix factorization, and compared to genome-wide mutation spectra obtained from rodent bioassay-derived FFPE tumors resulting from the same exposures (source: US National Toxicology Program).

Results: γH2Ax was markedly increased in the treated cells compared to untreated controls. The acrylamide-exposed cell lines exhibited TP53 mutations on purine residues and WES analysis identified the putative acrylamide-mutational signature manifested by a diffuse pattern with some transcription-strand bias. DNAs from ochratoxin A exposure-derived animal tumors are undergoing whole-genome sequencing to complement the in vitro experimental results.

Conclusion: Global profiles of genetic changes derived from well-controlled experimental exposure systems can provide mechanistic insights into the etiology of human cancers and may be applicable to carcinogen evaluation, classification and evidence-based cancer prevention measures.

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Poster # 92

Impact des adipocytes et de leur secrétome sur l'efficacité de l'hormonothérapie dans le cancer du sein en situation de surpoids/obésité

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Mots clés : cancer du sein, obésité, adipocytes, secrétome adipocytaire, hormonothérapie

L'obésité, facteur de risque établi de cancer du sein chez les femmes ménopausées, est aussi responsable de plus forts taux de récidives et de mortalité. Dans ce contexte, nos travaux évaluent l'impact du secrétome adipocytaire (SA) dans la moindre réponse à l'hormonothérapie. Pour cela, 1/ des cellules cancéreuses mammaires ont été soit co-cultivées avec des cellules souches adipeuses (CSAd) humaines (hMAD) différenciées en adipocytes matures (AM) soit cultivées en présence de milieux conditionnés (MC) par les hMAD, puis traitées avec un anti-estrogène (anti-E ; Tamoxifène Tx ou Fulvestrant Fv). La prolifération cellulaire a été mesurée par fluorescence à la résazurine (Fluoroskan Ascent FL®, n=3) et suivie en temps réel par impédancemétrie (iCELLigence, n=3). 2/ L'impact du surpoids a été évalué ex vivo en utilisant des AM différenciés à partir de CSAd de femmes minces ou obèses cultivées en présence de cellules MCF-7 et de Fv. Dans nos différents modèles, le SA est capable de majorer la prolifération des cellules MCF-7, récepteur aux estrogènes positives (RE+), et d'inhiber totalement l'effet antiprolifératif du Tx et du Fv. L'utilisation de cellules MDA-MB-231 RE- a montré que le SA majore la prolifération cellulaire, contrecarre l'effet antiprolifératif du Tx mais pas du Fv, suggérant que les effets observés ne passent pas exclusivement par la voie du RE. Par ailleurs, en utilisant des AM de femmes de poids variables, seul le SA des femmes d'IMC>30 amoindrit l'efficacité du Fv, cet effet s'accompagnant d'une augmentation de l'expression du gène OB-R. Ainsi, ces résultats préliminaires suggèrent que le SA limite l'efficacité de l'hormonothérapie et ce, de façon plus prononcée en cas de surpoids, ce qui pourrait contribuer au processus d'échappement tumoral. Projet financé par l'INCA, projet Mammadipo N°6666.

Poster # 93

Long-Term Exposure to Bisphenol A or Benzo(a)pyrene Alters the Fate of Human Mammary Epithelial Stem Cells in Response to BMP2 and BMP4, by Pre-Activating BMP Signaling

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Keywords: breast cancer, bisphenol A, bone morphogenetic proteins, pollutants, microenvironment, stem cells

Bone morphogenetic protein 2 (BMP2) and BMP4 are key regulators of the fate and differentiation of human mammary epithelial stem cells (SCs), as well as of their niches, and are involved in breast cancer development. We established that MCF10A immature mammary epithelial cells reliably reproduce the BMP response that we previously identified in human primary epithelial SCs. In this model, we observed that BMP2 promotes luminal progenitor commitment and expansion, whereas BMP4 prevents lineage differentiation. Environmental pollutants are known to promote cancer development, possibly by providing cells with stem-like features and by modifying their niches. Bisphenols, in particular, were shown to increase the risk of developing breast cancer. Here, we demonstrate that chronic exposure to low doses of bisphenol A (BPA) or benzo(a)pyrene (B(a)P) alone has little effect on SCs properties of MCF10A cells. Conversely, we show that this exposure affects the response of immature epithelial cells to BMP2 and BMP4. Furthermore, the modifications triggered in MCF10A cells on exposure to pollutants appeared to be predominantly mediated by altering the expression and localization of type-1 receptors and by pre-activating BMP signaling, through the phosphorylation of small mothers against decapentaplegic 1/5/8 (SMAD1/5/8). By analyzing stem and progenitor properties, we reveal that BPA prevents the maintenance of SC features prompted by BMP4, whereas promoting cell differentiation towards a myoepithelial phenotype. Inversely, B(a)P prevents BMP2-mediated luminal progenitor commitment and expansion, leading to the retention of stem-like properties. Overall, our data indicate that BPA and B(a)P istinctly alter the fate and differentiation potential of mammary epithelial SCs by modulating BMP signaling.

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Poster # 94

Contribution of Adipose Stem Cells from Obese Subjects to Hepato-or Breast-Carcinoma Tumorogenesis, through Promotion of Th17 Cells

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Keywords: obese adipose stem cells, Th17 lymphocytes, breast cancer, hepatic cancer

Introduction: As opposed with lean adipose tissues (AT), obese AT are heavily infiltrated with variety of inflammatory cells. Among them, Th17 cells are found not only within AT, but also in the periphery in obese subjects. We have demonstrated that AT-derived stem cells (ASC), or their progenitors, contribute to inflammation through promotion of Th-17 cells, provided that they are issued from obese-, but not lean-AT (Diabetes, 2015; Adipocyte, 2016). Because obesity is associated with increased prevalence of various cancers, including hepatic or breast cancer, we postulated herein that ASC-mediated promotion of Th17 cells might result in tumorogenesis progression.

Materials and Methods: Human ASC were isolated from WAT of obese donors (obASC). Mononuclear cells (MNC) were collected from blood donors. PHA-activated co-cultures of obASC/MNC, which increase secretion of IL-17A, IL-1¤ and IL-6, were performed. Conditioned media (CM) were collected from such cultures, and added to HuH7 (hepato-carcinoma cell line) or MCF-7 / MDA-MB-231 (breast carcinoma cell line) cultures for 24h. mRNA profiles were measured by qRT-PCR. Expression of CXCR4 was measured by flow cytometry.

Results: CM from 48 hr PHA-activated-ASC/MNC co-cultures enhanced IL-1¤, VEGF¤, IL-8 TNF-¤¤and IL-6 mRNA expression in HUH7 by almost 700, 2, 3, 3, and 6-fold, respectively. A putative effect of CM on HUH7 invasiveness was supported by 2a –fold, and 3-fold increase in MMP9, and CXCR4 expression, respectively. In addition, CM also increased IL-1¤, IL-6, IL-8 and VEGF-¤ mRNA expression in both MCF-7 and MDA-MB-231 cell lines.

Conclusion: Our results suggest that the interaction of ob ASC with immune cells contribute to an inflammatory environment, able to impact hepato- or breast-carcinoma cell secretion profile, and/or invasiveness, either through propagation of inflammatory cytokines outside adipose tissues, or ASC migration inside tumors.

Infections & Immunity

Poster # 95

Personalized Cancer Vaccines: Accelerated Selection of Patient-Specific Neo-Epitopes

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Keywords: neo-epitope, T cell, immunogenicity, cross-reactivity, tolerance, vaccine, Immunotherapy

T cell neo-epitopes derived from tumor sequences can be harnessed to stimulate focused immune responses against tumor cells. A large proportion of neo-epitopes prove to be non-immunogenic despite the application of computational algorithms for selecting neo-epitopes. Poor predictive performance may partially be due to erroneous inclusion of **self-epitopes** matching the TCR of regulatory, anergic or deleted T cells. Selection of self-epitopes can lead to weaker vaccine responses, active immune suppression, and immune-mediated adverse effects.

We have developed advanced tools, EpiMatrix and JanusMatrix that streamline the selection of **truly neo-non-self-epitopes**. These state-of-the-art tools have been extensively validated in prospective vaccine studies for infectious diseases [Moise et al. 2015 Human Vaccines & Therapeutics 11:9, 2312-2321]. Priority is given to neo-epitopes exhibiting reduced potential for inducing **regulatory T cells** (Tregs).

Re-analysis of published studies [e.g. **Strønen et al. 2016 Science 352(6291), 1337-41**] demonstrates that EpiMatrix and JanusMatrix select immunogenic neo-epitopes with 72% accuracy, as compared to 21% accuracy using publicly available tools. In addition, retrospective analyses of cancer vaccine efficacy studies performed in mice [**Kreiter et al. 2015 Nature 520, 692-696**] show that mutanome-directed vaccines effective at preventing tumor growth contain higher numbers of JanusMatrix-validated neo-epitopes with lower self-cross-reactive potential.

Two **prospective** studies have also confirmed that **direct application of JanusMatrix focuses candidate selection on higher value sequences** than conventional algorithms. Neo-epitopes with low Treg activation potential may then be used to support development of personalized therapies including vaccination and in vitro expansion of tumor infiltrating lymphocytes for adoptive cell transfer.

Poster # 96

High Incidence of Hepatitis B Virus PreS2 Deletion in West Africa Among HBV Chronic Carriers: Association with Hepatocellular Carcinoma

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Keywords: hepatitis B virus, hepatocellular carcinoma, variants, genotypes

Hepatitis B virus (HBV)-related cirrhosis and HCC are highly endemic in Sub-Saharan Africa (SSA). Previous results from the PROLIFICA (Prevention of Liver Fibrosis and Cancer in Africa) European Commission funded (FP7) project showed that although individuals often showed a low viral load (2,000 UI/mL) an early onset of cirrhosis and HCC with aggressive and large tumors were frequently found in the population leading to low survival levels. Additionally, a high proportion of occult HBV infections have been detected and HBV genomic variants with both clinical and biological impacts have been reported. Indeed, we have found a high incidence of PreS2 deletion mutants in >55% of individuals with HCC, 38% with liver cirrhosis and 24% of chronic HBV carriers. This PreS2 mutation affects the polymerase gene resulting in the deletion of several immune epitopes and accumulation of viral envelope proteins in the endoplasmic reticulum. Interestingly it has been reported (Hsieh et al. 2015) that in cell lines infected with such a mutated HBV, the NBS1 DNA repair enzyme is sequestrated in the cytoplasm and was associated with lower levels of homologous recombination DNA double strand break repair. As low levels of nuclear NBS1 expression have been associated with PARP inhibitor sensitivity this observation opens up the intriguing possibility that HCCs carrying preS2 mutations might be sensitive to the cytotoxic effects of PARP inhibitors and that PARP inhibitors could be a useful clinical option for HCC treatment. Further in-vitro and in-vivo studies are needed to investigate this possibility and to establish the role of PreS2 deletion mutants in the development of HCC in SSA.

Poster # 97

Mitochondria Associated Membranes in the Pathophysiology of Chronic Hepatitis C

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Keywords: hepatitis C virus, mitochondria-associated ER membrane, liver cancer

Infection by the hepatitis C virus (HCV) is a major risk factors for liver cancer development. Within the hepatocytes, viral replication will take place at the endoplasmic reticulum (ER). The ER and mitochondria, organelles that regulate cellular metabolism, are interconnected through highly specialized subdomains named the mitochondria-associated ER membranes (MAMs). In addition to their role in calcium and lipid exchange between the 2 organelles, several lines of evidence suggest that MAMs play an important role in HCV life cycle as well as in immune defense against the virus. However, the role of MAMs in disease progression of chronic hepatitis C remains ill defined. Using the Huh7.5 cell line infected with the JFH1 HCV strain, cell fractionation and molecular biology approaches allowed us to investigate how HCV alters MAM composition and function. In parallel, we evaluated the impact of altered MAM structure on calcium flux, respiration and oxidative stress in HCV infection. We found that several viral proteins localize specifically to MAMs and alter MAM structure, indeed, presence of the MAM resident protein VDAC which is involved in calcium flux decreases in infected cells. In turn, silencing of structural components of MAMs inhibits viral replication. Our results demonstrate that HCV increases oxygen consumption. This was due to a combination of altered substrate availability and increased specific activity of complex I. In addition, we observed a maintenance of the mitochondrial membrane potential and a concomitant increase in superoxide production. These studies show that mitochondrial activity is intact and even increased in infected cells and associated with increased oxidative stress, an observation consistent with the metabolic adaptations observed in cancer cells. HCV-induced MAM alterations may play a major role in these processes and are thus likely to play an important role in fibrosis progression of chronic hepatitis C.

Poster # 98

Molecular Basis of TLR3 Targeting in Lung Cancer

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Keywords:

Toll-like receptor 3 (TLR3) is a pattern recognition receptor that senses viral dsRNA and mediates innate immune responses characterized by the production of type I IFNs and pro-inflammatory cytokines. TLR3 targeting also has an anti-cancer therapeutic value because of the selective toxicity of dsRNA molecules against transformed epithelial cells. We have previously found that TLR3 behave like a typical death receptor to trigger caspase 8-dependent apoptosis in lung epithelial cancer cells. Formation of TLR3 death complex requires Receptor Interacting Protein Kinase 1 (RIPK1) which plays a key scaffold function linking TLR3 adapter TRIF to the caspases-mediated apoptotic machinery. This death complex formation is negatively regulated by the Cellular Inhibitor of APoptosis (cIAPs) ubiquitin ligases while Cellular FLICE-like Inhibitory Protein (cFLIP) blocks the apoptotic activity of caspase-8. However, pre-clinical data for tumor type selection are lacking, and resistance molecular mechanisms of normal vs tumor epithelial cells remain unclear. Hence, we investigated the respective role of cIAPs and cFLIP in the resistance of non-transformed versus transformed bronchial cells against TLR3-mediated death.

Our data revealed that c-FLIP, rather than cIAPs, primarily controls the susceptibility of cancer cells to TLR3-mediated, RIPK1/caspase-8-dependent apoptosis. On the other hand, both cFLIP and cIAPs exerted in non-transformed epithelial cells an efficient double brake against TLR3-mediated death. cFLIP invalidation was also achieved by use of the chemotherapeutic agent paclitaxel which strongly synergized with a TLR3 ligand to enhance apoptosis selectively in cancer cells in preclinical in vitro and in vivo models, further confirming the primarily role of cFLIP in cancer cells resistance. Finally, we found that human adenocarcinoma (n=53) and squamous cell carcinoma (n=47) lung cancers overexpress TLR3, indicating its potential therapeutic targeting.

Altogether, our observations suggest that TLR3 targeting in combination with cFLIP invalidation could represent an efficient and safe therapy against TLR3-expressing lung cancers.

Poster # 99

A Role for the Unfolded Protein Response (UPR) in Breast Cancer Immunosurveillance

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Keywords: Unfolded Protein response (UPR), Immunosurveillance, Breast cancer.

Breast cancer remains a public health problem despite scientific advances including recent emergence of immunotherapy. Indeed, established tumors arose thanks to an ability to escape the immune system but the immunosurveillance mechanisms bypassed in early cancerous transformation remain poorly understood. ER (endoplasmic reticulum) stress and especially UPR (Unfolded Protein Response) secondary could be one of the early intrinsic events induced by the oncogenic stress, and growing data support UPR's role in immunogenicity. Thus, we generated an in vitro model Human Mammary Epithelial Cells (hMEC) undergoing Ras-induced oncogenic stress. Pangenome transcriptomic analyses highlighted the deregulation of several UPR-related genes upon oncogenic stress, and our preliminary data suggested that two UPR branches are activated: the IRE1α/XBP1s and PERK/eIF2 α pathways. We therefore inferred that the UPR could participate in the cellular response induced by oncogenic stress in hMEC, and play a role in immunogenicity of mammary pre-neoplastic cells. Our long-term objective is to further characterize the immunogenic traits in pre-malignant mammary cells that may be regulated by these two UPR branches, and to identify the impact on immune detection by innate immune cells, such as dendritic cells and/or neutrophils. The global aim is to identify mechanisms of early immune detection of cell transformation, allowing the definition of novel therapeutic strategies restoring the immunosurveillance processes and inducing an effective anti-tumor immune response.

Poster # 100

EMT-Associated Immune Evasion Mechanism : Implication of Immune Checkpoints

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Keywords: EMT, breast, immune escape, immune checkpoints

EMT is a transdifferentiation process that allows the cells to acquire a fibroblast-like migratory phenotype. Its role in cancer has been well documented in the metastatic dissemination steps but also in the early phases of tumoral transformation. Tumors are also characterized by their ability to escape immune surveillance, and there is growing evidence that common mechanisms are involved in EMT and immune escape.

We have previously shown that EMT is induced in vivo upon immune pressure. Orthotopic implantation of an epithelial Her2+ tumor cell line in immunodeficient and immunocompetent mice leads to aggressive tumors that acquire EMT features only in the immunocompetent host during the immune escape phase.

In the present study, we are investigating the mechanisms allowing these EMT-tumor cells to escape immune surveillance. One of our hypothesis is that EMT might be associated with an increase in the expression of Immune Check-Points Ligands (ICP-L). Our results suggest that some of these ICP-L are upregulated on the Her2+ cell line following in vitro TGFβ1 induced EMT. Orthotopically injected in an immunocompetent mouse, this plastic cell line leads to the development of heterogeneous tumors and we showed by RNA sequencing on sorted tumor cells and by flow cytometry that ICP-L expression profile in epithelial or mesenchymal contingent of the same tumor differs, suggesting an association between ICP-L expression and EMT. We derived a mesenchymal cell line from one of those tumors, that express constitutively some ICP-L, and able to express others with IFNg treatment. Of importance, during the immune escape phase while tumor cells have acquired EMT features, all tumor Infiltrating CD8+ Lymphocytes expressed multiple ICP. The tumors are also infiltrated by regulatory T cells that express ICP as well.

These results suggest a first mechanism of EMT associated immune escape through ICP-L upregulation mediating resistance to T cell effectors and/or Treg engagement.

Poster # 101

In Situ Biomarker Analysis in Cancer Immunotherapy: Development of Quantitative Multiplex IHC

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Keywords: multiplex IHC, TIL, IHC quantification

There is growing evidence that immune contexture influences cancer progression and clinical outcome of patients. The tumor microenvironment (TME) is the bed of cancer progression and the target of developing drugs.

Objective is to develop and validate IHC panels to analyze the human immune TME. Panels developed allow to analyze the lymphocyte compartment (TIL), macrophage status (M1/M2) and Tumor Lymphoid Structures (TLS) in clinical samples. Methods are based on multiplex OPAL system with fluorescence labelling. For TIL analysis, we develop a panel with CD4, CD8 and CD20 markers. For TAM, we analyze the whole macrophages with CD68 and the M2/M1 with CD163/iNOs. The analysis of TLS is assessed with DC-LAMP, CD20 and CD3. After multiplex IHC, quantifications of all markers are performed using Calopix software (Tribvn). Methods are partially validated, including the accuracy of multiplex labeling as compared to simplex, the evaluation of repeatability and reproducibility with multi operators. These analyses are performed on human tumors: Non Small Cell Lung Carcinoma (NSCLC), Breast carcinoma (BC), Pancreatic carcinoma (PC), Glioblastoma (G) and Hepatocellular carcinoma (HCC). Results: We observe that accuracy of the methods for TIL is variable depending of the tumor. We observe a good concordance between simplex and multiplex quantification in BC, PC and G. This concordance is more difficult to obtain in NSCLC and HCC and further improvement need. TAM and TLS panels are at the end of development. The intra and inter operators variability evaluation are ongoing. Conclusion: Development of new in situ biomarkers is fundamental to understand the influence of TME on tumor progression for immunotherapeutic development. We describe here the development of multiplex IHC panels that allow the immune “phenotyping” of this TME.

Poster # 102

The Use of PARP Inhibitors to Radiosensitise Liver Tumours and the Impact of HBV Proteins

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Keywords: HCC, HBV, PARP inhibitors, radiotherapy

As over 50% of all cancer patients will receive radiotherapy (RT) there is considerable interest in the development of radiosensitisers that can replace chemotherapeutic agents without the associated dose-limiting toxicities. Potent and specific catalytic inhibitors of poly(ADP-ribose) polymerases (PARP) have been developed that enhance radiosensitivity in many in vitro and in vivo models. Such inhibitors allow the binding of PARP proteins to DNA strand breaks but inhibit their poly(ADP-ribosyl)ating activity resulting in the trapping of PARP proteins at DNA damage sites. These trapped proteins and the persistence of unrepaired DNA strand breaks both contribute to the cytotoxicity observed. Based on this strong rational and as RT is a promising approach for hepatocellular carcinoma (HCC) treatment, we carried out proof-of-concept experiments that showed in two cell lines enhanced radiosensitivity when irradiated in the presence of the PARP inhibitor Veliparib (Guillot et al., BMC Cancer, 14 (2014) 603). As hepatitis B virus (HBV) infection is a major cause of HCC these studies have been extended to include liver cells carrying the wild type (WT) or HBx deficient HBV virus (HBVΔX). A greater sensitivity was seen in cells carrying the WT virus. The HBx protein interacts with the host cell's DDB1/ E3 ubiquitin ligase complex to target the Smc5/6 complex for degradation (Decorsière et al., Nature, 531 (2016) 386). How Smc5/6 degradation contributes to the increased sensitivity is under investigation. In order to assess the variation in DDR proteins in HCC we have in parallel analysed the expression patterns of 210 DDR genes in the TCGA HCC data set. 7 DDR genes were significantly over-expressed, including PARP-1. PARP1 over-expression has been confirmed in a panel of French HCC and lends support to the concept that targeting PARP activity in HCC could be exploited for therapeutic benefit.

This study was supported by grants from the LNCC (National and Rhône Alpes).

Poster # 103

How does Cellular Plasticity Foster Immune Escape in Melanoma?

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Keywords: Melanoma, cellular plasticity, immune escape

Melanoma is the most aggressive type of skin cancer whose treatment at a metastatic stage with targeted and immunotherapy is still faced with severe problems of resistance. The epithelial to mesenchymal transition (EMT) is a major cancer cell plasticity process driving tumorigenesis, stemness and resistance to treatment in carcinoma. In melanoma, the EMT inducing transcription factor ZEB1 plays a major oncogenic role (Caramel et al, 2013), driving resistance to MAPK targeted therapies (Richard et al, 2016). We investigated its role in regulating the phenotype of melanoma cells and its capacity to escape the immune control. In vitro we are testing two putative immune escape mechanisms, namely the tumoral antigen presentation and the immune exhaustion. We demonstrated that ZEB1 drives a stem-like phenotype associated with the loss of differentiation-related antigen like tyrosinase and associated proteins. On the contrary high ZEB1 mRNA expression levels are associated with mRNA expression of cancer associated antigens. In parallel we are investigating the expression of immune checkpoints ligands (ICPLs) in melanoma cells. Extended mRNA analyses showed positive correlation between ZEB1 mRNA expression levels and ICPLs such as the Programm Cell Death Receptor ligands 1 and 2. Immunostaining of patients biopsies from the Hospital Lyon Sud will allow us to visualize interactions between tumor cells and the microenvironment. We are also examining the impact of targeted therapies on the ZEB1-associated immune escape, given that sequential combination approaches are currently tested in clinic, in order to take benefit from the rapid efficacy of targeted therapies and the long term response of immunotherapies. Preliminary results suggest that BRAF inhibitors may potentiate ZEB1-induced coinhibitory molecules expression fostering the immune escape. Overall, this will demonstrate the role of ZEB1-mediated cell plasticity in melanoma escape from immune control.

Poster # 104

Monitoring of Intrahepatic and Circulating Immune System Features in Patients with Hepatocellular Carcinoma by Multicolor Flow Cytometry

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Keywords: immunotherapy, liver, HCC, antitumor immune surveillance, checkpoints

During development of hepatocellular carcinoma (HCC), an effective antitumor immune surveillance in liver microenvironment is impaired. Thus, enhancement of antitumor immune responses by immune checkpoint modifications is a promising treatment strategy.

The aim of our project is to investigate intrahepatic and circulating immune system in advanced-stage HCC patients i) before treatment by performing direct immunomonitoring on fresh liver biopsies and fresh whole blood, ii) to follow the immunological changes induced during treatment by blood analyses 1 and 3 months after start of treatment and iii) finally to evaluate again intrahepatic and circulating immune system in case of tumor progression.

To date, 16 HCC patients are included in this study. Fresh liver biopsies from tumor and from non-tumor tissue are homogenized and stained for multi-parametric FACS analyses. Similarly, whole fresh blood is stained. Following markers are investigated: CD45, CD3, CD56, CD16, CD15, CD19, CD8, CD335, CD107, CD69, CD274 (PD-L1) and CD279 (PD-1),CD137 (4-1 BB), CTLA-4, LAG-3, CD134 (OX40), TIM-3.

We observed differences in checkpoint molecule expressions on peripheral immune cells compared to intrahepatic immune cells. For instance, frequency of PD-1^+ intrahepatic T lymphocytes is much higher compared to peripheral T cells ($p<0.0001$) and PD-1 is mainly expressed by $CD8^+$ T lymphocytes in the tumor tissue.

In conclusion, this study contributes to understanding of the inhibitory receptor network complexity that regulates immune system to generate an immunosuppressive microenvironment of HCC and represents an opportunity to derive predictive factors for response to treatment.

Social Sciences, Prevention

Poster # 105

Etude comparative des facteurs de risque hormonaux du cancer du sein chez les femmes ménopausique et non ménopausique

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Mots clés : cancer du sein, risque, ménopause, facteurs de reproduction

Objectif : Évaluer la différence entre le cancer du sein pré-et post-ménopausique concernant les menstruations et les facteurs de risque de reproduction.

Patientes et Méthodes : Nous avons mené une étude cas-témoins au sein de la population de Tlemcen. 320 cas de cancer du sein recrutés à CHU, EHS et EPSP de Tlemcen et 640 témoins recrutés dans les polycliniques de la wilaya, appariés aux cas selon l'âge et le lieu de résidence. Pour chaque facteur de risque, le rapport de cotes (OR) et l'intervalle de confiance (IC) à 95% ont été calculés par analyse de régression logistique conditionnelle, séparément pour les femmes avant et après la ménopause.

Résultats : Parmi les patients atteints de cancer du sein, 45,6% étaient pré-ménopausiques et 54,4% étaient post-ménopausiques.
L'âge à la ménarchie n'avait pas d'association avec le cancer du sein ni chez les femmes ménopausées, ni chez les femmes non ménopausées, même résultat est retrouvé pour l'âge au premier accouchement d'un enfant né vivant par contre la gestité était un facteur protecteur seulement pour les femmes non ménopausées [OR = 0,47, IC 95% (0,25-0,88)], ainsi que la parité notamment les femmes ayant eu plus de 3 enfants [(OR = 0,44, IC 95% 0,20- 0,95)], l'allaitement maternel constituait un facteur protecteur seulement pour les femmes non ménopausique (OR = 0,55, IC 95%: 0,32 à 0,94).

Conclusions : La majorité des facteurs de risque pour le cancer du sein chez les femmes pré-ménopausique sont retrouvés chez les femmes ménopausiques sauf pour la parité, et l'allaitement qui ont diminué le risque de cancer du sein chez les femmes non ménopausique.

Poster # 106

Définir les priorités pour la prévention du cancer en France : les cancers en 2015 attribuables à des facteurs de risque environnementaux ou comportementaux

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Mots clés : Facteurs de risque environnementaux et comportementaux, fraction attribuable, prévention

Contexte : Les études récentes sur la perception des risques de cancer montrent que les facteurs de risque environnementaux ou comportementaux établis sont mal connus des français, et une mise à jour de l'estimation de leur contribution au fardeau du cancer est nécessaire. Le but de ce projet est d'estimer la part des nouveaux cancers en 2015 attribuable à ces expositions.

Méthode : Les facteurs de risque étudiés étaient les suivants : consommation de tabac, d'alcool, alimentation, obésité, activité physique, infections, hormones, UV, expositions professionnelles et environnementales, pollution atmosphérique, facteurs socio-économiques, aspirine, vitamine D et radiations ionisantes. Des données d'expositions représentatives de la population française ont été recueillies, ainsi que les données de quantification des risques de cancer correspondantes, afin d'estimer la fraction de chacune des localisations de cancer concernées attribuable à chaque facteur de risque.

Résultat : En 2015, environ 40% des nouveaux cas de cancer pouvaient être attribués à l'ensemble des facteurs de risque étudiés. Le tabagisme représentait le plus fort contributeur, près d'un cancer sur cinq (19%) lui étant attribuable. Il était responsable de 80% ou plus des cancers du poumon (88%), du larynx (84%) et de la cavité orale (80%) chez les hommes, et de 40 à 68% des mêmes cancers chez les femmes. Les autres facteurs de risques majeurs étaient la consommation d'alcool, l'alimentation et l'obésité.

Conclusion : Une grande part des nouveaux cas de cancer en France en 2015 étaient attribuables à des facteurs de risque modifiables. Basé sur les meilleures données représentatives existantes, cette étude constitue un outil majeur : i) pour les décideurs pour cibler et optimiser de futurs programmes de prévention ; ii) pour la population pour mieux hiérarchiser les facteurs de risque de cancer et ainsi agir au niveau individuel pour réduire son risque.

Poster # 107

Le niveau de connaissances des médecins du secteur public des risques environnementaux liés au cancer dans la ville de Batna, Algérie

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Mots clés : cancer, environnement, médecins, public

Introduction : Les répercussions des modifications de l'environnement et des modes de vie sur l'incidence des cancers constituent une préoccupation majeure de santé publique. Les liens entre expositions environnementales et le cancer représentent une interrogation chez les individus et un sujet émergeant de la relation médecin-patient. L'objectif de l'étude était d'évaluer le niveau de connaissances des médecins vis à vis des risques environnementaux liés au cancer et leur capacité de répondre aux préoccupations du public et proposer des mesures pour améliorer ces connaissances.

Matériel et méthodes : Une enquête transversale portant sur les connaissances, attitudes et pratiques à travers un questionnaire auto- administré aux médecins du secteur public de la ville de Batna , présents le jour de l'enquête, le mois de janvier 2014.

Résultats : 76 médecins avaient participé à l'étude avec un sex-ratio de 0,46, répartis dans différentes spécialités, parmi eux, 46% rapportaient que les modes de vie étaient à l'origine du cancer, la capacité des médecins à informer leurs patients variait en fonction du thème abordé, ils étaient mieux impliqués à l'égard du tabac, de l'alcool et des expositions professionnelles. Les facteurs qui empêchaient les médecins d'aborder les mesures de prévention avec les malades étaient la surcharge des consultations, le manque d'informations fiables et actualisées. Face à cette demande des patients et des médecins l'engagement d'une réflexion autour de cette problématique s'avère nécessaire.

Poster # 108

Implementation of a Program Based on Adapted Physical Activity and Recommendations for Second Cancers Prevention for Adolescents and Young Adults with Cancer: PREVAPAJA

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Keywords: adolescents and young adults, cancer, physical activity, implementation; prevention

Context: About 700 new cases of cancer are recorded each year among Teenage and Young Adults with Cancer (aged 15-25 years old) (TYAC) in Rhône-Alpes region (France); more than 200 are treated and supported within the TYAC Department of the Institute of Hematology and Oncology Pediatrics (IHOPe, Centre Léon Bérard). These patients survive from their disease in 80% of cases; they have six times more likely to develop a risk of second primary cancer (SPC) than their peers. This risk of SPC is multifactorial and varies depending on the type of first cancer, treatment received and the prevalence of risk factors (smoking, overweight, sedentary lifestyle, environmental exposures...).

Objectives: To implement a clinical program based on adapted physical activity sessions (APA) and cancer prevention recommendations for TYAC during the active treatment period (4-6 months). To assess TYAC satisfaction regarding benefits in terms of exercise practice, and knowledge improvement on cancer prevention recommendations.

Methodology: This project is based on 3 steps during one year: 1 / a 4-month APA program during the active treatment period conditioned by fitness check-ups; 2 / an information interview on the brakes and facilitators in the pursuit of a physical activity at the end of the program, and the adoption of cancer prevention behaviors; 3 / a one-year phone call on the implementation of SCP's risk prevention measures.

Results: 47 AYACs participated in the study, representing 46% of the patients in the AYAC Department. They are affected by genital cancers (28%), sarcoma (27.5%), lymphoma (27.5%), neuroblastoma (9%), or benign tumors (8%), and are aged 18 to 25 years old. They performed an average of 4 sessions in the hospital [2-12] and 14 sessions at home [4-27] during their program; 40% benefited from an information interview. This is an ongoing study, scheduled on 2 years (2016-2017), and supported by the Regional Oncology Network “Espace Santé Cancer R-A".

Poster # 109

Evaluation économique du programme d'éducation thérapeutique « Mieux manger, mieux bouger à l'aide de l'éducation nutritionnelle » chez les patientes atteintes d'un cancer du sein

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Mots clés : cancer du sein, coût, éducation thérapeutique du patient, évaluation économique, micro-costing

Introduction : L'espérance de vie des femmes atteintes d'un cancer du sein ayant augmenté, la prévention tertiaire, à travers l'Éducation Thérapeutique du Patient (ETP) fait partie intégrante de l'accompagnement des patientes. Cette étude a pour objectif principal d'évaluer les coûts d'un programme ETP nutritionnel et d'activité physique dont le but est d'aider les patientes atteintes d'un cancer du sein à gérer leur poids.

Méthodes : Cette étude prospective est une description des coûts, de type micro-costing bottom-up, des deux premières années du programme (2014/2015), mené en ambulatoire, au Centre de Lutte Contre le Cancer Léon Bérard, Lyon, France. Les coûts directs ont été estimés selon la perspective hospitalière, en euros 2016. Des analyses de sensibilité déterministes et probabilistes ont été conduites.

Résultats : 65 patientes ont été incluses. L'âge moyen était de 52 ans, la majorité d'entre elles étant en activité ou en arrêt maladie (72%). Dans la plus part des cas, elles avaient subi une chirurgie (95%) et une chimiothérapie (71%). Hors coûts de structure, le coût moyen par patiente du programme s'est élevé à 541,04€ (écart type 88,44€ ; IC à 95% [520,06-562,03]), soit 687,13€ coût de structure compris. La variation du coût unitaire du diététicien impactait le plus le coût total du programme.

Conclusion : Cette étude de micro-costing, estimation précise des coûts de production, permet ainsi d'apprécier les changements à apporter pour optimiser l'organisation de cette activité et d'éclairer le décideur sur les budgets à allouer pour l'implémentation d'un tel programme.

Poster # 110

A Global View on Cancer Incidence and National Levels of the Human Development Index

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Keywords: cancer, Incidence, Human development, Socioeconomic, International

Socioeconomic factors are associated with cancer incidence through complex and variable pathways. We assessed cancer incidence for all cancers combined and 27 major types according to national human development levels.

Using GLOBOCAN data for 184 countries, age-standardized incidence rates (ASRs) were assessed by four levels (low, medium, high, very high) of the Human Development Index (HDI), a composite index of life expectancy, education, and gross national income.

A strong positive relationship between overall cancer incidence and HDI level was observed. When comparing the ASR in very high HDI regions to that in low HDI regions, we observed a positive association ranging from 2-14 and 2-11 times higher in males and females, respectively, depending on the cancer type. Positive dose-response relationships between the ASR and HDI level were observed in both sexes for the following cancer types: lung, pancreas, leukemia, gallbladder, colorectum, brain/nervous system, kidney, multiple myeloma, and thyroid. Positive associations were also observed for testicular, bladder, lip/oral cavity, and other pharyngeal cancers, Hodgkin lymphoma, and melanoma in males, and corpus uteri, breast, and ovarian cancers and non-Hodgkin lymphoma in females. A negative dose-response relationship was observed for cervical and other pharyngeal cancers and Kaposi sarcoma in females. Although the relationship between incidence and the HDI remained when assessed at the country-specific level, variations in risk within HDI levels were also observed.

We highlight positive and negative associations between incidence and human development for most cancers, which will aid the planning of cancer control priorities among countries undergoing human development transitions.

Poster # 111

Cancers' Risks Induced by Environmental Factors in Auvergne Rhone-Alps Region: Individuals' Perceptions and Health-Related Behaviors Declared Adopted

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Keywords: risk perceptions, health-related behaviors, cancers, Environmental factors, Auvergne-Rhone-Alps Region

Objective: To analyze individuals' perceptions of their cancers' risks induced by environmental factors in the AURA Region and their relationships with health-related behaviors declared adopted.

Methods: Cross-sectional survey: online auto-administrated questionnaire sent to a panel of respondents aged between 18 and 75. Quotas' method on gender, age, and socioeconomic status was used to ensure the sample's representativeness. Questionnaire's development was based on findings from a literature review and a qualitative study previously conducted by authors. Questions sections encompassed health-related behaviors, perceptions of cancers' risks in general and induced by 22 environmental factors, cancer history, cancer-related beliefs, perceived control, worry and salience. The questionnaire has been validated by a panel of experts and pre-tested among lay-individuals.

Results: Behaviors declared the most adopted were homes' aeration, no smoking, balanced diet, and regular physical activity while the least declared adopted behaviors were related to radon, WIFI and pesticides. Respondents perceived more often their cancers' risks related to 12 over 22 environmental factors as "rather low". However, 41% and 36% of respondents perceived "somewhat high" their cancers' risks related to outdoor pollution and stress while 44% and 37% perceived "very low" their cancers' risks related to secondhand smoking and alcohol consumption. Preliminary results showed that being a woman, being worried about cancer, and feeling concerned about cancers' risks induced by environmental factors increases the odd of perceiving own cancers' risks as high rather than low.

Conclusion: Further analyses will be conducted and presented at the conference especially regarding predictors of respondents' perceived cancers' risks related to the 22 environmental factors. However, our preliminary findings emphasized the influence of gender, worry, and salience on cancers' risks perceptions.

Poster # 112

Facteurs de risque du cancer du sein à Tlemcen : Etude cas-témoins

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Mots clés : cancer sein , cas-témoins, facteurs de risque, régression logistique, Tlemcen

Introduction : Le cancer du sein représente un problème majeur de santé publique dans le monde. En 2012, 8177 sont enregistrés en Algérie avec 3000 décès .Le dépistage est important mais d'application difficile, ce dernier se fait en fonction des facteurs de risque prédominant dans la population d'où l'intérêt de leur identification. L'objectif de notre étude est de déterminer les facteurs de risque chez la population de Tlemcen.

Matériel et méthodes : Il s'agit d'une étude de cas témoins au sein de cette population, 320 cas de cancer du sein incident recruté au niveau du CHU , EHS et les EPSP de la wilaya de Tlemcen et 640 témoins appariés selon l'âge et la commune de résidence des cas.

Résultats : Une association positive et significative a été détectée entre les antécédents du premier degré du cancer du sein et le cancer du sein [rapport de cotes (OR)= 4, 98 intervalle de confiance à 95%[($IC_{95\%}$ (1,52-16,26)]. Une association positive a été détectée entre le tabagisme passif et le cancer du sein OR= 3,58 [$IC_{95\%}$ (1,94-6,11)]. En outre une association positive a été détectée entre la survenue de la ménopause et le cancer du sein[OR= 2,84, $IC_{95\%)}$(1,11-7,21)].Une association positive a été aussi détectée entre le cancer du sein et la durée du cycle >28jrs[OR=6,73 ,$IC_{95\%)}$(3,50-12,94).Les femmes mariées ou ayant été mariées semblent être protégées contre le cancer du sein par rapport aux femmes célibataires [OR=0,21, $IC_{95\%)}$(0,09-0,47)].

Conclusion : Le dépistage précoce de la maladie reste le principal moyen de lutte contre le cancer du sein. Lorsqu'il est dépisté à un stade précoce, et si un diagnostic et un traitement appropriés sont disponibles, il y a de fortes chances qu'il puisse être soigné.

Poster # 113

Facteurs liés au diagnostic tardif du cancer du sein

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Mots clés : diagnostic tardif, âge avançé, niveausocioéconomique, dépistage

Introduction : Le cancer du sein est le premier cancer féminin en termes d'incidence et de mortalité. En Algérie , il vient au premier rang des cancers de la femme avant celui du col utérin Il constitue un problème de santé publique. Son pronostic est étroitement lié au stade auquel le diagnostic est posé. Il s'agit d'une pathologie dont les moyens diagnostiques sont de nos jours développés, allant de la détection précoce à la mise en évidence de lésions infra-cliniques, ce qui a nettement amélioré le pronostic dans les pays développés. Ce travail que nous présentons a pour objectif d'identifier chez notre population, les facteurs qui amènent les patientes à consulter à des stades tardifs.

Patientes et méthodes : Une étude rétrospective a été menée de janvier 2011 à Mars 2013 portant sur 320 patientes porteuses d'un cancer du sein au sein du service de gynécologie EHS mère enfant Tlemcen. Un questionnaire a été élaboré et dument renseigné en ayant recours aux dossiers des malades. Des analyses uni variées par des modèles de régression logistique nous ont permis d'analyser l'association entre les variables, prise une à une, et le risque de cancer du sein tardif par le calcul de l'odds ratio avec son intervalle de confiance à 95% .

Résultats : Ainsi 45,3% des patientes consultaient au-delà de deux mois et demi avec un délai moyen de consultation de 7,80±16,17 mois avec comme motif de consultation des lésions classées T4 dans 16,5%, et des tumeurs d'emblée métastatiques dans 18,4%. les femmes âgées de plus de 65 ans ont OR= 4,08(2,1-7,6), les femmes ayant un niveau socioéconomique élevé sont diagnostiqué précocement par rapport aux autres avec un OR= 0,09(0,01-0,74) de même que celles ayant un délai de consultation.

Nanomedicine, Health Technologies

Poster # 114

New High-Resolution, High-Sensitivity Dedicated Breast Positron Emission Tomography (PET) Scanner

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Keywords: nuclear medecine, PET Scan, mammography, nanotechnologies

Positron Emission Tomography (PET) is an imaging system used in nuclear medicine for the detection of cancer. The scintillating crystals of the PET scan detect the ▫-rays emitted by the radioactive marker absorbed by the carcinogenic cells of the patient. One of the main limits of current ▫-rays scintillator detectors is the small percentage of UV-visible photons extracted from the scintillating crystal. The low light output efficiency reduces the signal-to-noise ratio and limits PET spatial resolution.

Our solution relies on ground-breaking nano-photonics and advanced nano-manufacturing. The introduction of a periodic nano-pattern of a thin high refractive index material layer at the crystal surface boosts photon extraction thanks to the optical principles of diffraction [1] and refractive index matching (patent pending). The typical size of these structures is between 0.4 and 1 µm. Numerical simulations predict a potential gain of 70% of extracted light. However, a key challenge lies in the manufacturability of such nano-structures over large areas (5*5 cm2) of expensive and fragile crystals.

We have succeeded to fabricate several patterned scintillating “turbo” crystals. Preliminary in vitro results show a light extraction enhancement up to 35 %. A first batch of such “turbo” crystals will be mounted on a commercial ONCOVISION MAMMI PET scanner for mammography by March 2017. Clinical trials will follow at CHU-V in Lausanne.

Boosting light extraction efficiency will result in shorter acquisition time, higher quality images and lower dose of radioactive isotope injection needed. Perspectives include deployment to full-body PET scanners for oncology, Time of Flight PET systems and applications in neurology for early detection of Alzheimer’s disease. This work is financially supported by Eurostars (TURBOPET project E! 8974).

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Poster # 115

Vers un contrôle en ligne de la qualité des faisceaux en radiothérapie par modulation d'intensité

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Mots clés : contrôle qualité, radiothérapie par modulation d'intensité, détecteur en transmission, modélisation

Un détecteur innovant, nommé TraDeRa pour « Transparent Detector for Radiotherapy », a été développé par le groupe Physique pour les Applications Médicales du LPSC pour l'assurance qualité en radiothérapie par modulation d'intensité. Il est actuellement en phase de maturation, accompagné par la SATT Linksium Grenoble Alpes. A terme, un prototype intégrant une suite logicielle de caractérisation des défauts d'irradiation sera produit afin de démarcher les équipementiers dans le domaine du contrôle qualité en radiothérapie. Il est prévu de constituer une structure de type startup au printemps 2018 pendant la phase d'incubation qui suivra la maturation.

Le prototype repose sur une nouvelle version du détecteur qui comporte 1600 électrodes capables de mesurer en 2D et en temps réel les caractéristiques des faisceaux de rayonnement ionisants utilisés pour les traitements en radiothérapie externe. Des campagnes de mesures sont prévues en milieu hospitalier afin de relever les performances effectives du système dans des conditions d'exploitation clinique, sans que la présence de patient soit nécessaire.

En parallèle, une méthode originale basée sur la simulation Monte Carlo fait le sujet d'un travail de thèse afin d'estimer en quelques minutes les paramètres d'un accélérateur donné. Dans la suite de ces travaux, l'objectif sera de fournir une carte de dose dans un volume d'eau virtuel en se basant sur les caractéristiques du faisceau mesurées en ligne par le détecteur. Les développements du détecteur ont conduit à deux brevets.

Poster # 116

Assessment of Gold Nanoclusters (AuNC) in Head and Neck tumor mouse model

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Keywords: head and neck cancer, gold nanoclusters, fluorescence imaging, in vivo biodistribution, good renal clearance

Head and neck cancer is the sixth most common type of cancer worldwide, with a 5-years survival rate of approximately 40%. Thus improving early diagnosis and therapy is necessary. Over the past 10 years Gold Nanoclusters (AuNCs) appeared as excellent candidates for bioimaging owing to their intrinsic optical properties in the Near InfraRed (NIR), and for active targeting therapy as radiosensitizers. The goal of this study was to evaluate the uptake of different NC coupled with Zwitterionic ligands (AuZw) in Cal-33 cells, their cytotoxicity and their in vivo biodistribution and pharmacokinetics in a CAL33-Luc orthotopic head and neck tumor mouse model.

AuNCs can passively accumulate within tumors after intravenous injection with a well spectrally contrast from background with maximum emission wavelength at about 800nm for AuZW 1:2 and 705nm for AuZW 1:5. AuZW 1:5 had a better uptake and biocompatibility than those 1:2 and their half-life was also much longer. Furthermore, in vivo and ex vivo fluorescence imaging showed that NIR AuZW can strongly accumulate in tumors and are mainly eliminated by kidneys. We also demonstrated an increase of half-life AuZW 1:5 compared to those 1:2. These results indicate that AuNC 1:5 seems to be promising contrast imaging agents for in vivo and ex vivo fluorescence tumor imaging with a good renal clearance.

Poster # 117

Lipid Nanoparticles as Delivery Vehicle for siRNA Therapeutics

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Keywords: lipid nanoparticles- nanomedicine- siRNA therapeutics

Nanomedicine, the application of nanotechnology to medicine, is a growing field for decades. Several nanoparticles are already on the market, especially for tumour diagnosis or therapy, such as Doxil. Their applications are based on their ability to reach the tumour tissue due to the well-known “enhanced permeability and retention” (EPR) effect where they can accumulate locally in the tumour at very high levels and increase thus the efficiency of their contents, i.e. anti-cancer agents. Most of these drugs are hydrophobic small molecule compounds whereas new therapeutic strategies in development are based on larger molecules from biological origin such as peptides, proteins or nucleic acids. Novel nanoparticle formulations are now highly anticipated for delivery of these bio-macromolecules, in particular for applications beyond cancer therapy. Here, we designed lipid nanoparticles able to deliver nucleic acids. The resulting particles presented not only a high colloidal stability, but also a good safety profile. Moreover, their efficacy for the transfection of siRNA were evidenced by using various cellular models. Further preclinical evaluation of these siRNA lipid particles in different animal models is now ongoing and will contribute to their successful clinical translation.

Poster # 118

Comparison Between Anger and Compton Cameras for Medical Imaging: A Monte Carlo Simulation Study

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Keywords: SPECT, gamma camera, simulation, medical imaging

Single Photon Emission Computed Tomography (SPECT) is at present one of the major techniques for non-invasive diagnostics in nuclear medicine. Almost the whole clinical routine is based on collimated cameras, originally proposed by Anger. Due to the presence of mechanical collimation, detection efficiency and energy acceptance are limited and fixed by the system geometrical features. In order to overcome these limitations, the application of Compton cameras for SPECT is being investigated for several years.

We propose in this study to compare a commercial SPECT-Anger device, the GE Healtcare Infinia system with HEGP collimator, and the Compton camera prototype under development by our collaboration (Krimmer et al, NIM A787, 2015, p. 98-101), through Monte Carlo simulations (GATE v7.1 and Geant4 9.6 respectively). Given the possible introduction of new radio-emitters at higher energies intrinsically allowed by the Compton camera detection principle, the detectors are exposed to point-like sources at increasing primary gamma energies, from actual isotopes already proposed for nuclear medicine applications. The detector performances are studied in terms of radial event distribution, detection efficiency and final image, obtained by gamma transmission analysis for the Anger system, and with an iterative LM-MLEM algorithm for the Compton reconstruction.

Preliminary results show for the Compton camera a detection efficiency increased of a factor greater than an order of magnitude, associated with an enhanced spatial resolution for energies beyond 500 keV. We discuss then the proven advantages of Compton camera application with particular focus on dose delivered to the patient, examination time and spatial uncertainties.

Poster # 119

Detection of Cancer Cells by Smart Targeting of Nucleoli

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Keywords: Nucleolin,AS1411,lateral flow,rapid test

Paper-based lateral flow bioassays (LFB) are inexpensive, rapid, flexible, and easy-to-use analytical tools for point-of-care diagnosis. The best-known success of lateral flow test is the pregnancy strip for home testing that was invented in 1980s.

The goal of my thesis research project is to set up a LFB allowing the detection of circulating cancer cells. This sensitive biosensor will be based on gold nanoparticles conjugated with AS1411 which is an aptamer specific for the cell surface Nucleolin protein.

The proposed bio-sensing approach consist of a paper-based lateral flow immunoassay, which is an integrated technique enabling a detection device on a simple nitrocellulose strip.

Gold nanoparticles are used as the visualizing agent and the red colour of them is followed. AS1411 gold nanoparticle complex (AGC) and biotinylated AS1411 aptamer will be stored in conjugate pad. When the sample is loaded on the sample pad AS1411 labelled with GNPs will bind to the cells and flow along the LFB.

Deposition of streptavidin on test zone, cancer cells which will carry biotinylated aptamer and AGC referring specific interaction of AS1411 aptamer with Nucleolin will be captured in the test zone because of strong interaction of biotin and streptavidin. Qualitative or semi-quantitative detection of various analyt can be carried out by colorimetry either by eyes or with a strip reader. The signal related to AGC is a characteristic red band and will enable visual detection of cancer cells in test zone without instrumentation.

Because streptavidin is deposited on test zone. In the absence of cancer cells, biotinylated aptamers and not ACG can be captured in test zone but as biotinylated aptamer is not carrying gold nanoparticles no red band will be observed.

Poster # 120

Modified 5FU-Loaded Lipid Nanocapsules as a Novel Drug Delivery System for Pancreatic Cancer Treatment

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Keywords: nanomedicine, lipid nanocapsules, modified 5-FU, pancreatic cancer

The Enhanced Permeability and Retention (EPR) effect of nanosystems has been considered the gold standard principle for cancer drug delivery systems. However, due to the high heterogeneity, high interstitial fluid pressure and impaired blood supply of tumors, the accumulation of nanomedicines is strongly hindered [1]. Pancreatic cancer is an example of inaccessible tumor, characterized by an irregular vascular permeability and by formation of lymph node metastases [2].

The aim of this work was to prepare 5FU-loaded nanocarriers and to target them to immunocompetent organs such as lymph nodes, which are where tumor cells rapidly spread. Firstly, the drug was modified with a lauric acid chain to obtain a mono-lauroyl-derivative (5-FU-C12, MW of about 342 g/mol). Then this novel hydrophobic compound, 5FU-C12, was encapsulated into LNCs. The average size for blank and drug loaded systems was approximately 60 nm, with a low polydispersity index (<0.1) and a neutral surface charge. Due to the high hydrophobicity of 5-FU, the rate of encapsulation efficiency inside LNCs was above 90%. Further, *in vitro* studies on B6KPC3 (pancreatic cancer cell line) were performed to investigate the efficacy of prepared 5-FU-C12-loaded LNCs in inducing cytotoxicity. Following 48h of incubation, 5-FU-C12-loaded LNCs showed a toxic effect comparable to the free 5FU water and 5FU-C12 acetone solution with IC50 values of 4.53 µM (CI 3.83, 5.35),1.48 µM (CI 0.95, 2.27) and 2.75µM (CI 1.47, 4.05), respectively. Further *in vitro* and *in vivo* therapeutic efficacy evaluations would disclose the full potential of these LNC encapsulating a novel 5-FU derivative.

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Poster # 121

Audiological measurements to assess malignant gliomas development

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Keywords: malignant glioma, intracranial pressure, otoacoustic emission, cochlear microphonics potential, noninvasive monitoring

Malignant gliomas are brain tumors with fast evolution which are known to relapse during the first year of the diagnosis. These tumors growth is generally accompanied by intracranial hypertension due to several reasons: inflammatory reaction around the tumor, tumor mass effect, secondary vascular changes and change in the circulation of the cerebrospinal fluid (CSF). Thus, the development of gliomas inevitably modifies intracranial pressure (ICP). Over the last decade, the laboratory has developed two audiological methods to monitor ICP in a non-invasive way: the otoacoustic emissions (OAE) and the cochlear microphonics potential (CM). These techniques are based on the principle that the pressure variations of the CSF are transmitted within seconds through the cochlear aqueduct to cochlear fluids, thereby modifying the impedance of the ear. In this way, OAE and CM phase shift in proportion to ICP variation. 20 patients enrolled after a partial surgical treatment of their malignant glioma were monitored with CM and/or OAE, every three months during one year, in comparison with MRI and clinical signs. The preliminary results show a good efficiency of the CM and OAE techniques in that a good agreement is found between phase shift and significant tumor growth or regression detected on MRI. Different or reduced effects of ICP may also be observed depending on tumor localization relative to the monitored side. The OAE method showed some variability on repeated measurements, attributed to calibration changes. A correction has been brought, the implementation of which offsets the effect of calibration drifts. Phase shift of auditory responses to sound is a promising tool to identify the development of malignant gliomas, and suggests a fast, painless and user-friendly monitoring that may complement current controls and avoid repeated MRI tests.

Poster # 122

Signaux mécaniques et cancer; de la Preuve de Concept à la Preuve d'Efficacité

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Mots clés : physical oncology, mechanical cues, in vivo, pancréas, magnetic gradient field, constraint field

Il a été démontré depuis plus de dix ans qu'il est possible de « normaliser » in vitro des tumeurs cancéreuses, et vice versa en utilisant des signaux mécaniques et non pas biologiques(MJ Paszek, 2005; F Montel, 2011, M Olcum, 2014 et autres).
Nous appliquons les mêmes principes de l' « oncologie physique » mais in vivo pour montrer l'effet d'un «champ de contrainte» sur la croissance tumorale.

Une lignée cellulaire de cancer du sein humain MDA MB 231 mélangée avec des nanoparticules ferriques a été greffée par voie sous cutanée chez la souris. Les nanoparticules entourent rapidement la tumeur en raison de la grande différence en énergie libre de surface. Nous avons appliqué un champ de contrainte, et par conséquence des signaux biomécaniques, dans la tumeur transplantée in vivo en utilisant deux aimants permanents situés de part et d'autre de la tumeur entourée par des nanoparticules ferriques. Le rôle des nanoparticules est de transformer une partie de l'énergie magnétique en énergie mécanique ; on parle de « BioActionneurs ».
Ce traitement biomécanique a été appliqué 2 heures / jour pendant 21 jours.
Il y avait une différence très significative ($p=0,015$) entre le volume des tumeurs traitées et témoins non traités. En plus des différences significatives du volume in vivo, il y avait une différence très significative entre les tumeurs traitées et les groupes témoins quand on mesurait la surface de la tumeur vivante sur des coupes histologiques des tumeurs.
La prochaine étape sera l'utilisation d'un champ de contrainte sur du cancer humain du pancréas greffé dans le pancréas de souris.

Cette première preuve de l'action d'une contrainte sur la croissance tumorale in vivo signifie que l'intervention biomécanique peut avoir un potentiel de translation élevée comme outil thérapeutique spécialement dans les tumeurs localement évoluées telles que le cancer du pancréas, le carcinome hépatique primitif, le glioblastome, … qui sont des 'unmet needs'.

Poster # 123

Nanoparticles Enhance Anticancer Activity of Calcitriol, the Active Metabolite of Vitamin D3

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Keywords: calcitriol, nanoparticles, vectorization, cancer

Calcitriol, the active metabolite of vitamin D3 plays a role in many types of cancer including breast and prostate cancer, by controlling growth, differentiation and survival of cancer cells [1]. However, calcitriol antitumoral activity requires supraphysiological doses (>10−9 M in vivo) associated with a high risk of hypercalcemia, which led to the failure of clinical studies [2]. Indeed, the last published clinical phase III trial (ASCENT-2, 2006), which was conducted in patients with a prostate cancer showed inferior survival in the calcitriol arm.
Calcitriol vectorization may present a potential strategy to avoid calcemic side effect by targeting cancer cells and hence improving calcitriol specific delivery.

In this line, Poly-D-lactic acid, a biodegradable polymer, was used for the preparation of 200 nm nanoparticles by the nanoprecipitation method. Different formulations were developed showing a good encapsulation efficiency higher than 85% with variable release profiles. The growth inhibitory efficiency of calcitriol-loaded NP was evaluated in vitro on human breast adenocarcinoma cells (MCF-7) using an MTT assay. Incubation for 24 hours with 10−6 M of calcitriol showed a significant difference at day 7 between free and encapsulated calcitriol with 79% cell survival for free calcitriol and 49% for calcitriol-NP.

In conclusion, PLA based NP maintain and extend calcitriol antiproliferative activity in vitro. These findings will be investigated in vivo on a MCF-7 xenograft mouse model in order to confirm calcitriol-vectorization benefit.

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Poster # 124

Applicateur instrumenté pour le contrôle qualité in vivo en curiethérapie à Haut Débit de Dose

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Mots clés : curiethérapie HDR, dosimetrie, contrôle qualité, applicateur instrumenté

La curiethérapie représente actuellement près de 5% des traitements de radiothérapie en France et plus de 50% des traitements sont réalisés avec un haut débit de dose (HDR).
Les traitements en curiethérapie HDR sont délivrés en un nombre réduit de fractions avec des doses importantes par fraction (>5Gy) et avec des profils de dose présentant de forts gradients. Les conséquences peuvent être importantes en cas d'erreur lors du traitement (mauvais positionnement de la source, temps d'exposition incorrects, …). Au cours des années 2013-2014, 23 événements significatifs pour la radioprotection des patients (ESR critère 2.1) en curiethérapie ont été déclarés à l'ASN.

Pour sécuriser les procédures de curiethérapie HDR gynécologiques, nous avons développé un applicateur instrumenté qui à terme permettra un contrôle in vivo et en temps réel des principaux paramètres physiques du traitement, conformément aux recommandations ESTRO (Booklet n°8).

Les études de caractérisation de ce système menées dans un fantôme sur des installations cliniques avec des protocoles de référence sur une période de 15 jours et plus de 800 positions de la source HDR (192Ir) ont montré que le système permet de suivre temps réel la position d'arrêt de la source (dwell position) et le temps d'exposition associé (dwell time) sans biais (avec un écart type sur les mesures de 0.42mm et 100 ms, respectivement). Les taux de détection en cas d'erreur de positionnement de la source atteignent 96% pour des erreurs comprises entre 0.7 et 1mm et 100% au-delà de 1.5mm (ce qui remplit les exigences ESTRO où le seuil est défini à 2mm). Le système permet également d'estimer la dose accumulée en des points d'intérêt (POI). La précision a été estimée en 4 POI sur 10 réalisations d'un protocole typique de traitement, et l'écart entre les doses mesurées et planifiées reste inférieure à 5% pour l'ensemble de ces points. Dosilab va démarrer des études complémentaires dans différents centres en France et en Europe

Poster # 125

i-Particles Functionalized with TLR Agonists for Cancer Therapy

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Keywords: cancer therapy, TLR ligands, Toll-like Receptor, particles

Toll Like Receptors (TLR) are a family of pattern recognition receptors (PRR) which recognize distinct structures in microbes, referred to as PAMPs (pathogen associated molecular patterns). Some TLR agonists have a strong antitumor activity by different mechanisms as inducing apoptosis, indirect activation of tolerant host immune system to destroy cancer cell and/or potentiating the effect of chemotherapy.

However, systemic administration of such TLR agonists leads to toxic effects thus limiting injectable doses, associated with suboptimal delivery to the target site and consequently lack of effect. Hence, strategies must be developed to limit their systemic dissemination, focus their delivery to the targeted tissues and protect them from degradations, in order to increase their efficacy while decreasing their toxicity.

Adjuvatis develops, manufactures and formulates biodegradable i-Particles®, for drug delivery. Only made of poly(lactic acid), a polymer used since more than 20 years with a strong safety records in human, those fully metabolizable particles are produced by a surfactant-free, eco-friendly process. It results in sub-micron particles around 200 nm in diameter, which facilitate their acceptance from regulatory authorities as they are not classified as nanomaterial in Europe nanomedicine, associated with a high safety profile. Active Pharmaceutical Ingredients can be encapsulated within the polymeric core of the particles, which can then be administrated by several routes of administrations (sub-cutaneous, intravenous, mucosal or by skin applications). The encapsulation of Nod ligands, TLR-4, TLR-9 or TLR-8 agonists was shown to potentiate their efficacy as evaluated in vitro on reporter cells and in vivo in mice. Finally, the co-encapsulation of TLR agonist and a fluorophore, offers a potent way to investigate the biodistribution of those particles.

Poster # 126

3D Silicon Coincidence Avalanche Detector (3D-SiCAD) for Hadrontherapy and Proton Tomography : Preliminary Results on Charged Particle Detection

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Keywords: charged particle detector, SPAD, hadrontherapy, proton tomography

Ce travail vise à démontrer les capacités d'un nouveau type de détecteur de particules chargées, bénéficiant des progrès récents en intégration microélectronique 3D, pour des applications médicales avancées telles que l'hadronthérapie (ex. réduction des incertitudes dans la fluence pour délivrer la bonne dose, étiquetage précis d'un temps d'arrivée des ions pour la sélection en temps de vol des rayons gamma prompts) et la radiographie - tomographie proton (ex. filtrage des neutrons et des rayons gamma, amélioration potentielle de la résolution spatiale).
Ce détecteur intégré en technologie CMOS est appelé 3D-SiCAD (3D Silicon Coincidence Avalanche Detector).
Il met en œuvre un mode de coïncidence interne innovant, objet d'une forte activité R&D au niveau international, basé sur un empilement vertical de pixels de type SPAD -Single Photon Avalanche Diode (mode Geiger) permettant d'améliorer significativement la discrimination entre les évènements liés au passage de particules (déclenchement simultané de deux pixels) et le bruit intrinsèque. L'originalité de notre démarche consiste en l'application de ce concept innovateur aux applications médicales.
Les étapes de conception du premier prototype ainsi que les principaux résultats obtenus seront présentés avec notamment :
i) les performances de la diode SPAD seule réalisée en technologie CMOS 0.35µm avec par exemple un taux de comptage dans l'obscurité moyen de 70Hz/µm^2,
ii) la démonstration expérimentale de la diminution du bruit apparent (taux de comptage de faux événements) grâce à la détection en coïncidence dans une courte fenêtre temporelle. En pratique, le taux de réjection du bruit peut atteindre un facteur x103 dans les conditions optimales,
iii) la mise en œuvre de cette solution technologique pour la mesure de l'activité d'une source de strontium.
Finalement les performances actuelles, les pistes d'amélioration possibles ainsi qu'une revue des travaux en cours et futurs seront brièvement discutés.

Poster # 127

Development of 3D Cell Culture from Basal-Like Triple-Negative Breast Cancer Cell Lines: An Excellent Preclinical Tool for Resistance Study to Chemotherapy

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Keywords: 3D cell culture, Basal-Like Triple-Negative Breast Cancer, BLTN, SUM1315, MDA-MB-231

"Basal-Like Triple-negative" (BLTN) tumours represent 15 to 20% of breast cancers and are very aggressive. Indeed, because of their phenotype (ER, PR and HER2 negative) neither hormone therapies nor conventional chemotherapy protocols are efficient, leading to rapid metastatic development. More, they are frequently associated with hereditary mutations on BRCA1/2 genes. Thus, targeted therapies were developed in preclinical and clinical studies, such as anti poly-ADP-ribose polymerases (anti-PARP) using the concept of synthetic lethality. For this, monolayer preclinical in vitro cell culture studies were performed as they represent the high throughput gold standard for toxicity screenings of chemotherapeutics. However, this type of culture does not reproduce the three-dimensional properties of tumours. Therefore, over the last decades, the three-dimensional (3D) cell culture has increasingly been integrated into the drug development process. Indeed, this innovative technique consists in forming aggregated and compact cell clusters also called spheroids. Those in vitro micro-tumours would exhibit metabolical and biochemical heterogeneities that could reproduce the inconstancy of tumours with respect to response to treatments. In this context, these works were focused on the determination of 3D cell culture conditions of BLTN breast cancer cell lines SUM1315 and MDA-MB-231, using the "liquid-overlay" technique. Cell concentration, metabolic activity and proliferation as well as topology and ultra structural characteristics of spheroids were firstly determined. Then, sensibility profiles for several anti-cancer agents were then assessed and compared to 2D cell culture conditions. This developmental work will allow more predictive tumours' drug resistance characterization in order to improve patients' prognosis.

Poster # 128

Délivrance de doxorubicine dans des cellules tumorales par ultrasons sans agent de nucléation

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Mots clés : cavitation, ultrasons, chimiothérapie, bulle

La doxorubicine (DXR) est l'agent chimiothérapeutique le plus utilisé pour le traitement de cancers. Les études antérieures ont établi la capacité des ultrasons (US) focalisés en combinaison avec des agents de contraste (AC) à augmenter significativement la délivrance de DXR. Notre objectif est de montrer la faisabilité de potentialiser l'effet de la drogue par US en l'absence d'AC *in-vitro*.
Nous proposons l'utilisation d'un dispositif US de sonoporation [PLOSONE 2015, Chettab &all] paramétré comme suit: F=1.1MHz ; PRF=250Hz DC=15% ; Pression crête =3.2MPa, durée: 35s. Ces critères sont choisis pour initier et entretenir la cavitation dans un régime stable. Le système d'écoute du bruit US permet de contrôler la présence de bulles oscillantes (régime stable; émergence spectrale de F/2: I(F/2)) et le niveau de bruit spectral (régime inertiel avec l'implosion de bulles) caractérisé par la moyenne du signal spectral CI (dB).Le traitement va porter sur 2 lignées cellulaires CEM et MDA à raison de $2x10^6$ cell/ml par échantillon de 650 µl d'OPTI-MEM additionné respectivement de 0.1 et 2 µM de DXR. Immédiatement après le traitement US et 72 h après, une analyse par FACS a permis de caractériser respectivement l'internalisation de DXR par les cellules et la viabilité cellulaire (annexine-PI). Les analyses ont porté sur 4 essais réalisés en triplicata pour chaque lignée.

Le niveau de cavitation inertiel est faible 1.8±1.5dB alors que l'intensité d'émergence de F/2 est I(F/2)=12.3±2.7 dB (CEM). Pour chaque lignée, la combinaison US-drogue améliore de manière significative l'internalisation de la drogue dans les cellules ($p<0.002$) et diminue notablement la viabilité ($p<0.0001$) par rapport à la drogue seule (test Wilcoxon). Aucune corrélation entre I(F/2) et les critères d'évaluation biologique n'a pu être mis en évidence.

Ces travaux ont montré qu'il est possible de s'abstenir de l'utilisation d'AC pour potentialiser l'internalisation de DXR dans des cellules par ultrasons.

Poster # 129

Cell Line-Dependant Resistance to 5-Fluorouracyl in 2D versus 3D Multicellular Tumor Spheroids

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Keywords: spheroids-cancer-colorectal-5-FU-imaging

Introduction and Objectives: Few available methods can predict the response to conventional chemotherapeutic drugs for a particular patient. This lack of personalized medicine is the main cause for metastases and low survival of Colorectal Cancers (CRC). Multicellular Tumor Spheroids (MCTS) which mimic the 3-Dimensions (3D) organization of a tumor are considered as a strong model to study cancer cell biology and to evaluate the response to conventional chemotherapeutic drugs in a more physiological context than conventional 2D cultures. Our aim is to develop a 3D microfluidic platform compatible with soluble factor exchanges (drug, product secretion analysis) and live fluorescence imaging to probe MCTS growth, invasion and drug response.

Methods: Using the three CRC cell lines, HT29, HCT116 and SW480, and the chemotherapeutic drug 5-Fluorouracyl (5-FU) at various concentrations, image analysis methods are developed in order to explore the antiproliferative drug response on the MCTS growth and integrity (live and dead staining and volume quantification). For comparison, we also measure the 5-FU response on cell growth by videomicroscopy and impedimetric methods (xCELLigence) on 2D cultures.

Results: We report the dynamics of CRC spheroids growth during five days and we describe quantitatively and qualitatively the cellular response to the chemotherapeutic drug 5-FU using biological parameters extracted from time-lapse microscopy. A cell line-dependant resistance to 5-FU with different dynamics of growth in 2D versus 3D was observed. Perspectives: This project will have potential benefits through the development of new screening assays for CRC. We plan to implement an agarose-based microfluidic device enabling the perfusion of drugs around samples and the collection of secreted molecules, coupled with Single Plane Illumination Microscopy (SPIM) to perform long term 3D live imaging of MCTS.

Poster # 130

Large Area Polycrystalline Diamond Detectors for Online Hadron Therapy Beam Tagging Applications

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Keywords: synthetic diamond detectors, on-line monitoring of hadron therapy, charge collection efficiency, time resolution, integrated fast read out electronics

The MoniDiam project of the LPSC laboratory is part of the French national collaboration CLaRyS for the on-line monitoring of hadron therapy. It relies on the imaging of nuclear reaction products that is related to the ion range. The goal here is to provide large area detectors with a high detection efficiency for carbon or proton beams giving time and position measurement at count rates greater than 100 MHz (beam tagging hodoscope). High radiation hardness and intrinsic electronic properties make diamonds reliable and very fast detectors with a good signal to noise ratio. Commercial Chemical Vapor Deposited (CVD) diamonds were studied starting with plasma etch thinning and contact metallization done at the LPSC laboratory. Their applicability as particle detector was investigated using α and ß radioactive sources, 95 MeV/u carbon beams and short-bunched 8.5 keV photons from the European Synchrotron Radiation Facility (ESRF). This facility offers unique capability of highly focused beams. The energy deposition is uniform in the irradiated detector volume. It permits us to mimic the interaction of single ion beams. A time resolution ranging from 20 ps up to 40 ps and an energy resolution ranging from 7 % up to 10% were measured. It permitted us to conclude that polycrystalline CVD diamond detectors are good candidates for our beam tagging hodoscope development. The final detector will consist in a ~15×15 cm2 mosaic arrangement of stripped diamond sensors read by a dedicated integrated fast read-out electronics (~1800 channels).

Poster # 131

Interventional Imaging in Neurosurgery of Gliomas: Use of Several Fluorescence Emission Spectra of 5-ALA Induced Protoporphyrin IX to Guide Neurosurgeons

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Gliomas are infiltrative tumors of the central nervous system that account for more than 50 % of primitive brain tumors [1]. Their current treatment is based on surgery when possible but a tradeoff needs to be found by neurosurgeons between removing the maximum amount of tumor cells and preserving functionality of the brain. To help removing the maximum amount of tumor cells, a clear definition of tumor margins is required and yet the identification of infiltrative parts is still a challenge [2]. To assist neurosurgeons, fluorescence techniques provide high expectations since aminolevulinic acid (5-ALA) induced protoporphyrin IX (PpIX) accumulates in tumor cells and emits a reddish fluorescence when excited with UV/blue light. Intraoperative fluorescence microscopy enables therefore neurosurgeons to visualize some tumor cells [3] but its sensitivity is limited, particularly in tumor margins or in low grade gliomas. Moreover, this is a qualitative technique. In parallel, the relevance of 5-ALA induced PpIX fluorescence spectroscopy to increase the sensitivity has been shown [4,5] and allows quantification of PpIX concentration. Previous studies on human beings concentrated on the fluorescence emission spectrum of PpIX with a main peak at 634 nm. However, we demonstrated the presence of second fluorescence emission spectrum of PpIX with a main peak at 620 nm. We showed that the use of the two spectra of fluorescence enables to discriminate solid part of the tumor against tumor margins ex-vivo [6]. We now want to see how the two spectra can help disciminate tumor margins against healthy tissue during neurosurgery.

To reach our goal, we developed a portable spectroscopic device ending with a probe that neurosurgeons put on the brain during surgery. Excitation light is lead to the tissue through the probe and emitted fluorescence is collected through the same probe and sent to a spectrophotometer. Fluorescence measurements are then fitted with a linear combination of characteristic spectra. Results of the fit are then compared with anatomopathological results. We present the development and use of our intraoperative device and preliminary results comparing fluorescence measurements and anatomopathological results.

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Plateforms

Poster # 132

Preclinical Tools to Accelerate Drug Discovery

In 2007, the Léon Bérard Center (CLB), the Hospices Civils de Lyon (HCL), the National Institute of Health and Medical Research (INSERM) and the University Claude Bernard Lyon 1 (UCBL) have partnered to create Synergie Lyon Cancer (SLC) foundation and to offer Lyon medico scientific actors cutting –edge technologies. The foundation developed various platforms, thank to multidisciplinary collaborations and by unifying mutual resources.

We currently propose expertise in:

- **Drug discovery and development** at the C3D (Center for Drug Discovery and Development)
- **Tumor animal models** development at the LMT (Laboratoire des Modèles Tumoraux)
- Monitoring **treatment efficacy on ex vivo models** at the Ex vivo platform
- Analysis of **tumor histology** at the Pathology Research Platform
- **Analysis and understanding of biological data** through specifically developed pipelines of analysis at the Bioinformatics platform – Gilles Thomas

These preclinical packages will help you to validate your targets and biomarkers, to identify and develop lead compound, to study the mechanisms of action and to perform the POC of your candidate molecules (antibody and small organic compound).

From project elaboration to technical processes and data analyses, our qualified staff can help accelerate your research. According to your needs, we can design customized investigations by offering all possible options available in the different platforms. Our support goes beyond technical services by scientific collaborations to help you define the best strategy to pursue your project. We work together for the fight against cancer.

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