17èmes

Journées Cancéropôle Grand Sud-Ouest

17 au 19 Novembre 2021

Centre des Congrès / Carcassonne

SEMINAR BOOKLET

Cancéropôle Grand Sud-Ouest

www.canceropole-gso.org

INSTITUT NATIONAL DU CANCER

L'équipe du Cancéropôle Grand Sud-Ouest remercie vivement

- **les conférenciers et les modérateurs des sessions,**
- **les patients partenaires et particulièrement Sabine Dutheil,**
- **les coordonnateurs et les membres des Comités de Pilotage des Axes,**
- **les membres du Comité de Pilotage Scientifique,**

pour leur participation et leur implication dans l'élaboration du programme de ces 17èmes Journées et la qualité scientifique des présentations.

Comité de Pilotage Scientifique

JC. Bernhardt, JP. Bleuse, P. Clavère, P. Cordelier, A. Evrard, A.M Gué, B. Jacques, L. Karayan-Tapon, M. Khatib, S. Krouri, F. Lalloué, G. Laurent, M. Lutzmann, V. Moreau, J. Pannequin, P. Rochaix, C. Sardet, P. Soubeyran, D. Tougeron

Comités de pilotage des Axes

Axe 1 - Signalisation cellulaire et Cibles thérapeutiques
B. Bessette, G. Bossis, N. Bourmeyster, S. Britton, N. Larmonier, V. Moreau, **J. Pannequin**, M. Poupot, D. Santamaria, C. Sirac, F. Vergez

Axe 2 - Dynamique du Génome et Cancer
JC. Andrau, F. Chibon, E. Julien, G. Legube, L. Linarès, **M. Lutzmann**, D. McCusker, S. Millevoi, V. Pancaldi, E. Pinaud

Axe 3 - Recherche translationnelle, de la biologie à la clinique
N. Bakalara, T. Chardès, E. Chatelut, E. Deluche M. Dufresne, V. Gigoux, **AM. Khatib**, F. Lalloué, L. MBacthi, MA. Poul, I. Soubeyran, D. Tougeron

Axe 4 - Cancers : enjeux individuels et collectifs
D. Alabarracin, F. Cousson-Gélie, S. Darquy, **C. Delpierre**, P. Gorry, S. Gourgou, I. Ingrand, **B. Jacques**, A. Sasco, F. Sordes, B. Trétarre

Axe 5 - Technologies pour la santé
S. Bégu, L. Cognet, A. Collin, P. Cordelier, D. Cornu, M. Delarue, A. Ferrand, JL. Feugeas, M. Gary-Bobo, **AM. Gué**, D. Kouamé, S. Lecommandoux, C. Llacer, S. Papot, A. Pothier, JP. Pouget, MP. Rols, O. Sandre, H. Seznec, V. Sol

Bienvenue à cette 17ème édition des Journées Annuelles du Cancéropôle Grand Sud-Ouest.

En tant que nouvelle Directrice du Cancéropôle Grand Sud-Ouest, je suis très heureuse de vous accueillir à Carcassonne, d'autant plus heureuse que ces Journées marquent la reprise tant attendue des actions du Cancéropôle en présentiel, qu'il s'agisse de séminaires ou de formations.

Cette année, nous avons choisi de nous réunir sur un nouveau site, celui de Carcassonne. Choisir un nouveau lieu pour ce retour en présentiel, c'est aussi une façon de relancer les dynamiques et de redécouvrir les différentes équipes de notre territoire dans un cadre nouveau, propice, pourquoi pas, à redécouvrir le travail des uns et des autres.

A nouveau, les Axes scientifiques du Cancéropôle GSO se sont largement impliqués dans la construction du programme de ces Journées Annuelles, tant au niveau des sessions des Axes que pour l'organisation des plénières. Ce programme, riche en interventions pour la plupart par des chercheurs et des cliniciens de notre interrégion, a aussi pour ambition de favoriser le croisement des disciplines et l'émergence de nouvelles collaborations.

Nos Journées sont aussi comme chaque année l'occasion d'accueillir des conférenciers invités de grande qualité que nous remercions vivement.

Je vous remercie d'être présents et réunis pour ces Journées que j'espère riches en informations et en discussions. Je suis sûre qu'elles seront aussi l'occasion de rencontres informelles et de moments de convivialité, pour poursuivre la dynamique qui nous anime depuis plusieurs années.

Je vous souhaite à tous de très bonnes Journées du Cancéropôle Grand Sud-Ouest !

Nadine Houédé
Directrice du Cancéropôle Grand Sud-Ouest

LE PROGRAMME DE SOUTIEN A L'EMERGENCE DU CANCEROPOLE GSO

OUVERTURE DEBUT FEVRIER 2022 - SOUMISSION EN LIGNE

EMERGENCE DE PROJETS

OBJECTIFS	Valider les premières étapes d'un projet ou une étude de faisabilité indispensables pour une soumission à un AAP national
CRITERES	Approche nouvelle et originale, nouvelle voie d'exploration ou arrivée d'une équipe dans un nouveau champ disciplinaire
FINANCEMENT	20 k€ maximum par projet

EMERGENCE DE MODELES ET OUTILS

OBJECTIF	Soutenir la mise en place de modèles et outils innovants avec une visée technique, allant des modèles biologiques à la modélisation et au traitement des données en lien avec le cancer, afin de favoriser la mise à disposition de nouveaux modèles pour la communauté du GSO et servir de tremplin pour l'obtention de financements plus importants
CRITERES	Approche nouvelle et originale, impact du développement d'un tel modèle/outil en cancérologie
FINANCEMENT	20 k€ maximum par projet

EMERGENCE DE CONSORTIUM

OBJECTIFS	Soutenir le développement de projets pluri-équipes au sein du GSO qui, avant de postuler à des appels à projets nationaux, doivent disposer de données préliminaires qui valorisent la complémentarité de leurs compétences
CRITERES	Initiation d'un nouveau consortium au sein du GSO (pas de publication ni de co-financement préalable). Inscription dans une dynamique de mutualisation des expertises (trans- ou inter-axes).
FINANCEMENT	20 k€ maximum par projet

NOUVEAUTES 2022 : EMERGENCE DE PROJETS EN INTELLIGENCE ARTIFICIELLE

EMERGENCE DE PROJETS COLLABORATIFS AVEC DES START-UPS LOCALES

LES PROGRAMMES DE SOUTIEN, SOUMISSION EN LIGNE AU FIL DE L'EAU

MOBILITE TECHNOLOGIQUE

OBJECTIF Acquérir une technologie originale, qu'elle soit ou non déjà présente dans le GSO.

PUBLIC ELIGIBLE Statutaires, doctorants en 1ère et 2ème année et non-statutaires (les non statutaires doivent s'engager à rester dans leur équipe de recherche au minimum pendant 12 mois après la fin de la mobilité).

SEJOUR 3 mois maximum **FINANCEMENT** 4 k€ maximum

ORGANISATION DE SEMINAIRES

CRITERES Séminaires organisés sur le territoire du GSO ou par des chercheurs du GSO et ouverts à l'ensemble de la communauté scientifique du GSO.

FINANCEMENT 2 k€ maximum sous forme de subvention, de prise en charge d'un conférencier ou d'inscriptions d'étudiants et de jeunes chercheurs.

SOUMISSION AU MINIMUM 4 MOIS AVANT LA DATE DE L'EVENEMENT

CANDIDATS ERC "STARTING GRANT" ET "CONSOLIDATOR GRANT"

OBJECTIF Améliorer le dossier de candidature.

PUBLIC ELIGIBLE Candidats classés A en 1ère phase puis B après l'audition par le jury ERC

FINANCEMENT 20 k€ maximum destinés à financer des travaux ou de la mobilité

COLLABORATION TRANSFRONTALIERE

OBJECTIF Organiser la réunion d'équipes de recherche afin de construire un projet de recherche et servir de tremplin pour l'obtention de financements.

PAYS ELIGIBLES Pays du Sud-Ouest européen : Espagne et Portugal.

FINANCEMENT 4 k€ maximum pour un déplacement ou un cycle de déplacements sur une période d'un an impliquant plusieurs chercheurs du GSO.

EMERGENCE DE COLLABORATIONS AXE 4

Organiser la réunion d'équipes afin de construire un projet de recherche et servir de tremplin pour l'obtention de financements.

CRITERES Exploration de thématiques encore peu développées nécessitant des collaborations interdisciplinaires. Les attendus sont l'identification des équipes clés dans le domaine, la pertinence des collaborations présentées, la possibilité de rassembler les équipes.

FINANCEMENT 3 k€ maximum

API-K - INCITATION A LA RECHERCHE EN CANCEROLOGIE - GSO/GIRCI SOHO

Le **Cancéropôle GSO** et le **GIRCI SOHO** organisent annuellement un AAP Interrégional Cancer

OBJECTIF Inciter les jeunes cliniciens à la recherche clinique et/ou translationnelle

FINANCEMENT 40 k€ par projet (maximum)

SOUMISSION DEBUT 2022 AUPRES DE LA DRCI DE L'ETABLISSEMENT PARTENAIRE

LES FORMATIONS DU CANCEROPOLE GRAND SUD-OUEST

LES TRANSLATIONNELLES DU GSO

Les Translationnelles réunissent de jeunes médecins (internes et chefs de cliniques) et de jeunes chercheurs (fin de thèse et post-doctorants) afin de les former à la recherche translationnelle sur une thématique donnée et de les inciter aux échanges transversaux.

PRECEDENTES EDITIONS :

- **Oncodermatologie** (ROCHE) en 2014 sur le mélanome et 2015 sur le carcinome épidermoïde
- **Immuno-oncologie** (BMS), **Métastases hépatiques des cancers colorectaux** (SANOFI), **Oncologie thoracique** (BOEHRINGER INGELHEIM) en 2016
- **Immuno-Oncologie : l'immunothérapie anti-cancéreuse** (BMS) en 2018
- **Cancer du poumon** en 2019

PROCHAINE EDITION 2022

L'ECOLE D'IMAGERIE DU PETIT ANIMAL APPLIQUEE AU CANCER

L'Ecole d'Imagerie du Petit Animal Appliquée au Cancer a été mise en place sur l'initiative du Club "Imagerie clinique et In Vivo " du Cancéropôle Grand Sud-Ouest. Elle présente les différentes modalités d'imagerie anatomique, fonctionnelle et moléculaire du petit animal. Elle s'appuie sur les plateformes et expertises régionales et met en avant les récentes innovations technologiques et méthodologiques en imagerie préclinique. Alternant cours et ateliers pratiques sur les plateformes d'imagerie, elle a lieu tous les 2 ans.

OBJECTIFS :

- Aborder les principes théoriques et les aspects pratiques de chaque technique d'imagerie,
- S'initier aux dernières technologies,
- Evaluer les potentialités et les limites des différentes techniques d'imagerie,
- Intégrer un réseau de scientifiques régionaux intéressés par l'imagerie médicale.

*PROCHAINE EDITION DU 12 AU 16 JUIN 2022 A TOULOUSE. INSCRIPTIONS SUR **imagerie.canceropole-gso.org***

DEVELOPPEMENT D'UN MEDICAMENT

Organisée en alternant cours et ateliers, cette formation a pour objectif de former ensemble des jeunes médecins (internes, chefs de clinique), pharmaciens (internes) et chercheurs (fin de thèse, post-doctorants et titulaires) sur les différents aspects du développement d'un médicament en cancérologie. Elle a lieu tous les 2 ans et bénéficie du soutien institutionnel de plusieurs entreprises du médicament.

PRECEDENTES EDITIONS :

- **2015 : Développement d'un médicament, de la biologie à la clinique**
- **2017 : Développement d'un médicament : les anticorps thérapeutiques et l'immunothérapie**

PROCHAINE EDITION 2022

WORKSHOP JEUNES CHERCHEURS

Le Workshop Jeunes Chercheurs a objectif d'améliorer la qualité des travaux et des publications de jeunes chercheurs. Il réunit des experts de renom et des jeunes chercheurs (post-doctorants seniors et jeunes titulaires) sélectionnés sur leurs travaux, en format résidentiel, afin de favoriser les échanges et de permettre aux jeunes chercheurs de bénéficier d'un coaching de qualité.

PRECEDENTES EDITIONS :

- **2014 : Genomic instability in Cancer**
- **2015 : Signaling in Cancer**
- **2017 : Nanomedicine in Cancer**
- **2017 : Genome dynamics and Cancer**
- **2018 : Signaling in Cancer**
- **2020 : Biofabrication and Cancer**

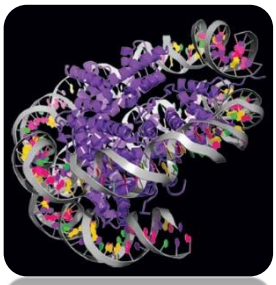
PROCHAINE EDITION : GENOME DYNAMICS AND CANCER, DU 14 AU 15 MARS 2022 A CARCASSONNE

PROCHAINEMENT

YOUNG SCIENTISTS WORKSHOP
GENOME DYNAMICS AND CANCER

March 14-15, 2022, Carcassonne

Objectives: The Cancéropôle GSO is pleased to announce the 8th edition of its coaching workshop for young researchers. This 2-day scientific event will involve presentation and discussion of recent findings and future projects in the scope of Genome Dynamics in Cancer. The format of the meeting (focused scientific research themes, fewer than 30 participants, full board retreat in a nice environment) is designed to foster exchanges and communication between junior and senior scientists and strengthen research networks between the Great Southwest (GSO) labs.
Selected young researchers will have the opportunity to enhance the quality of their scientific and career projects by receiving feedback from the following renowned experts in the field:



Richard HEIDEMANN, *Director Immunobiology at Charles River Laboratories, Edinburgh*

Sarah LAMBERT, *Group leader at Curie Institute, Orsay*

Thomas LEMBERGER, *Deputy Head of Scientific Publications at EMBO ; Head, SourceData ; Associate Editor, Review Commons, Heidelberg*

Michel WERNER, *Director of Institut Jacques Monod, Paris*

Meeting format

- Scientific sessions opened by lectures by invited experts
- Projects presented by selected young researchers and discussion
- Training session on scientific publications
- Free accommodation for the participants, conditional on attendance

Deadline: January 10 2022

Call for applicants

The main objective of this workshop is to allow young scientists to improve their scientific and career projects and enhance the quality of their publications (confidentiality respected).

Audience

- Around 10 post-docs and young scientists (CR and MdC), affiliated to a Greater Southwest lab, will be selected for the coaching of their projects and careers.
- Additional young researchers, affiliated to the teams of the selected candidates, may be invited to attend the workshop and benefit from such a unique experience.

Scientific scope

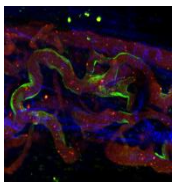
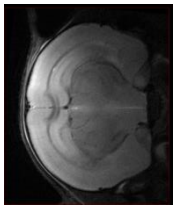
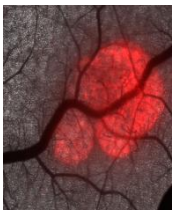
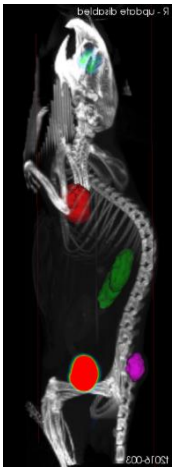
Abstracts related to Cancéropôle GSO Axis 2 themes are welcome including: Genome organization and (in)stability in DNA replication, repair and cell cycle; Chromatin & Epigenetics; Gene expression : from transcription to translation ; Mutagenesis & Genomic alterations.

Applicants selection

- Respect of the scientific scope of the call.
- Scientific quality of the candidate's project(s).
- Candidates' resume.

Submit your application: wsjc2022.canceropole-gso.org

6EME ECOLE D'IMAGERIE
DU PETIT ANIMAL APPLIQUEE AU CANCER
12 au 16 juin 2022, Toulouse

Lieu : Toulouse (Institut Universitaire du Cancer – Oncopole, CHU Purpan, CREFRE-Oncopole, IPBS)

Objectifs :
L'imagerie préclinique *in vivo* permet de mettre en évidence de nouvelles cibles thérapeutiques et d'évaluer rapidement de nouvelles stratégies médicales. Ces techniques sont mises à la disposition de la communauté scientifique sous forme de plateformes ouvertes aux académiques comme aux industriels. La 6ème édition de cette école, organisée par le Cancéropôle Grand Sud-Ouest, le CREFRE et l'IPBS (plateformes GenoToul) abordera toutes les modalités d'imagerie in vivo (anatomique, fonctionnelle et moléculaire) ainsi que le suivi des animaux. Les principes théoriques des différentes modalités seront présentés par des chercheurs et médecins experts dans le domaine. En complément, des ateliers d'imagerie en situation réelle au sein des plateaux techniques permettront une analyse exhaustive des potentialités et champs d'applications de chaque technique.

Responsables scientifiques : Elisabeth BELLARD, Muriel GOLZIO et Carine PESTOURIE

Intervenants : Caroline DELMAS, Magali JACQUIER, Elisabeth MOYAL, Laure PARENT, Anne-Sophie SALABERT, Aymeric BLANC, Neal BURTON, Franck COUILLAUD, Frédéric COURBON, Philippe DAVAULT, Franck DESMOULIN, Tim DEVLING, Emmanuel GRAS, Renaud LEBRUN, Gilles RENAULT, Guillaume REVEILLON, Pierre SICARD, Philippe TROCHET

Conférencier invité : **Mickaël TANTER** (Institut Langevin, Paris) - « ***Nouvelles technologies d'imagerie en échographie*** »

Prérequis : Avoir un projet d'imagerie *in vivo*

Public : Chercheurs, Ingénieurs, Techniciens, Post-doctorants, Doctorants

Programme :

- **Formation théorique** : Optique, Bioluminescence, Fluorescence, Microscopie intravitale multiphotonique, Imagerie Nucléaire, Radiochimie et radiopharmacie, IRM, Echographie, Microtomographie Rayons-X, Irradiation guidée par imagerie, Endomicroscopie confocale laser, Anesthésies et confinement des animaux et des locaux, Règlementation et bien-être animal.
  Tables rondes, visite du cyclotron et du service de radiochimie PiR2.
- **Formation pratique** : des ateliers par petits groupes sur toutes les modalités d'imagerie avec des modèles murins.

Nombre de participants : 20

Tarifs (sans/avec hébergement) :
Académique : 700€ / 1100€
Privé : 1400€ / 1800€

Le tarif « sans hébergement » inclut les repas du soir

Prise en charge possible dans le cadre de la ***formation professionnelle continue***

Validation d'unité de formation continue en expérimentation animale

Inscription avant le 15 avril 2022
Plus d'infos : imagerie.canceropole-gso.org

Program

Wednesday 17th November

12h30 – 13h45 Welcome lunch

13h45 – 14h00 Opening ceremony

Cancéropôle Grand Sud-Ouest Director

14h00 – 16h00

Chairs: Anne-Marie GUE & Fabrice LALLOUE

Lecture (virtual): **Caroline DIVE**, *Cancer Research UK, Manchester (United Kingdom)* - **Liquid Biopsy to support better treatment for small cell lung cancer**

- **Sandrine DABERNAT**, *Biotherapy for genetic diseases, inflammation and cancer (Bordeaux)* - Extracellular vesicle liquid biopsy: the next generation cancer biomarker ?
- **Camille DANTZER**, *Bordeaux Research in Translational Oncology* - Mutated β-catenin regulates extracellular vesicles machinery in hepatocellular carcinoma

Lecture: **Valérie TALY**, *Centre de Recherche des Cordeliers, Inserm U1138, CNRS SNC5096 (Paris)* - **Tracking circulating tumor DNA for cancer patient follow-up**

16h00 – 17h00 Poster session & Coffee break

17h00 - 18h30

Chair: Bruno CONSTANTIN

Lecture: **Albrecht SCHWAB**, *Institute of Physiology II, Münster (Germany)* - **The role of KCa3.1 channels in non-small cell lung cancer**

- **Bruno CONSTANTIN**, *Laboratoire STIM - CNRS ERL 7003 - EA 7349 (Poitiers)* - Calcium signaling in cancer stem cells
- **David MOREAU**, *École des Mines de Saint-Étienne* - Manipulation of ionic channels and calcium signaling in glioblastoma with infrared radiation
- **Catherine LECLERC**, *Molecular, cellular and developmental biology Unit, CBI-MCD (Toulouse)* - Quiescence status of glioblastoma stem-like cells involves remodelling of Ca2+ signalling and mitochondrial shape

Chair: Majid KHATIB

- **Alexandra FAUVRE**, *Montpellier Cancer Research Institute* - Platinum-based chemotherapy induce inflammation via a non-canonical STING pathway dependent on ATM activation
- **Alexia FRANCOIS**, *Angiogenesis and Cancer Microenvironment Laboratory (Bordeaux)* - Proteolytic protein repression mediates tumor T cells infiltration and anti-tumoral immune response: Drug-repurposing approach
- **Amandine AMALRIC**, *Institute for Functional Genomics (Montpellier)* - "Epitranscriptomics": a promising source of biomarkers for personalized medicine
- **Céline HERVIEU**, *CAPTuR (Limoges)* - Colorectal cancer stem cells respond differently to chemotherapies depending on their original location
- **Elsa FRISTOT**, *Structural Biochemistry Center (Montpellier)* - Programming lactic acid bacteria for colorectal cancer therapy
- **Jean DESCARPENTRIE**, *Angiogenesis and Cancer Microenvironment Laboratory (Bordeaux)* - Repression of liver colorectal metastasis by the naturally occurring inhibitor of Furin (ppFurin)

- **Johanna MARINES**, *AZELEAD (Montpellier)* - Modelling 3D tumour microenvironment in vivo using live imaging technique: a tool to predict cancer fate
- **Kévin BIGOT**, *Montpellier Cancer Research Institute* - New Imidazo[1,2-a]quinoxalines compounds for Pancreatic Ductal Adenocarcinoma treatment: mechanism of action and target identification
- **Marine BRUCIAMACCHIE**, *Montpellier Cancer Research Institute* - Association of oxaliplatin-based chemotherapy and ATR inhibitor in pancreatic cancer
- **Soha SALLOUM**, *Institute of Human Genetics (Montpellier)* - Characterization of β-catenin translation factories
- **Zeinab TARHINI**, *Limoges University* - The effect of metformin on the survival of colorectal cancer patients with type 2 diabetes mellitus

Chair: Anne-Marie GUE

Lectures: **Sylvain CUSSAT-BLANC**, *Institut de Recherche en Informatique de Toulouse* - **ISiCell: a web-oriented platform to model cellular processes**

Ovidiu RADULESCU, *Laboratory of Pathogen Host Interactions (Montpellier)* - **Computational Models of Heterogeneity in Melanoma: Designing Therapies and Predicting Outcomes**

5 selected talks for “Ma techno en 180 secondes” (flash-posters):

- **Malvina MARKU**, *Cancer Research Center of Toulouse* - On the dynamics of TAM formation in Chronic Lymphocytic Leukaemia: a multi-scale approach
- **Chloé BESSIERE**, *Institute for Regenerative Medicine and Biotherapy (Montpellier)* - Towards a novel framework for large scale RNAseq data analysis in human health
- **Alexia BRUNEL**, *Cancer Research Center of Toulouse* - Bioinformatical analysis of tumor cell with stroma crosstalk that impact aggressiveness of pancreatic ductal adenocarcinoma
- **Elisa LAMBERT**, *XLim (Limoges)* - Microfluidic Lab-On-Chip for UHF-Dielectrophoresis Discrimination of Glioblastoma Undifferentiated cells
- **Justine JOURNAUX**, *Cancer Research Center of Toulouse* - Nanotherapy of pancreatic adenocarcinoma by targeted magnetic hyperthermia: efficacy and mechanisms

18h30 – 19h30 Icebreaker & Poster session

Thursday 18th November

08h00 - 08h30 Welcome coffee

08h30 – 10h00

Session 3 – Myeloid cells in cancer **33**

Chairs: Mary POUPOT & Nicolas LARMONIER

Lecture: **Antonio SICA**, *University of Piemonte Orientale 'A. Avogadro' (Novara) and Humanitas Clinical and Research Center, Milan (Italy)* - **Therapeutic targeting of myelopoiesis in cancer**

- **Frédéric LAGARRIGUE**, *Institute of Pharmacology and Structural Biology (Toulouse)* - Integrin signaling in tumor-associated macrophages
- **Julien FAGET**, *Montpellier Cancer Research Institute* - Lessons from the remote alteration of granulopoiesis in NSCLC: what is neutrophil homeostasis telling us on immune checkpoint blockade ?
- **Céline BLAYE**, *Institut Bergonié and ImmunoConcept Lab (Bordeaux)* - The tumor-promoting myeloid landscape in breast cancers

10h00 – 11h00 Poster session & Coffee break

11h00 - 12h45

Session 4 – Preneoplasia & early dissemination **39**

Chairs: Julie GUILLERMET-GUIBERT & David SANTAMARIA

Lecture (virtual): **Daniel MUÑOZ-ESPÍN**, *CRUK Cambridge, Department of Oncology, Hutchison/MRC Research Centre, University of Cambridge (UK)* - **Impact of cellular senescence in lung precancerous lesions: senotherapeutic opportunities**

- **Julie GUILLERMET-GUIBERT**, *Cancer Research Center of Toulouse* - Role of extrinsic factors in cancer initiation: importance of the stressed niche in pancreatic cancer
- **Zeinab HOMAYED**, *Institute for Functional Genomics (Montpellier)* - Unravelling the role of early dissemination in colorectal cancer
- **Dimitri HAMEL**, *Digestive Health Research Institute (Toulouse)* - Epithelio-stromal relationships in physiology & IBD: osteopontin, a key factor for epithelial regeneration and tumor initiation ?
- **Hamid-Reza REZVANI**, *Biotherapy for genetic diseases, inflammation and cancer (Bordeaux)* - Metabolic and immune features as predictive biomarkers of risk stratification of skin carcinoma

12h45 – 14h15 Lunch break

14h15 - 16h05

Session 5A – Cell signaling and therapeutic targets **45**

Chairs: Violaine MOREAU & Julie PANNEQUIN

- **Damien GREGOIRE**, *Institute of Molecular Genetics of Montpellier* - Ras/MAPK signalling intensity defines subclonal fitness in a mouse model of primary and metastatic hepatocellular carcinoma
- **Lucile BANSARD**, *Institute for Functionals Genomics (Montpellier)* - JMV7048, First-in-class PROTAC degrader of PXR

Lecture: **David SANTAMARIA**, *Inserm U1218 Action (Bordeaux)* - **Novel mechanisms controlling KRAS oncogenic output: impact on tumour fitness and cancer vulnerabilities**

- **Coralie CAYRON**, *Cancer Research Center of Toulouse* - PI3K signaling and tumoral metabolism in pancreatic cancer
- **Thomas NAVES**, *CAPTuR (Limoges)* - Sortilin exhibits tumor suppressor-like activity by limiting EGFR function

- **Marcin DOMAGALA**, *Cancer Research Center of Toulouse* - Characterization of a novel monoclonal antibody targeting tumor-associated macrophages

Chairs: Gaëlle LEGUBE & Malik LUTZMANN

Lecture (virtual)**: Joanna MORRIS**, *Birmingham Centre for Genome Biology and Institute of Cancer and Genomic Sciences, University of Birmingham (United Kingdom)* - **Back-up pathways that support BRCA1 deficient cells**

- **Sébastien BRITTON**, *Institute of Pharmacology and Structural Biology (Toulouse)* - BRCA1 prevents R-loop-associated centromeric instability
- **Israa AL JAMAL**, *CRIBL (Limoges)* - IgH Locus Suicide Recombination (LSR) in Chronic Lymphocytic Leukemia (CLL): Prognosis Indicator ?
- **Yvan CANITROT**, *Molecular, cellular and developmental biology Unit, CBI-MCD (Toulouse)* - Control of Homology directed repair of DNA double strand breaks by the KDM8 histone demethylase
- **Francesco CALZAFERRI**, *Institut des Biomolécules Max Mousseron (Montpellier)* - New strategies to target DNA methylation: from the discovery of novel DNMT inhibitors to the identification of novel epigenetic targets
- **Vera PANCALDI**, *Cancer Research Center of Toulouse* - Network approaches to dissect the epigenome-phenotype connection in immune cell

Introduction et modération : Béatrice JACQUES

Conférence (à distance) : **Arnaud HALLOY**, *Université de Nice* - **Savoirs expérientiels et partenariat patient: une alliance indispensable**

- **Frédéric BOUHIER**, *Chargé de mission Société Française de Santé publique* - VIH/Sida et Empowerment : « Quels enseignements pour la mobilisation des patients en cancérologie ? »
- **Sabine DUTHEIL**, *Patiente partenaire Bordeaux* - Le cancer du sein, une maladie chronique pas (tout à fait) comme les autres ?

16h05 – 17h00 Coffee break

17h00 – 18h30

Chairs: Aubin PENNA & Gilles FAVRE

- **Anne MONTFORT**, *Cancer Research Center of Toulouse* - Combining TNF inhibitors to anti-PD-1 and anti-CTLA-4 for the treatment of advanced melanoma patients
- **Xavier ORLIK**, *ONERA (Toulouse)* - Real time in vivo observation of the human skin microvascularization
- **Léa DOUSSET**, *Biotherapy for genetic diseases, inflammation and cancer (Bordeaux)* - Vitiligo under PD-1 inhibitors for melanoma patients: what's new and how we can go further ?

Lecture: **Daniel PEEPER**, *The Netherlands Cancer Institute, Amsterdam (The Netherlands)* - **Rational concepts for tumor & immune cell therapy combinations**

Friday 19th November

08h00 - 08h30 Welcome coffee

08h30 – 10h15

Session 7A– Poor prognosis Cancers: Brain tumors **75**

Chairs: Norbert BAKALARA & Fabrice LALLOUE

- **Thomas DAUBON**, *Institute for cellular biochemistry and genetics (Bordeaux)* - Glioblastoma Metabolic Symbiosis: When Lactate Takes The Lead
- **Charles DUPIN**, *Biotherapy of genetic diseases, inflammatory disorders and cancers (Bordeaux)* - Glioblastoma, radiotherapy and 5-AminoLevulinic Acid: a dead end ?
- **Gabriel MARSERES**, *Immunology from concept and experiments to translation (Bordeaux)* - Targeting glioblastoma with in vitro amplified Vδ2neg γδ T cells: preclinical study
- **Alexandra GARANCHER**, *Montpellier Cancer Research Institute* - Understanding the immune microenvironment to improve immunotherapy for pediatric brain tumors
- **Jean-Philippe HUGNOT**, *Institute for Functional Genomics (Montpellier)* - Insight on the tumoral cell diversity, their formation and proliferation in IDH1-mutant diffuse low brain gliomas: a key role for Notch1 signaling

Session 7B – Extracellular matrix & cancer **81**

Chairs: Christine BEZOMBES & Anne-Marie GUE

Lecture: **Audrey FERRAND**, *Digestive Health Research Institute (Toulouse)* - **Extracellular Matrix Mechanical Properties and Regulation of the Intestinal Stem Cells: When Mechanics Control Fate**

- **Christine VARON**, *Bordeaux Research in Translational Oncology* - The bacterial genotoxin, cytolethal distending toxin, modulates cell differentiation and elicits epithelial to mesenchymal transition
- **Laure GIBOT**, *Molecular Interactions and Chemical and Photochemical reactivities (Toulouse)* - Electric fields as powerful tools to transiently modulate cutaneous extracellular matrix
- **Clément AUGER**, *CAPTuR (Limoges)* - Using nanoparticle tracking analysis (NTA) for appreciation of up and down extracellular vesicle secretion
- **Léa NORMAND**, *Bordeaux Research in Translational Oncology* - Tumor cells-released tracks promote cell migration on type I collagen

Session 7C – Le patient formateur **87**

Introduction et modération: Béatrice JACQUES

- **Olivia GROSS**, *LEPS UR 3412, Université Sorbonne Paris Nord* - L'expérience de Bobigny | Intervention à distance
- **Marie-France KOLTES**, *Institut des métiers de la santé* et **Patrick LARTIGUET**, *aidant partenaire formateur (Bordeaux)* - Du paternalisme médical au partenariat en santé: accompagner le changement auprès des établissements de formation des professionnels de santé
- **Sandra ALLAIRE de la FUENTE**, *patiente partenaire en oncologie (Bordeaux)* – Du témoignage bénévole à la co-construction d'une séquence pédagogique rémunérée
- **Guy KANTOR**, *Ligue contre le cancer en Aquitaine* - Lancement d'un dispositif de formation par les patients au sein de la ligue contre le cancer de Gironde | Intervention à distance

10h15 – 11h00 Coffee break

11h00 – 12h30
Session 8A – Poor prognosis Cancers: Pancreatic cancer **93**

Chairs: Marlène DUFRESNE & Véronique GIGOUX

Lecture: **Núria MALATS & Francisco X. REAL**, *Spanish National Cancer Research Centre (CNIO), Madrid (Spain)* - **Learning from biology: The challenge of pancreatic cancer prevention**

- **Guillaume TOSATO**, *Montpellier Cancer Research Institute* - Discovery of Soluble Pancreatic Cancer Biomarkers Using Innovative Clinical Proteomics and Statistical Learning
- **Yvan MARTINEAU**, *Cancer Research Center of Toulouse* - Translatome-based classification reveals a dual metabolic dependency of a new tumor subtype of pancreatic cancer
- **Pierre CORDELIER**, *Cancer Research Center of Toulouse* - Targeting cancer cells with basal-like, mesenchymal phenotype with oncolytic virus to inhibit the growth of pancreatic cancer
- **Corinne BOUSQUET**, *Cancer Research Center of Toulouse* - Towards the identification of Cancer-Associated Fibroblast signaling underlying pancreatic cancer aggressiveness

Introduction et modération : Béatrice JACQUES

- **Lise MOLIMARD** et **Nathalie CAPLET**, *SIRIC Brio* - Implication des patients en recherche : retours d'expériences et réflexions sur différentes modalités
- **Julien BIAUDET**, *Cancéropôle CLARA et* ***Raymond MERLE*** *Département Universitaire des Patients Grenoble Alpes* - Construire un appel à projets avec et pour les patients : retour d'expérience du Cancéropôle CLARA
- **Pascal RAGOUET**, *Centre Emile Durkheim (Bordeaux)* - Recherche profane et autothérapie. Quelques pistes sur le concept de recherche translationnelle centrée patient

12h30 – 12h45 Poster award session

12h45 – 14h00 Lunch

Session 1 – Liquid biopsies

1 / 1
Liquid biopsies to support better treatment for small cell lung cancer

Caroline DIVE

Cancer Biomarker Centre, CRUK Manchester Institute, University of Manchester, United Kingdom

Small cell lung cancer (SCLC) is an aggressive neuroendocrine tumour that disseminates early and has a 5-year survival rate of <7%. Acquired resistance to platinum-etoposide chemotherapy is almost universal after initial treatment responses and whilst immunotherapy brings benefit to an undefined subset of patients with SCLC, it is of short duration. Personalised medicine for patients with SCLC is urgently required. However, progress in recent years in understanding SCLC biology and phenotypic heterogeneity has led the definition of molecular subtypes that, in preclinical models, have differing vulnerabilities raising the possibility of stratified approaches.

Historically, clinical trials in SCLC have not included predictive or disease monitoring biomarkers largely because of a lack of understanding of what to measure and the considerable difficulty of obtaining tumour specimens, particularly longitudinally. Circulating Tumour Cells (CTCs) are prevalent in SCLC and circulating tumour DNA (ctDNA) is also readily detectable in most patients with extensive stage SCLC (1,2). Therefore, repeatable liquid biopsies based on CTCs and ctDNA hold significant potential to enhance or direct future clinical trials in SCLC.

SCLC CTCs express EpCAM and are thus detectable using the CellSearch (CS) platform. CS CTC number is prognostic at baseline, has a wide dynamic range making it useful as a pharmacodynamic biomarker, and also acts as a surrogate of tumour response to chemotherapy (1). The prevalence of CS CTCs also allows CTC-based predictive biomarker assay development. In this regard we showed that a single CTC-based copy number alteration (CNA) signature can predict pre-treatment whether a SCLC patient will be chemorefractory (progress within 90 days of chemotherapy treatment) or initially chemosensitive (3). In 2014, we pioneered the development of CTC derived patient explant (CDX) models in immune compromised mice (4,5) which faithfully recapitulate the donor patients' tumour morphology and genomic landscape, and mimic their response to cisplatin-based chemotherapy. The models also display patient-relevant metastatic tissue tropism and cell subpopulations are competent for vasculogenic mimicry (6). We have recently developed CDX that metastasise to the brain, a significant clinical issue, and will use these unique CDX to study brain metastasis (5). We are currently interrogating phenotypic heterogeneity in our panel of 45 CDX models and using them to test novel drug combinations including DNA damage repair and cell cycle checkpoint inhibitors (7). CDX cells can be cultured ex vivo for short periods, genetically manipulated and re-implanted in vivo for hypothesis testing studies. Finally, we recently developed approaches to directly culture a SCLC patient's CTCs opening up the future possibility of using CTC cultures as patient 'avatar' for real time therapy testing to inform their treatment. These applications of SCLC CTCs will be discussed and reviewed.

Assessment of ctDNA CNA provides a readily applied disease monitoring tool, and I will also discuss our recent development of a methylated ctDNA assay that is more sensitive, detecting even stage 1 disease and show promise to molecularly subtype SCLC from a blood draw, with the horizon of stratified medicine trials.

1.Hou et al J Clin Oncol. 2012.
2. Mohan et al, J Thoracic Oncol, 2020
3. Carter et al Nature Medicine, 2017.
4. Hodgkinson et al, Nature Medicine, 2014.
5. Simpson et al, Nature Cancer, 2020.
6. Wiliamson et al, Nature Comms, 2016.
7.Lallo et al. Clin Cancer Res. 2018.

1 / 2

Extracellular vesicle liquid biopsy: the next generation cancer biomarker ?

Samuel AMINTAS[1,2,3], Etienne BUSCAIL[4,5], Véronique VENDRELY[1,2,3], Laurence CHICHE[1,2,3], Christophe LAURENT[1,2,3], Aurélie BEDEL[1,2,3], François MOREAU-GAUDRY[1,2,3], **Sandrine DABERNAT[1,2,3]**

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Extracellular vesicles (EVs), produced by healthy and tumor cells and released in various bodily fluids, including the blood, carry a rich content in biomolecules packaged in bilayer phospholipidic membranes.

Considered as waste bags for a long time, they actually count active subtypes, including exosomes, driving functional local and distant cell-cell communication and molecular exchange. The last decade has seen research intensification in the field of cancer because EVs were found to support tumor control and progression, both at the level of the tumor cells themselves but also through tumor microenvironment and metastatic niche. Because of their specific active cargo, they are being evaluated as biomarkers for liquid biopsy. Compared to soluble circulating biomarkers, their complexity might enrich information on tumor and metastases status.

We will see how specific surface proteins can help diagnose pancreatic cancer and will discuss the future use of EV detection/quantification in the management of cancer.

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Mutated ß-catenin regulates extracellular vesicles machinery in hepatocellular carcinoma

Camille DANTZER[1], Aude BRUNEL[2], Jean-Frédéric BLANC[1], Mireille VERDIER[2], Barbara BESSETTE[2], Fabrice LALLOUE[2], Violaine MOREAU[1], Clotilde BILLOTTET[1]

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Hepatocellular carcinoma (HCC) is the most common primary liver tumor in adults and represents the sixth most common cancer in the world and the fourth leading cause of cancer-related death. HCC is a pathology with a poor prognosis: the diagnosis remains challenging because of its difficulty to be detected early in disease's progression and due to the lack of effective therapies. Since 2008, Sorafenib (multi-kinase inhibitor) has been the first-line reference for the treatment of HCC. More recently, several clinical trials based on immunotherapy have shown their effectiveness on overall survival. Thus, the combination Atezolizumab (anti-PDL1 antibody) with Bevacizumab (anti-VEGF antibody) is now the first-line treatment for advanced HCC. Despite this therapeutic advance, clinical data suggest that immunotherapy could be less effective in patients with β-catenin-mutated HCC. These tumors are characterized by an environment devoid of immune infiltrates, leading to resistant-immunotherapy tumors. However, how the β-catenin oncogene promotes this immune escape and how tumor cells trigger immunosuppressive cascades is not yet fully understood. Our project focuses on the involvement of β-catenin signalling in tumor cells/immune cells communication through extracellular vesicles (EVs).

Using a transcriptomic analysis performed in HepG2 cells, an alteration of the EVs machinery upon knock-down of mutated β-catenin has been identified. In the same model, we also found a defect in the secretion of EVs when β-catenin is mutated. We further identified two target genes of the EVs machinery whose expression is dependant on β-catenin signalling. These results were confirmed in two other cell lines, and in HCC human samples mutated for β-catenin. Thus, these results suggest that β-catenin mutations may inhibit EVs formation and/or secretion in liver tumor cells. As EVs and their contents (chemokines, mRNAs, miRNAs…) are essential factors for intercellular communication, we now hypothesized that this decrease in the production of EVs could lead to defective recruitment of leukocytes, making these tumors poor in immune infiltrates and resistant to immunotherapy.

Our results provide new knowledge on the impact of β-catenin mutations on the tumor microenvironment and may allow the development, from liquid biopsies, of a new tool for stratifying patients with HCC for the response to immunotherapies.

1 / 4
Tracking circulating tumor DNA for cancer patient follow-up

Valérie TALY

Personalized medicine, pharmacogenomics and therapeutic optimization team (MEPPOT), Centre de Recherche des Cordeliers, « Equipe labélisée Ligue Contre le Cancer », UMRS1138, CNRS SNC 5096, Sorbonne Université, Université de Paris, INSERM

Droplet-based microfluidic has led to the development of highly powerful tools with great potential in High-Throughput Screening where individual assays are compartmentalized within aqueous droplets acting as independant microreactors. Thanks to the combination of a decrease of assay volume and an increase of throughput, this technology goes beyond the capacities of conventional screening systems. Added to the flexibility and versatility of platform designs, such progresses in the manipulation of sub-nanoliter droplets has allowed to dramatically increase experimental level of control and precision.
We will present how by combining microfluidic systems and clinical advances in molecular diagnostic we have developed an original method to perform millions of single molecule PCR in parallel to detect and quantify a minority of mutant sequences within a high quantity of non-mutated sequences in complex mixture of DNA with a sensitivity unreachable by conventional procedures [1]. Droplet-based digital PCR (dPCR) assays allowing analysis of several genetic and epigenetic cancer-specific alterations have been developed and validated for the detection of tumor DNA in patient samples [2-4]. In particular, droplet based digital PCR has allowed to reach unprecedented sensitivity and accuracy for rare sequences detection. Complementarity of ddPCR and highly sensitive optimized NGS technologies will be discussed. These technological developments have greatly facilitated the tracking of circulating cell-free nucleic acids in body effluents. We will present the pertinence of these approaches to monitor circulating tumor DNA in plasma (so-called liquid biopsy) for the follow-up of patients with localized or advanced cancers. Results of several prospective clinical studies will be presented [5-12].

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Session 2A – Ions channels & Cancer

2A / 1

The role of KCa3.1 channels in non-small cell lung cancer

Albrecht SCHWAB, Luca Matteo TODESCA, Etmar BULK

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KCa3.1 channels play an important pathophysiological role in non-small cell lung cancer (NSCLC). Their overexpression and/or the hypomethylation of their promoter predict a poor prognosis of NSCLC patients. Since cancer patients usually die of the sequelae of cancer metastasis we reasoned that KCa3.1 channels must contribute to steps of the metastatic cascade. Indeed, we could show that KCa3.1 channels promote NSCLC cell aggressiveness by modulating processes such as migration, proliferation and tumor cell extravasation. Recent data show that KCa3.1 channels are not only expressed in the plasma membrane, but that they are also found in the inner membrane of mitochondria. Mitochondrial KCa3.1 channels regulate ROS production. We will discuss which functional properties of NSCLC cells are regulated by this mechanism. Taken together, our findings lend support to viewing KCa3.1 channels as potential therapeutic target in NSCLC. It is particularly noteworthy in this context that the KCa3.1 channel blocker senicapoc has already been tested in a phase III clinical trial for another indication. Thus, senicapoc could be repurposed for treating NSCLC patients.

2A / 2
Calcium signaling in cancer stem cells

Bruno CONSTANTIN

Laboratoire Signalisation et Transports Ioniques Membranaires, Université de Poitiers, CNRS ERL 7003

Cancer stem cells are a subpopulation of tumor cells that proliferate, self-renew and produce various tumoral cells building-up the tumor. Responsible for the sustained growth of malignant tumors, cancer stem cells are proposed to play significant roles in cancer resistance to standard treatment and in tumor recurrence. Among the mechanisms that are dysregulated in neoplasms, those related to intracellular Ca2+ signaling play a significant role in various aspects of cancers. Ca2+ is a ubiquitous second messenger whose fluctuations of its intracellular concentrations are tightly controlled by membrane ion channels, pumps, exchangers and Ca2+ binding proteins. These components support the genesis of Ca2+ signals with specific spatio-temporal characteristics that define the cell response. Being involved in the coupling of extracellular events with intracellular responses, the Ca2+ toolkit is often hijacked by cancer cells to promote notably their proliferation and invasion. Growing evidence obtained during the last decade pointed to a role of Ca2+ handling and mishandling in cancer stem cells.

This lecture is highlighting the complex roles of Ca2+ toolkit and signaling in cancer stem cells and shows that numbers of Ca2+ signaling actors promote cancer stem cell state and are associated with cell resistance to current cancer treatments.

Part of our ongoing studies are directed towards identifying the function and regulation of store-operated calcium entry, the transient receptor potential channel, TRPC1, the calcium channel, Orai1, and the ER regulator of calcium channels, STIM1 in glioblastoma stem cells, and leukemic progenitors. We have provided evidence that expression of TRPC1 and STIM1 is dependent on Bcr-Abl oncogene and that Store-Operated Calcium Entry (SOCE), a downregulated influx in leukemic progenitors, is an essential component of the agonist-stimulated Ca2+ influx mechanism that is required for sustained NFAT nucleus translocation (Cabanas et al., 2018). We have also identified calcium entry as an important component of cell signaling controlling human glioblastoma stem cells activity. Inhibition of SOC dependent calcium entries decreases proliferation impairs self-renewal and reduces expression of pluripotency proteins of CSC from GBM (Terrier et al., 2021). Our data showing the ability of Store-Operated Calcium Channels (SOCC) inhibitors to impede CSC self-renewal pave the way for a strategy to target the cells considered as responsible for conveying GBM resistance to treatment and tumor relapse.

2A / 3

Manipulation of ionic channels and calcium signaling in glioblastoma with infrared radiation

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Mines Saint-Etienne, Centre CMP, Département BEL, Gardanne

Infrared neural stimulation (INS) is an optical neurostimulation technique successfully used to excite peripheral nerves and the central nervous system. The main advantage of this method is the spatial selectivity achieved without any need of adding exogenous labels or photoabsorbing molecules. Although at present, this phenomenon has primarily been explored in the neurosciences, we discovered that the impact of infrared radiation is not restricted to excitable cells and may have general utility for manipulating cell signaling.
To understand these effects, we must first consider the several hypotheses on the mechanism mediating INS. It is agreed that the effect is likely photothermal [1], given the absorption of infrared (IR) radiation by water at the wavelengths employed in INS experiments (1470; 1860; 2120 nm), so the subsequent effect of the temperature increase is still under consideration. The first hypothesis, proposed by the Montpellier/Nimes group, implicates the temperature-sensitive Transient Receptor Potential Vanilloid (TRPV) cation channels, whose work focused on TRPV4 channels in primary sensory neurons [2]. A more general electrostatic mechanism was later suggested, where infrared exposure depolarizes the lipid membrane bilayer through a direct physical change in the membrane capacitance induced by the induced temperature gradient [3,4]. Intracellular effects have also been demonstrated, where an increase of intracellular Ca^{2+} ion concentration was reported in multiple targets. They were shown to originate from mitochondrial stores in cardiac cells or from the endoplasmic reticulum in our research, via activation of the phospholipase C and the inositol trisphosphate (IP3) signaling pathway in glioblastoma cells [5], later also confirmed in chinese hamster ovary cells, neuroblastoma-glioma cells [6], and in cultured spiral ganglion neurons [7].

Even though the underlying mechanism of infrared-induced calcium signaling is not yet fully elucidated, this method can nevertheless be applied in more complex biological models and cancer research. Here I will present and discuss our findings on the impact of infrared exposure on calcium signals in human glioblastoma cell spheroids transfected with the genetically encoded indicator GCaMP6f. The possibility of using this direct interaction between infrared radiation and biological signaling for other applications is an exciting new direction that may open new potential therapeutic avenues for cancer.

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2A / 4

Quiescence status of glioblastoma stem-like cells involves remodelling of Ca2+ signalling and mitochondrial shape

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Glioblastoma multiforme (GBM) are the most aggressive primary brain tumors with a survival period rarely exceeding 2 years after initial diagnosis. GBMs display significant heterogeneity within the tumor mass, among which a small sub-population of tumor cells with stem-like properties (GSLCs) is responsible for tumor growth, resistance to therapies and tumor recurrence. Within the tumor mass GSLCs localized to hypoxic and acidic microenvironments have been found to be in a quiescent state and to represent the most aggressive form of GSCs. Although cellular quiescence is one option for cancer stem-like cells to evade killing, the functional characterization of quiescent GSCs remain poorly understood. Through RNAseq analysis we identified Ca2+ signalling genes differentially expressed between proliferating and quiescent GSLCs. Using the bioluminescent Ca2+ reporter EGFP-aequorin we observed that the changes in Ca2+ homeostasis occurring during the switch from proliferation to quiescence is controlled through store-operated channels (SOC). We showed that this switch is characterized by an increased capacity of GSLCs' mitochondria to capture Ca2+ and by a dramatic and reversible change of mitochondrial morphology from a tubular to a donut shape. Our data suggest that the remodeling of the Ca2+ homeostasis and the reshaping of mitochondria during the transition proliferation to quiescence constitute a protective mechanism that favors cancer stem-like cells survival and its aggressiveness in GBM.
Funding: This work was supported in France by the Centre National de la Recherche Scientifique (CNRS), Université Toulouse 3, Université de Strasbourg and by a joint grant from the Agence Nationale de la Recherche (ANR) given between France and Hong Kong to CL, JH and MM (CalciumGlioStem ANR-13-ISV1-0004)

Session 2B – Translational research, from biology to the clinic : Flash Posters

2B / 1

Platinum-based chemotherapy induce inflammation via a non-canonical STING pathway dependent on ATM activation

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[5] CHU de Nîmes

Upper Tract Urothelial Cancers (UTUC) are aggressive tumours of ureter or renal pelvis. They are treated as bladder cancer with more than 50% of relapse justifying the need of new therapeutic options. For this purpose, we study therapeutic combinations to stimulate the immune system by platinum-based chemotherapy in order to potentiate the effect of an anti-PD-L1 (Durvalumab). Indeed, UTUC usually present low tumour immune infiltrate that may limit their response to immunotherapy. The aim is to compare the effects of three combinations (cisplatin + gemcitabine, carboplatin + gemcitabine and oxaliplatin + gemcitabine) and to determine the best inducer of tumoral immunogenicity. Using UTUC cell line(UM-UC-14) we have evaluated the cytotoxicity effects of the chemotherapies combinations in 2D and 3D cell cultures. We have assessed their potential (i) to induce DNA damage using image cytometry, (ii) to induce PD-L1 expression using flow cytometry, (iii) to activate the cGAS/STING pathway using qPCR, and finally (iv) to stimulate the anti-tumor immunity by in vitro and in vivo experiments.

Our results demonstrate that all three chemotherapies combinations present synergistic effects in UM-UC-14 (UTUC cell line) spheroid cultures. These treatments also induce DNA damage pathway activation in UM-UC-14 cells demonstrated by an increase of γH2AX, phospho-ATM, phospho-CHK1 and phospho-CHK2 positive cells. We found an increase of PD-L1 membrane expression after treatment in UTUC cell line. Moreover, RNA Seq analyses indicates that the major pathways induced by these combinations are the inflammatory pathways. We could observe an immune cell death induction demonstrated by an increase of ATP and HMGBA release and calreticuline exposure. We showed cGAS/STING pathway activation as evidenced by an increase of P-IRF3 protein level and interferon stimulated genes (ISGs) expression. We demonstrated an inhibition of the ISGs induction after treatment by our chemotherapies when cells are treated with an ATM inhibitor or in UMUC-14 deleted for STING.

These results indicate that the combination of platinum salts + gemcitabine induces inflammation via a non-canonical STING pathway dependent on ATM activation in UMUC-14.

2B / 2

Proteolytic protein repression mediates tumor T cells infiltration and anti-tumoral immune response: Drug-repurposing approach

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Immune checkpoints, such as programmed death (PD-1), play important roles in regulating T cell responses. They were proven to be effective targets in treating various cancers; however, prolonged stimulation of T cells due to chronic infections or cancer results in gradual suppression of the cell's effector function. The discovery that inhibitory receptors serve as an immune checkpoint, which regulates T cell effector function, was rapidly exploited for the treatment of various solid and hematologic cancer. Although therapies targeting PD-1 were clinically effective in various preclinical models and cancer patients, several patients with solid tumors are refractory to these treatments. Solid tumors evade anti-cancer immune control by establishing immune-privileged niches within the tumor microenvironment that reduce proliferation, viability, and/or activity of cytotoxic T lymphocytes (CTL). Interestingly, a wide range of proteins involved in these pathways require proteolytic cleavage of their protein precursors by furin to be biologically active.

Using computer-aided virtual screening and repurposing approved drugs against furin, we generated a collection of molecules acting in different therapeutic areas that we tested in vitro and in vivo. We found that 14 drugs were able to inhibit the activity of the furin in vitro using enzymatic digestion assay. In cells they were able to repress the cleavage of the known furin substrate PDGF-A. Of these molecules, two namely I0 and I13 induced a potent repression of PD-1 expression in T cells activated by PMA/Io or CD3. Subcutaneous inoculation of mice with syngeneic cancer cells revealed the anti-tumoral efficacy of these two drugs that associated increased intratumoral T cells infiltration in the developed tumors. In addition, the treated mice showed improved overall survival while compared to controls.

These and other findings highlight the potential use of drug repositioning process for the identification of safe furin inhibitor able to repress PD-1 expression in T cells and FDA-approved drugs as a novel immunotherapeutic approach to inhibit tumor progression.

2B / 3

"Epitranscriptomics": a promising source of biomarkers for personalized medicine

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Diffuse gliomas are among the most common tumors of the central nervous system, with a high morbidity and mortality and very limited therapeutic possibilities. They are characterized by a variability in the histological and molecular features, the ability to transform into a higher grade and/or to disseminate, and response to treatment. Most particularly, grade II (low-grade) and III (high grade) gliomas cannot be easily distinguished, as intratumor heterogeneity of the tumor grade is not rare in patients treated with extensive surgical resection.
Identification of accurate biomarkers, through molecular profiling in healthy and cancer patient samples, could improve diagnosis and promote personalized medicine. While genetic and epigenetic alterations of DNA are currently exploited as cancer biomarkers, their robustness is limited by tumor heterogeneity. Further, defining a set of biomarkers instead of one would maximize the prediction performance. Recently, cancer-associated alteration of RNA marks has emerged as a promising source of diagnostic and prognostic biomarkers.
RNA epigenetics (a.k.a "epitranscriptomics") is an emerging field that encompasses more than 150 chemical modifications in all types of RNA. These modifications fine-tunes gene expression and play a role in key cellular processes in both physiological and pathological contexts. Therefore, it comes at no surprise that a growing number of studies have connected variations of specific modified nucleoside levels in solid/liquid biopsies with cancer onset and progression.

Others and we have associated several chemical marks with cancer evolution, adaptation as well as response to conventional therapy. Building on these observations, epitranscriptomics landscape may evolve along with cancer progression and grading. Our goal is to exploit multiplex targeted mass spectrometry in order to establish "epitranscriptomics signatures" that could be used for diagnosis and/or prognosis.

Total RNA was extracted from a cohort including 59 RNA samples from tumor biopsies (glioma / glioblastoma patients at different stages of the disease (grades II, III, IV)) as well as 19 "control samples" using TRIzol reagent (Invitrogen). RNA was digested into nucleotides and dephosphorylated into nucleosides. The nucleosides were quantified by using a LCMS-8060 mass spectrometry in MRM mode.

Among the 35 RNA modifications implemented in our LC-MS/MS method, we successfully detected 25 modifications. We designed an experimental pipeline dedicated to feed a bioinformatics process with both experimental and clinical data. MS data was merged with that containing the grade information. Then we applied statistical analysis methods to (1) assess the variability of any chosen nucleoside quantity with the tumor grade (0 for controls; II, III or IV for glioma patients according to WHO classification) and (2) investigate whether the variation of nucleoside quantities among samples reflect the distinct tumor grades, for instance using Principal Component Analysis (PCA). We could distinguish three categories of chemical marks -decreased, increased and unchanged. Remarkably, three-dimensional PCA was capable of separating all three grades of glioma, most particularly grade II from grade III. Finally, a machine learning based approach (Support Vector Machine) demonstrated that epitranscriptomics-based grading prediction is accurate over 90%.
In the coming years, cancer studies in the blossoming field of epitranscriptomics will definitively translate into opportunities for clinical applications. Easy to perform, fast, cost-effective, sensitive and reproducible methods are needed to evaluate the power of these potential biomarkers. RNA modification profiling by mass spectrometry could become a powerful tool for identifying biomarkers signatures in cancer which, coupled with machine-learning approaches, may help disease diagnosis as well as clinical decision-making.

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Colorectal cancer stem cells respond differently to chemotherapies depending on their original location

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Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths worldwide. Treatment options for CRC include surgical resection, chemotherapy, targeted therapy and immunotherapy. Unfortunately, even after well-directed curative treatment, some patients experience treatment failure and relapse due to multi-drug resistance, which can be attributed to cancer stem cells (CSCs). With their self-renewal and multi-lineage differentiation capabilities, CSCs play a key role in tumor initiation, therapeutic resistance and metastasis development. However, CSCs represent less than 5% of the tumor mass, which is a challenge for their isolation. The sedimentation field-flow fractionation (SdFFF) technique allows the sorting of homogeneous populations of poorly differentiated or undifferentiated cells according to biophysical characteristics.

The objectives of this research project are: (1) to isolate CSCs from cell lines and primary cultures, representative of different stages of CRC using SdFFF, (2) to characterize phenotypically and functionally the sorted fractions (CSC-enriched vs differentiated), and (3) to analyze the response to chemotherapy of these fractions in 2D and 3D models.

Our phenotypic and functional characterization results confirm the relevance of SdFFF to isolate CSC-enriched fractions. Furthermore, our preliminary results have demonstrated a difference in chemotherapy sensitivity between CSC-enriched and control fractions, as well as between cell lines derived from primary tumors and those derived from CRC metastases, in 2D. Chemosensitivity tests are underway in 3D models and the regulatory pathways that may be associated with chemotherapy resistance will be analyzed. The ultimate goal of this project is to study the therapeutic response of CSC-enriched fractions from CRC patient samples in order to predict treatment efficacy.

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Programming lactic acid bacteria for colorectal cancer therapy

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In the recent years, bacteria have been genetically engineered to detect and treat several pathologies in vivo, including infections, metabolic disorders and inflammatory bowel diseases. Recently, numerous studies have been conducted to modify bacteria to treat cancer. The strategy of anti-cancer bacteria consists in genetically modifying bacteria in order to make them recognize, colonize, and proliferate in the tumor microenvironment and finally produce in situ therapeutic molecules in a controlled manner. A potential advantage of using bacteria as cargo is to counter the side effects of chemotherapy and immunotherapy treatments, which are still generally delivered systemically.

Our project is to engineer *Lactobacillus gasseri* as a cargo to treat cold solid tumors using colorectal cancer as model. As precision engineering of LAB is currently limited by the lack of tools enabling reliable control of gene expression, a part of this project aims at building a collection of well-characterized genetic parts to control transcription, translation and secretion levels. In parallel, we are optimizing the production of cytotoxic (*Cytolysin A, Azurin*) and immunomodulatory proteins (*VHH- aPDL1, VHH VEGF*, interleukins,...) in *Lactobacillus gasseri*. Ultimately, bacterial therapeutic activity will be controlled by sensors responding to signals from the tumor microenvironment. In order to test, improve and validate our recombinant strains, we are combining in vitro spheroid-based screening with animal models.

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Repression of liver colorectal metastasis by the naturally occurring inhibitor of Furin (ppFurin)

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At present, surgical resection is the only curative option for liver colorectal metastasis, and it produces roughly between 20% 5-year disease-free survival rate. Indeed, although the liver represents a common site of spread from many tumor types isolated hepatic metastases most commonly occur from colorectal tumors. Thereby, a better understanding of the cellular and molecular biology of colon cancer and its hepatic metastases will facilitate the development of new effective prognostic and/or therapeutic strategies that may be used alone or with conventional treatments.

To date, the proteolytic processing and activation of various precursor proteins involved in colon cancer are known to be mediated by the convertase Furin and its targeting by gene therapy are suggested in ongoing clinical trials. In this study, we evaluated the repression of the malignant and metastatic phenotype of cancer cells by the prodomain of Furin (or ppFurin), a natural inhibitor of this convertase. The overexpression of ppFurin in cancer cells considerably reduces the furin enzymatic activity and furin ability to activate substrates involved in cancer such as PDGF-A and IGF-1R. Inhibition of the cleavage of these substrates affects their signaling pathways, as well as invasion of tumor cells while increasing their sensitization to apoptotic agents. In mice, intrasplenic inoculation of colon cancer cells induces the formation of hepatic metastases and this effect is repressed by ppFurin. Similarly, the use of synthetic ppFurin of 83 amino-acid mediated the inhibition of tumor cells proliferation and migration/invasion. In parallel, the analysis of Furin expression in colorectal and/or liver metastasis patients revealed high expression of this convertase in colon tumors, and this expression is further upregulated in their corresponding liver metastasis. Taken together, these findings demonstrate that ppFurin may constitute a potential strategy for the prevention of liver colorectal metastases.

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Modelling 3D tumour microenvironment in vivo using live imaging technique : a tool to predict cancer fate

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The tumour microenvironment (TME) characterisation has become over the years a major topic in the understanding of tissue tumorigenesis and novel treatments development. The tumour niche is a very dynamic region where different cell types coexist and interact with cancer cells, conditioning their fate. Among them, vessels play an important role as a pathway for cancer dissemination and oxygen and nutrient supply. Similarly, the recruitment and infiltration of immune cells in the tumour mass, such as macrophages, have been described as having a direct impact on cancer progression. Moreover, the composition of the tumour niche is nowadays used as a mean of diagnosis and indicator of good or bad prognosis. Therefore, the 3D dynamic visualization of the TME appears as a predictive tool of cancer cell behaviour in terms of intravasation, invasiveness and metastasis. However, to study in detail the molecular and cellular mechanisms of cancer progression, innovative methods based on in vivo models are essential. In recent years, zebrafish embryos have emerged as a relevant tool in oncology. Among many advantages, its transparency allows to image and visualize in real time conserved cellular processes involved in tumour dissemination. We report for the first time a cutting-edge methodology to visualize in 3D live imaging the interaction between TME and cancer cells to study cancer fate. Through a xenograft model of human glioblastoma and melanoma cancer cells in zebrafish embryos, we highlight two major actors of TME, macrophages and vessels. The 3D reconstructions of the tumour niche show a massive macrophage recruitment and infiltration in the tumour mass. Simultaneously, they demonstrate the active role of vasculature in tumour progression through neo-angiogenesis and vessel co-option events. This method can be implemented to study different cancer types and TME players. Finally, with the exponential interest in precision medicine, this method will represent a powerful tool to predict tumour fate in patients and to screen new efficient therapies.

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New Imidazo[1,2-a]quinoxalines compounds for Pancreatic Ductal Adenocarcinoma treatment : mechanism of action and target identification.

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In 2020, the survival rate for patients with Pancreatic Ductal Adenocarcinoma (PDAC) is dramatically poor with a five-year survival rate less than 10%. The research of new treatments, which could complement the current therapeutic arsenal constitued only by non-specific treatments such as gemcitabine or FOLFIRINOX, associated with innovative diagnostic strategies, is a major challenge.

IBMM F16 team's developed new Imidazo[1,2-a]quinoxaline compounds with potent activities against cancer cells. Among them, EAPB02303 exerts nanomolar activities in pancreatic cancer cells. The objective of the IRCM and IBMM collaboration project is to elucidate the precise mechanism of action of EAPB02303 and to assess its anticancer potential in pancreatic cancer.

We characterized EAPB02303 effect on cell viability on a panel of pancreatic cancer cells including Patient Derived Xenograft (PDX) and Cancer Associated Fibroblasts (CAF). Moreover, we looked for synergy with other pancreatic cancer treatments and found a potent synergy with paclitaxel. Now, these results will be confirmed in pancreatic Patient Derived Xenografts models. A pharmacokinetic study will help us to define the best way of injection of EAPB02303.

Furthermore, we found by flow cytometry and immunofluorescence that EAPB02303 induces mitosis arrest and mitotic catastrophe after 24h treatment. We also showed that cells undergo apoptosis after 48h treatment.

We used chemical proteomic methods for target identification of EAPB02303 based on affinity purification using compounds-immobilized beads. This quantitative proteomic technique helped us to identify potential targets of EAPB02303. Now we are analyzing mRNAseq and Reverse Phase Protein Array data of pancreatic cancer cell lines treated with EAPB02303 6 or 24h. These data will allow us to identify signaling pathways and key proteins implicated in EAPB02303 effect. All these potential proteins and pathways will be confirmed by knockdown models and western blot.

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Association of oxaliplatin-based chemotherapy and ATR inhibitor in pancreatic cancer

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Pancreatic Ductal Adenocarcinoma (PDAC) is an extremely aggressive disease with no efficient treatment. Recently, a new polychemotherapy oxaliplatin-based (FOLFIRINOX) has been approved but associated with toxicity and limited efficiency. Most of the drugs induce their toxicity by provoking DNA damages and replication stress leading to the activation of DNA repair pathways. Recently, a PARP inhibitor has been approved by the FDA for patients with BRCA mutated PDAC showing the potential of this type of therapy. Therefore, in this project, we added an ATR inhibitor (ATRi) to FOLFIRINOX to increase responses and analyzed the effect of this combination on the tumor microenvironment.
Viability matrix in 2D & 3D co-culture of tumor cells with primary CAFs were carried out and DNA damage repair pathways, cell death and autophagy were analyzed. In vivo, immunodeficient mice xenografted with ATCC and Patient Derived Xenograft models were treated with FOLFIRINOX and ATRi to evaluate the effect on tumor progression.
A synergistic effect of the combination was demonstrated in pancreatic models in co-culture with CAFs independently of the DDR deficiency. A higher apoptosis and DNA damages were observed in tumor cells treated with the combination associated with a decrease of DNA repair pathways and an inhibition of the autophagy flux. A protective effect of the CAFs on tumor cells was observed and secretome of CAF analysed. In vivo, the combination inhibits significantly the tumor growth compared to each treatment alone.
Now, a validation of this polychemotherapy in vivo using co-culture models in immunodeficient mice is crucial to confirm the therapeutic potential of this new treatment for PDAC.

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Characterization of β-catenin translation factories

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The Wnt / β-catenin pathway plays important roles in embryogenesis and in tissue homeostasis in adults. It is also very important in cancer. Indeed, nearly all colorectal tumors have an alteration of this pathway and about a third have a strong β-catenin signature. β-catenin is known for its dual function as a transcription factor in the nucleus and in adherent junctions at the cell membrane. This protein is tightly regulated in terms of its stability. β-catenin located at adherent junctions is stable whereas the one present in the cytoplasm is rapidly degraded by the proteasome. However, this protein is stabilized in the presence of a Wnt signal, and it can then be transported to the nucleus where it activates transcription. The key factors involved in the degradation of β-catenin are APC, Axin and the kinases CK1a and GSK3b. Altogether, these proteins form the "destruction complex". In the absence of Wnt, this complex binds β-catenin and degrade it. In the presence of Wnt, this complex binds to the Wnt receptor at the plasma membrane and no longer interactions with β-catenin, which is thus stabilized.
We have discovered a new regulatory mechanism in which β-catenin is translated in specialized cytoplasmic foci concentrating β-catenin mRNA, which we have called "translation factories". These foci concentrate the destruction complex and allow a co-translational degradation of β-catenin. They thus play an important role in the control of its expression.

The aim of this project is to study in detail these β-catenin translation factories and to address their possible roles in tumorigenesis. For this, we used quantitative proteomic approaches in order to characterize the biochemical composition of these factories as well as the polysomes translating β-catenin. Using APC as a bait in HEK293 cells, we found that the interaction of APC with β-catenin is translation dependent. This result confirms our hypothesis about the co-translational degradation of β-catenin.

Moreover, we found that APC interacts with many members of the CTHL complex. This complex is known for its E3 ubiquitin ligase function. This suggests a possible role of this complex in β-catenin foci formation and maybe in its degradation.

Since Wnt pathway is related to colorectal cancer, we tried to determine if these foci are also present in colonic cells. Using smFISH essays on mouse tissue sections, we found that these foci are present in wild-type colonic tissue. Moreover, these factories seem to have a specific localization pattern among the colonic crypt. Many are found to be formed in the differentiated epithelial cells, whereas stem cell and proliferative cells may lack them.

This completion of this project will provide detailed knowledge of β-catenin translation foci and it will clarify the role of these foci in normal or cancer cells. In particular, our experiments could determine a new function for the tumor suppressor APC, which would be to organize these translation foci and thus to control the synthesis and the fate of the nascent β-catenin protein.

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The effect of metformin on the survival of colorectal cancer patients with type 2 diabetes mellitus

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Introduction: Colorectal cancer (CRC) is the second most deadly cancer worldwide and the third most diagnosed globally. Evidence from previous studies suggests a protective effect of metformin in patients with CRC. The aim of this study was to examine the associations between metformin use and overall survival (OS) and disease-free survival (DFS) in CRC patients with type 2 diabetes mellitus.

Method: This was a historical cohort study. It included diabetic patients who underwent surgery for CRC at Limoges' University Hospital between 2005 and 2019. Data on the characteristics of patients, CRC, comorbidities, and drug exposure were collected from the patients' electronic medical records. The exposure was the use of metformin. Patients were followed for two years after surgery. The outcomes were overall survival (OS) and disease-free survival (DFS). All cases of CRC recurrence were confirmed by computed tomography (CT) scan or magnetic resonance imaging (MRI) and verified by biopsy. Multivariate analysis using the Cox model was performed to adjust various confounding factors (age, sex, body mass index (BMI), tumor site, cancer stage, Charlson comorbidity index, diabetes complication, the use of hypolipidemic and antihypertensive drugs) and all statistical analyses were done with IBM SPSS Statistics 22.

Results: Of the 1605 patients operated for CRC, 290 patients were identified with type 2 diabetes mellitus. Half of the diabetic patients were treated with metformin (49.7%).

The 2-year OS rate for metformin users was 86.9±2.9% and 71.0±4.0% for metformin non-users (p=0.001). The Cox regression model showed a 64.0% reduction in all-cause mortality (adjusted hazard ratios (aHR), 0.36; 95% confidence interval 95%CI 0.17-0.73) among metformin users compared with non-users. Furthermore, metformin users had better DFS than non-users (aHR, 0.31; 95%CI 0.19-0.52).

Conclusion: The use of metformin may improve OS and DFS in diabetic patients with CRC. Further prospective studies are also recommended to better explore the effect of this drug.

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ISiCell : a web-oriented platform to model cellular processes

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While developing agent-based models with biologists in the past 5 years (1, 2), we came to the conclusion that the design process of these models was not optimal for two main reasons. First, the biologists are unfortunately often kept outside of the design loop of the model because of the technicalities implied by the development of the model (programming language used in particular). It is actually very hard for a nonmathematician or a non-computer scientist to understand the inner mechanisms of a in silico model: to do so, it is necessary to understand the code produced by the modeler. Secondly, each modification of the model implies modification in the source code of the model, potentially meaning hours or days of work. Therefore, the duration necessary to produce the final model is long, taking months to obtain a stable theoretical model.

To overcome these two limitations, we have developed a new platform, named ISiCell, which is aimed at generating on-the-fly all the code necessary to implement an agent-based model. On this platform, the users (biologists and modelers together) are drawing diagrams that are representing the different states of the cells and their transitions from a state to another. For example, states can be "random walk", "differentiated", "in suspension", etc. Transition can be linked to environmental conditions (e.g. "sensing molecules", "contact with other cells", etc.) or internal conditions (e.g. "cell age", "concentration of molecules", etc.). Each state is described by a sequence of actions that the cells will accomplish when in this state. For example, the actions can be "divide", "move", "emit molecules", etc. Standard actions such as the one given in examples are already implemented in the platform and can be reused as is. If an action is not specific enough for a model or does not yet exist in the platform, the modeler can implement this new action in an editor dedicated to code production. This allows a strong versatility of the platform, by rapidly implementing new actions necessary for a specific model.

Once implemented, the code of model is automatically generated and compiled on-the-fly. The model can be visualized via a 2D or 3D graphical interface that displays initial simulations and allows preliminary parameter explorations with the biologists. Statistics can be generated via the interface in order to study in live the biological system represented in the model. They can be (but not limited to): number of cells per state in function of the time, spatial distribution of the cell, average age of the cells, etc. Finally, new experimental protocols can be designed and tested in live in order to evaluate the outcome of this new protocol.

The platform we propose is a web-oriented platform meaning that only a web navigator (such as Chrome or Firefox) is necessary to use it: this will allow any academic on earth with an internet connection to use our platform very simply without any installation required on their machine. Additionally, this could allow in term to improve the platform by allowing users to add new development to the platform and providing access to the community to the newly developed features.

This platform will also be improved over the next few years to, in term, allow the biologists to use it with as minimal as possible knowledge necessary in computer science. We are also planning to develop tools to simplify the calibration process of the developed model. Furthermore, we are exploring the possibility to plug optimization algorithms that would explore experimental protocols automatically to improve existing ones or create them from scratch.

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Computational Models of Heterogeneity in Melanoma: Designing Therapies and Predicting Outcomes

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Cutaneous melanoma is a highly invasive tumor and most patients with advanced melanoma have a poor clinical outcome.

The most frequent mutations in melanoma affect the BRAF oncogene, a protein kinase of the MAPK pathway. Therapies targeting BRAF/MEK are effective for only 50% of the patients and almost systematically generate resistance. Genetic and non-genetic mechanisms of drug resistance associated with the strong heterogeneity of melanoma cells are still poorly understood.

Recently we have introduced a novel mathematical formalism allowing to cope with the relation between tumor heterogeneity and drug resistance and proposed several models for the development of resistance of melanoma to treatment with BRAF/MEK inhibitors. These models predict that order of treatments in combination therapy matters. For instance, when combining dabrafenib and ipilimab therapies, treating with ipilimumab first and with dabrafenib later is the optimal protocol schedule.

In this paper we investigate the validity conditions of this prediction, for a variety of models. We are also studying the potentialities and limitations of adaptive therapy, consisting in maintaining a stable tumor burden containing a pool of sensitive cells by alternating between drug administration and holiday. Finally, we use spatially- and structurally-extended computational models to predict the outcome of different therapeutic strategies in terms of tumor heterogeneity.

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On the dynamics of TAM formation in Chronic Lymphocytic Leukaemia: a multi-scale approach

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Mathematical and network modelling of individual cells and cell populations offer a powerful approach to analyse complex biological systems at a multi-scale level, integrating theoretical and experimental knowledge into a single representation. In tumours, identifying the mechanisms that establish the immune cell - cancer cell interactions is crucial in understanding the system behaviour and dynamics. In this work, we focus on the ecology of the tumour microenvironment, and particularly on the differentiation of monocytes into tumour-associated macrophages (TAMs) in Chronic Lymphocytic Leukaemia (CLL). TAMs are known to play a critical role in the survival of cancer cells. In CLL, they protect the leukemic B cells from spontaneous apoptosis and contribute to their chemo-resistance. Here, we propose an integrated modelling approach of monocyte differentiation into TAMs in in-vitro monocyte-CLL co-culture, including molecular and inter-cellular interactions. Firstly, we apply a Boolean model on a macrophage gene regulatory network to identify the molecular pathways that lead to TAM formation in the presence of tumoural signals. Secondly, we build an agent-based model to explore the cell population spatio-temporal dynamics and identify the key processes controlling the stability of this multi-cellular system, in which cell behaviour is determined both by cell interactions and their internal molecular regulation. With this coupled approach we recapitulate a broad spectrum of macrophages, ranging from pro- to anti-tumoural phenotypes, while highlighting the effect of TAMs protecting role in CLL cell population dynamics.

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Towards a novel framework for large scale RNAseq data analysis in human health

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With its ability to reveal both altered gene expression levels and the production of aberrant transcripts, RNAseq is popular in the field of precision medicine. An increasing number of clinical trials uses this technology in order to discover functionally relevant alterations. Driven by myriads of projects, public RNAseq databases are exploding, to date, there is over 164,000 RNA-seq on SRA for human. This huge body of publicly available RNAseq libraries is a precious resource to identify specific transcriptional events. However, the challenges lie in the complexity of RNA biological content and the exponential increase in data volume. We want to make RNAseq data easily accessible, providing a capture of the whole transcriptome complexity, in the context of human health applications. Therefore, we developed a new framework based on a k-mer approach, constructed with several modules: 1/ a new RNAseq indexing structure that will serve as an efficient platform to request any transcribed information, 2/ a complete module to generate unique k-mers as signature of transcripts, 3/ a supporting web site to facilitate the queries for the biologists.

The indexing step uses Reindeer, a new k-mer based indexation structure. To our knowledge, it's the first method capable of performing fast mapping-free quantification of variant transcripts in thousands of RNAseq libraries [1]. The methodology is already efficiently implemented for several biological applications based on public datasets (from ten to thousand of RNAseq corresponding to 100Go to 10To of data). The k-mer designing module uses Kmerator, a tool developed to extract specific k-mers (https://github.com/Transipedia/kmerator) [2]. Finally, the web application is already available to facilitate large RNAseq datasets queries by the biologists with their sequences of interest as input (fasta format).

Concerning medical applications, we already requested and identified in selected public datasets, genes co-expressions, tissue specific biomarkers, as well as tumor specific signatures comparing normal and tumoral samples. As an example, we recovered known translocations and mutations in RNAseq Acute Myeloid Leukemia (AML) samples and identified new specific biomarkers (long non-coding RNAs…).

With the addition of advanced Machine Learning approaches, our framework could be used to select the best signatures and to improve diagnosis and prognosis models in cancers.

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Bioinformatical analysis of tumor cell with stroma crosstalk that impact aggressiveness of pancreatic ductal adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive cancers due mostly to its high metastatic and chemoresistance features. Hence, 5-year life expectancy is below 9% with a 6-month mean survival after diagnosis. PDAC is characterized by highly invasive pancreatic cancer cells, immerged in an exuberant stroma, which represents up to 80% of the tumor volume. Both tumor and stromal cells have to interact in order to survive in this harsh micro-environment.

Two major molecular (transcriptomic) subtypes of PDAC were clearly described through bio-informatical sequencing analysis of RNA extracted from patient tumor regions enriched with cancer epithelial cells: a dedifferentiated and more aggressive (associated with poor outcome) "basal" subtype, and a "classical" subtype presenting more general differentiation features and a better outcome. Molecular heterogeneity of the PDAC stroma was also studied through bio-informatical deconvolution analyses of bulk RNA sequences (extracted from tumor samples composed of both cancer epithelial and stromal cells); these analyses also stratified PDAC within two prognostic stromal subgroups, which however don't mirror the two identified epithelial (basal / classical) subgroups.

By performing bioinformatical analysis on transcriptomic data (RNAseq) from PDX (Patient Derived Xenograft), a hybrid tumor model from which human-derived tumor cell sequences are distinguishable from murine-derived stromal cell sequences, we searched for different stromal behaviours linked to the aggressiveness of the tumor, to be then validated in published PDAC patient databases.

According to NMF (Non-negative matrix factorization) and GSEA (Gene Set Enrichment) analyses, we identified two components allowing us to classify the samples according to a stromal gradient. Interestingly, these components are characterised by distinct functional signatures of aggressiveness and they are highly prognostic (correlated with survival). In addition, these components stratify patients independently on the described molecular tumor (basal / classical) classification. The results obtained (functional signatures as well as the impact on survival) were then validated on other PDAC published databases. Moreover, ligand-receptor bioinformatics analyses identified novel players of the TGFB superfamily in the most aggressive component.

Further analyses (bioinformatical and experimental) will now enable us to identify specific stromal therapeutic targets to be functionally tested using already available patient-derived cell models, including patient-derived tumor cell organoids and cancer-associated fibroblasts. Therapeutic targeting of the stroma must take into account its functional heterogeneity, and the functional validation of the newly identified players should define a promising axis for targeting the most aggressive PDAC.

2C / 6

Microfluidic Lab-On-Chip for UHF-Dielectrophoresis Discrimination of Glioblastoma Undifferentiated cells

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Glioblastoma (GBM) is one of the most aggressive solid tumors, particularly due to the presence of cancer stem cells (CSCs). Today the characterization of this type of cells with an efficient, fast and low-cost method remains an issue. Hence, we have developed a microfluidic lab-on-a-chip based on dielectrophoresis (DEP) single cell electro-manipulation to measure the two crossover frequencies: fx01 in low frequency range (below 500 kHz) and fx02 in Ultra High Frequency (UHF) range (above 50 MHz). Experiments were performed on ex vivo GBM cells from patients' primary cell culture in order to reflect clinical conditions. We demonstrate that the usual exploitation of low frequency range DEP does not allow the discrimination of the undifferentiated from the differentiated phenotypes of GBM cells. However, the presented study highlights the use of UHF-DEP as a very promising tool with the great potential to discriminate cell according to their internal biological properties. Our microfluidic system allows the identification and the discrimination of aggressive and resistant cells from a tumor. In the future, the early detection of CSC subpopulation in glioma tumor with UHF-DEP approach could have a prognosis value on therapeutic response and might allow to adapt therapeutic strategy following diagnosis.

2C / 7

Nanotherapy of pancreatic adenocarcinoma by targeted magnetic hyperthermia: efficacy and mechanisms

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Magnetic nanoparticles (MNPs) are already widely used in nanomedicine, notably as MRI contrast agents or in magnetic hyperthermia therapy. A clinical trial was conducted to treat high-grade brain tumors in 2011, and a clinical trial is currently being conducted in the United States on prostate cancer, in combination with radiotherapy. However, the benefit on life expectancy remains minimal and neither radiotherapy nor magnetic hyperthermia can distinguish between normal and cancerous tissues, leading to adverse effects.

Our strategy is based on the design of iron oxide MNPs capable of specifically recognizing target cells and therefore specifically treating cancerous tissue with targeted magnetic hyperthermia via the application of an external high frequency alternating magnetic field (AMF), while minimizing damage to healthy tissue. This targeted magnetic hyperthermia consists of specifically delivering MNPs into the lysosomes of target cells. The AMF exposure will then specifically eradicate these cells, without macroscopic temperature elevation. We have chosen as a model pancreatic adenocarcinoma, particularly resistant to conventional therapies and characterized by the presence of a large and dense microenvironment with a key role of CAFs (Cancer-Associated Fibroblast) cells secreting extracellular matrix proteins (collagen) that limits the penetration and efficacy of treatments (chemotherapy and radiotherapy). Cancer cells as well as pancreatic CAFs can overexpress the CCK2 receptor (membrane receptor that internalizes after binding of its specific agonist: gastrin).

We have developed MNPs with high thermal power, termed "NanoFlowers" (NF), decorated with fluorophore and gastrin molecules allowing respectively their detection and targeting. These MNPs (NF@Gastrin) specifically target cancer cells (MiaPaca2-CCK2) and CAFs (CAF-CCK2) expressing the CCK2 receptor, internalize and accumulate in their lysosomes. The AMF application (275 kHz, 30 mT) kills up to 45% of cancer cells and CAFs that have internalized NF@Gastrin, slows down their proliferation, and sensitizing them to Gemcitabine (a chemotherapy used for the treatment of pancreatic adenocarcinoma), without affecting cells lacking the nanoparticles. In parallel, we are studying the mechanisms that cause cell death. The hypothesis is that the MNPs temperature rise induced by AMF may trigger the release of ferric ions (Fe3+) from the MNPs, which catalyze the production of ROS (reactive oxygen species) in the lysosomes; then, ROS peroxide the proteins and lipids of the lysosomal membrane, induce the lysosome permeabilization leading to lysosomal cell death.

Our results confirm this hypothesis since NF@Gastrin generate ROS, induce the lysosome permeabilization and cell death after AMF exposure, while NF-SiO2@Gastrin covered with a silica shell preventing Fe3+ ion release do not induce cell death. We are now studying the impact of the targeted magnetic hyperthermia on cell migration, as well as on the expression of Damage-Associated Molecular Pattern (DAMP) proteins able to induce an anti-tumor immune response. We will then validate the efficacy of this new therapeutic strategy in a pre-clinical study on mouse models of pancreatic adenocarcinoma.

Session 3 – Myeloid cells in cancer

3 / 1
Therapeutic targeting of myelopoiesis in cancer

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Tumors promote immunological stresses that induce alterations in myelopoietic output, a process termed emergency myelopoiesis that leads to the generation of diverse myeloid populations endowed with tumor-promoting activity. New evidence indicates that the acquisition of this tumor-promoting phenotype by myeloid cells is the result of a multistep process, which includes initial events originating in the bone marrow and subsequent phases operating in the tumor microenvironment. Further understanding of these maturational steps in myeloid cell expansion might offer new potential therapeutic opportunities. I will discuss the prognostic and therapeutic significance of new mechanisms and molecules in myeloid cell evolution, as well as their role in cancer-associated immune dysfunctions.

3 / 2
Integrin signaling in tumor-associated macrophages

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Macrophages are massively recruited in the vast majority of solid tumors. Their abundance correlates with a poor prognosis. They enhance tumor progression and interfere with the efficacy of various anticancer therapies. Not surprisingly, therapies that aim to either deplete or reprogram macrophages merit considerable attention. However, caution has to be taken when modulating the immune responses as not to interfere with essential macrophage functions.

Integrin adhesion receptors play essential roles in leukocyte trafficking, activation and function to shape a successful immunity. Hence integrins are attractive targets for recalibrating immune responses within local microenvironments. They are proven therapeutic targets in many diseases but direct targeting of their extracellular domain, to interfere with their function, often leads to adverse effects limiting the use of integrin antagonists. Instead, we intend to target intracellular signaling events that orchestrate integrin activities in immune cells. In this regard we investigate the diversity and specificity of these signaling pathways in macrophages and investigate their potential to selectively manipulate specific macrophage subsets for therapeutic purposes in cancer disease. Our studies endeavor to devise novel and unanticipated avenues for blockade of macrophage migration and tumor infiltration to synergize with combination therapies that simultaneously target both protumor stromal cells and cancer cells.

3 / 3

Lessons from the remote alteration of granulopoiesis in NSCLC: what is neutrophil homeostasis telling us on immune checkpoint blockade ?

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Immune checkpoint inhibitors (ICIs) represent the latest revolution in the care of patients with cancer, providing long-term survival benefit in a subset (25 to 40%) of patients with melanoma, lung, colorectal carrying microsatellite instability (CRC-MSIhi) and bladder cancer. This implies that a better identification of predictive markers and the characterization of innate and adaptive resistance mechanisms became an important challenge to further improve the clinical advantage of ICIs. Our recent work together with findings from others highlighted the critical involvement of tumor-associated neutrophils (TANs) in resistance to ICIs in lung cancer. Especially, we observed that in lung adenocarcinoma, a massive TAN infiltration is associated with tumor immune exclusion, a key feature of resistance to ICIs and showed, in a mouse model of lung cancer, that neutrophil depletion remodels the tumor microenvironment (TME) in favor of a more efficient response upon anti-PD1 treatment.

TAN and circulating neutrophil diversity was demonstrated by single-cell transcriptomic analysis in samples from patients with lung cancer and from KrasLSLG12D/WT; p53fl/fl (KP) mice with lung cancer. Furthermore, the accumulation of the sub-population of tumor-promoting TANs characterized by their expression of SiglecF (but not total TAN infiltration) in mouse lung tumors requires a remote modification of osteoblasts behavior leading to osteopetrosis. Today an increasing number of publications points to a remote programing of neutropoiesis associated with cancer development, governing the emergence of tumor promoting TAN. Hence, TAN functional heterogeneity in each patient and ultimately TAN related resistance to ICI should be predictable by analyzing circulating neutrophil diversity and quantity.

Because identifying predictive markers of the response became critical in pathologies that receive first line ICI, we have initiated a study on a cohort of NSCLC patients (ALCINA2-03) aiming at performing a qualitative and quantitative characterisation of circulating immune cells and granulopoiesis alteration from peripheral blood samples at diagnostic.

Scientific rational and preliminary results from this ALCINA2-03 ancillary clinical study will be presented.

3 / 4
The tumor-promoting myeloid landscape in breast cancers

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Immunotherapies such as immune checkpoints inhibitors have represented a major therapeutic advance for many cancers, often with a very poor prognosis. However, the majority of breast cancer patients have yet to experience these therapeutic breakthroughs, with the exception of triple-negative breast cancers patients. Luminal or hormono-dependent breast cancers are associated with few tumour-infiltrating lymphocytes and respond poorly to immunotherapies, which has led to describe them as "low-immunogenicity" tumors or "cold" tumors. This view has been questioned by many studies demonstrating that not only breast cancers rarely fall in the "immune depleted" immunological classifications of tumors, but also that the breast cancer microenvironment is strongly infiltrated with myeloid cells such as tumor-associated macrophages, neutrophils, "myeloid-derived suppressor cells" (MDSCs) among others. Myeloid cells have been widely described for their immunosuppressive properties and their ability to inhibit anti-tumor immune response, and thus represent major obstacles for efficient immunotherapies. In breast cancers, two main challenges related to myeloid cells stand out. First, many phenotypes used to describe these cells are overlapping, as illustrated by the difficulty of distinguishing "granulocytic-MDSCs" from neutrophils in cancer patients. A better identification and characterization of myeloid subsets is thus much needed. We discuss existing data on myeloid cell phenotypes, and the recent advances made by single-cell RNAseq or mass cytometry approaches in the characterization of these cells in breast cancer. The second challenge is to more clearly decipher the many tumor-promoting, "non-immunological properties" of myeloid cells, including their ability to promote cancer growth, invasion and metastasis. In this context, the role of myeloid cells in the induction of a stemness phenotype in cancer cells is currently emerging. Cancer stem cells represent a subpopulation within tumors endowed with self-renewal capacity and asymmetrical division, that have drawn attention these last years for their resistance to treatments and their ability to form metastasis. We present data indicating that human monocyte-derived suppressor cells are endowed with the capability to promote the formation of tumorspheres in a 3-D coculture milieu, and that they could confer cancer cells chemoresistance properties.

Session 4 – Preneoplasia & early dissemination

4 / 1

Impact of cellular senescence in lung precancerous lesions: senotherapeutic opportunities

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Lung cancer is the world-leading cause of cancer-related deaths. One opportunity to prevent the development of advanced, incurable non-small cell lung cancer (NSCLC) is to abrogate the progression of precancerous lesions to more malignant phenotypes. Importantly, a subset of patients is diagnosed with multifocal premalignant lesions, and this population will grow with increases in lung cancer screening programmes. Most of these patients have a chronic smoking history and accumulate multiple lesions at different precancerous stages, and require close monitoring to prevent progression to adenocarcinomas. Unfortunately, surgery is usually impracticable due to the high numbers of accumulated lesions and currently there are no available pharmacological interventions.

Senescence is a cell autonomous response to damage and oncogenic stress featured by stable proliferative arrest and intense pro-inflammatory paracrine secretion, termed SASP, affecting nearby tissue. In neoplasia, senescence can be a tumour suppression mechanismn and it is a defining feature of a wide variety of premalignant lesions, both in humans an in mice, including lung adenomas. However, when senescent cells are not cleared by the immune system and accumulate in tissues they can drive a variety of tumour-promoting effects by both cell autonomous and cell non-autonomous activities. Here, we have used a senotherapy, based on a pharmacologically-active compound preferentially removing senescent cells by Bcl-2 inhibition, as a novel treatment modality to target precancerous lesions. We employed a Kras-driven lung cancer murine model that closely mirrors NSCLC in humans, exhibiting multifocal premalignant lesions at early post-tumour induction stages and similar gene expression profiles and phenotypes. We show that senolytic treatment prevents malignant transformation and reduces the tumour burden in mice, as assessed by CT scans, and gain insights into the crucial cellular types undergoing senescence in the lung cancer niche. Importantly, senolytic management significantly increases mouse survival. These results were validated in parallel by the development of a novel mouse model that allows the visualisation, identification, isolation and pharmacogenetic ablation of p16-positive senescent cells. Our results show that senescent cells that avoid immunosurveillance and persist in oncogenic lesions display potent tumour-promoting effects. Altogether, this work offers an innovative intervention to hamper progression of lung premalignant lesions to adenocarcinomas, and hence might result in a translational opportunity to prevent cancer. Additionally, our results emphasize the importance of detecting and/or identifying senescent cells as key players of the early tumour microenvironment.

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Role of extrinsic factors in cancer initiation: importance of the stressed niche in pancreatic cancer

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Pancreatic cancer is projected to become the third leading cause of cancer-related death by 2030. Understanding its initiation is critical to prevent the observed recent rise in pancreatic incidence and to develop interception strategies. While genetic alterations at the origin of pancreatic cancer development from exocrine cells are well known, the importance of stressed tissue microenvironment is less understood. Indeed, tissue microenvironment appears as an emerging factor regulating cancer-initiating cells by various mechanisms.

We previously showed that exocrine pancreatic acinar cells promote proliferation of exocrine duct-like cells, key iniating cancer cells, when cultured in 3D (PMID: 32131058). Identifying key proteins that are involved in acinar cell homeostasis is important. In this process, we found that inactivation in adult murine acinar cells of a central and conserved enzyme, that promotes autophagy and vesicular trafficking, called Vps34 led to progressive disappearance of acinar cells in profit of a phenotype mimicking steato-pancreatitis, associated with long-term alteration of mitochondrial metabolism in remaining acinar cells. We identified that the various sub-populations of acinar cells were differently impacted by Vps34-inactivation, suggesting that there is a spatial heterogeneity in the response to stress. Finally, the inactivation of Vps34 in the genetic context of Kras mutation accelerated pancreatic cancer formation and dissemination to distant organs.

Pancreatitis, pancreatic inflammation, is a recognized risk factor for pancreatic cancer; the molecular mechanisms associated with pancreatic steatosis and their impact on pancreatic cancerogenesis are less understood. This knowledge is necessary to explain the recent and massive rise in pancreatic incidence as well as implement relevant prevention strategies in France and in Europe. This project involves a multidisplinary consortium with complementary skills from different fields (fundamental cancerology, anatomopathology, bioprinting and toxicology).

I will mostly present work from Fernanda Ramos-Delgado (funded by Europe ITN PhD), Benoit Thibault (funded by ANR Radiance) and Camille Guyon (funded by Hopitaux de Toulouse, Fondation Toulouse Cancer Santé) from my lab. On this project, we collaborate with C Joffre, M Dufresne (CRCT), Biotis Bioimpression Art platform in Bordeaux (H de Oliveira) and E Boutet-Robinet (Toxalim).

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Unravelling the role of early dissemination in colorectal cancer

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Introduction:
Over 8 million people die from cancer every year almost invariably due to metastasis but despite more than 100 years of research, tumor dissemination remains poorly described.
In the classical model of metastasis, tumor cell dissemination occurs at late stages of tumor development. However, some studies performed in different types of cancer such as in melanoma, breast and pancreatic cancers; suggest that rare tumor cells spread to distant sites much earlier than previously believed. Despite colorectal cancer's CRC high incidence and mortality, worldwide, early tumour dissemination has not yet been studied in this cancer.

Our aims were first to demonstrate that early tumour dissemination process occurs in CRC using a genetically engineered mouse model, to assess the roleof early disseminated cells eDTC in distant organs as well as to validate data on patient blood samples.

Material and methods:
We have generated an inducible mouse model that enables us to lineage trace dissemination at the very early stages of tumoral development thanks to the expression of tdTomato and deletion of APC gene specifically in the intestinal epithelium. eDTCs were searched in the liver, the main organ that is prone to get colonized by metastatic CRC cells, using tissue clearing, intravital live imaging, and immunolabelling. The impact of eDTCs in the liver was assessed using CyTOF/Hyperion and validated with immunostainings and confirmed with in vivo using adapted assays.

Results and discussion:
In the liver of these mice, we first demonstrate the presence of eDTCs along with a strong infiltration of macrophages and cancer-associated fibroblast CAFs suggesting a microenvironmental remodelling. Concomitant with the theory of seed and soil, this liver remodelling has a strong impact on metastatic colonization where we have demonstrated that it enhances the welcoming of future waves of metastatic cells harboring powerful mutations.

Conclusion:
In conclusion, this project functionally validates for the first time the existence of an early dissemination process in CRC in mice and proposes a causal role of these cells in an early pre-metastatic niche preparation. Further studies to deeply characterise the liver remodelling are ongoing in order to identify potential actionable targets to prevent this early pre-metastatic niche preparation.

4 / 4

Epithelio-stromal relationships in physiology & IBD: osteopontin a key factor for epithelial regeneration and tumor initiation ?

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Chronic inflammatory bowel disease (IBD) is characterized by inflammation of the inner lining of the digestive tract: the mucosa. This pro-inflammatory environment is responsible for tissue damage that affects both the underlying stromal compartment and the epithelium (lack of renewal, differentiation and impaired permeability). Under physiological conditions, the maintenance of homeostasis and epithelial renewal are provided by complex mechanisms that control the phenotype and capacities of intestinal stem cells (ICS). Stromal cells, and in particular fibroblasts, actively participate in the establishment of a specific microenvironment (namely, the "niche") essential for this control. Our knowledge on the impact of fibroblast remodeling on the phenotypic control of altered epithelial cells in IBD patients remains limited. Thus, we studied the impact of the microenvironment changes induced by inflammatory fibroblasts on the epithelial compartment.

After establishing human fibroblasts primary cultures from either normal (NAF) or inflammatory (IAF) colon areas of respectively healthy or IBD patients, we characterized their morphologies and the activation markers expressions by a high content screening (HCS) approach. We confirmed that IAF keep their activated phenotype compared to NAF in vitro according their physio(patho)logy origin. Then, we characterized the cytokines and growth factors secretion patterns of these two populations. We established distinct secretory signatures, and identified a factor specifically secreted by IAFs: the osteopontin (OPN).

OPN is a pro-inflammatory cytokine promoting cell stemness and proliferation, reported to modulate the colitis severity in mouse*via* its interactions with the immune system. Nevertheless, its direct impact on the regenerative of normal epithelium has never been studied.

The OPN effects on the epithelial compartment were investigated by treating colic organoids established from normal mucosa (NORG) with different doses of recombinant OPN. Established from adult colorectal stem cells seeded in a 3D Matrigel© matrix, this organoid model represents a gold-standard to study the stem cell capacities to generate a functional epithelium composed of different epithelial subpopulations. Based on HCS morphological analysis, we found that OPN increases NORG area and promotes immature structures enrichment, suggesting a direct effect on immature epithelial cells (*i.e* ISC and/or progenitors). To decipher which cell population(s) is targeted by OPN, we first tested the aldehyde dehydrogenase (ALDH) activity in NORG after 15 days culture. Although ALDH activity was not significantly increased by OPN treatment, a transcriptomic analysis confirms that OPN upregulate the immatures markers expression while repressing differentiation-associated markers. NOTCH pathways were found upregulated suggesting that OPN promote progenitor population enrichment. Interestingly, EMT markers and OPN receptors were overexpressed. This gene signature expression on normal epithelium stimulated by OPN suggests some tumor initiating capacities. Further investigations are in progress to decipher whether OPN promotes normal epithelial cells phenotype switch into tumor-initiating cells.

To conclude, we identified OPN as a specific overexpressed factor by IAF in IBD fibroblasts, and its putative role in the phenotype shift from normal progenitor cells to tumor-initiating cells.

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Metabolic and immune features as predictive biomarkers of risk stratification of skin carcinoma

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Cutaneous squamous cell carcinomas (cSCCs) are one of the most frequent malignancies worldwide, with substantial associated morbidity and cost. A better understanding of the molecular changes involved in the transformation of this UVB-induced carcinoma, from precancerous lesions (actinic keratosis (AK)) to localized tumors and then to metastasis will aid in early detection, development of biomarkers and future targeted treatment strategies. Here we aimed to characterize the molecular and metabolic features of cSCC at different stages of carcinogenesis and their immunologic landscapes in order to uncover clinically relevant biomarkers predicting the cSCC evolution risk.

Concerning metabolic features, we first characterized the large-scale changes in the bioenergetic machinery of human skin at different stages of carcinogenesis using a quantitative label-free differential proteomic analysis of human skin hyperplasia (initial phase), actinic keratosis (AK, promotion phase), peritumoral skin, cSCCs (progression stage). We found that the changes in the topology of the metabolic pathways involved in the energy metabolism were very similar between hyperplasic, AK, and peritumoral tissue. Interestingly, the metabolic profile of tumors was significantly different from that of pre-cancerous lesions. Of note, there was a dramatic downregulation of the enzymes involved in lipid biosynthesis and a substantial upregulation of several enzymes involved in glycolysis. These results reveal that specific metabolic modifications, of which some persist throughout tumor development, occur at a very early stage of skin carcinogenesis. We then perform xenografts of cSCC and noted different tumor behavior with aggressiveness and metastasis.

To explore immunologic landscapes of cSCC at different stages of carcinogenesis, single cell RNA sequencing (scRNAseq) was applied on a premalignant and an aggressive sample of cSCC form the same patient. We uncovered several immune cell subsets depleted in cSCC compared to AK, particularly B cells and plasma cells, and a specific cluster of macrophages and T cells enriched in the tumor compared to AK. To conclude, our promising data suggest that metabolic and immune features of cSCC have a crucial role in the development and the aggressiveness of cSCC, and could be used as pertinent biomarkers for stratification of cSCC.

Session 5A – Cell signaling and therapeutic targets

5A / 1

Ras/MAPK signalling intensity defines subclonal fitness in a mouse model of primary and metastatic hepatocellular carcinoma

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Background

While it is well established that signal intensity and duration can lead to drastically different outcomes during development, little is known about its impact on tumorigenesis. We studied such quantitative heterogeneity of signal intensity in the context of MAPK pathway activation in hepatocellular carcinoma (HCC), the most frequent primary liver cancer, whose incidence is rising and whose clinical outcomes remain particularly grim. We hypothesized that variations in the signaling intensities of the MAPK Erk, which is activated in 30-50% of HCC, would trigger selective pressures that generate functional heterogeneity in otherwise genetically identical tumor cells.

Methods

In order to study population dynamics and intercellular interactions during tumor growth and dissemination, we developed a murine model of HCC that combines intrahepatic xenografts and lineage tracing. To model the activation of MAPK we used retroviral transduction of a constitutively active form of the Ras oncogene - $HRas^{G12V}$. The use of a bi-cistronic vector expressing fluorescent reporter proteins allows to determine the oncogene dosage, which is proportional to fluorescence intensity in individual cells. Populations of cells expressing defined range of the oncogene were purified by cell sorting, characterized ex vivo and injected as orthotopic allografts. The resulting tumors were characterized by RNAseq, flow cytometry and IHC.

Results

Our experimental model gave rise to both primary and extrahepatic (peritoneal) tumorigenesis. We showed that strong selective pressures operate in both tumoral locations, giving rise to tumors expressing an optimal, narrow ranges of the oncogene dosage, reflective of MAPK Erk signaling activity. Strikingly, the signal intensities compatible with tumorigenesis at the primary and metastatic sites were significantly different. Indeed, significantly higher Ras expression was observed in primary as compared to metastatic tumours, despite the evolutionary trade-off of increased apoptotic death in the liver that correlated with high Ras dosage. Functionally, we identified several components of the immune stroma that participate in the definition of cellular fitness in these two host microenvironments, among which NK cells appear to play a key role in defining the selective milieu controlling tumor growth dynamics.

Overall, our work describes a quantitative aspect of tumour heterogeneity and highlights potential vulnerability of a subtype of hepatocellular carcinoma as a function of MAPK Erk signalling intensity.

5A / 2
JMV7048, First-in-class PROTAC degrader of PXR

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Colorectal cancer is the second deadliest cancer in the world and tumor recurrence have been reported in 30 to 50 % of patients.Therapeutic failure is now explained by the existence of a subpopulation of cancer cells called cancer stem cells (CSCs). Notably, these CSCs over-express the transcription factor PXR, controling the expression of a large gene network involved in drug metabolism and detoxification, including ALDH1A1 and CYP3A4. In a xenograft model, we previously shown that PXR knockdown by shRNA inhibits tumor relapse of patient derived cells after chemotherapy treatment. To date there is no clinically relevant PXR antagonist. PROteolysis TArgeting Chimeras (PROTACs) have become a promising and appealing technology for modulating a protein of interest (POI) by degradation, including undruggable targets. PROTACs are hetero bifunctional molecules that connect a POI ligand to an E3 ubiquitin ligase recruiting ligand with an optimal linker. They give multiple advantages such as a long-lasting effect, because it requires a de novo protein synthesis, and a catalytically mode of action due to their successful dissociation after promoting polyubiquitination of the POI. Our first innovative demonstration consists in turning a high PXR affinity agonist (6m, Kd< 1nM, JMV6845) into a PXR PROTAC. By TR-FRET competitive and gene reporter assays we first identified a 6m-linker scaffold able to bind to the ligand binding domain (LBD) (kd50= 18.36nM) and activates PXR. X-ray crystallography studies reveal that the core of the molecule resides within the ligand binding cavity while the linker tail emerges from the LBD surface. Based on this scaffold we synthetized and tested several PROTACs targeting a variety of E3 ubiquitin ligases (CRBN, IAP, VHL, etc.). We finally identified 3 molecules, including out lead molecule JMV7048, leading to a significant PXR protein degradation and PXR signalling pathway in colon cancer cells. The use of ubiquitin E3 or 26S proteasome inhibitors confirmed its mode of action. Finally, despite poor pharmacokinetic properties (Tmax<5 minutes and AuCt=5189 ng/mL*h), we observed that intravenous injections of JMV7048 (25mg/mg) in SCID mice reduced the PXR expression in human xenografted tumours. The final objective is to improve JMV7048 bioavailability and test this molecule as an adjuvant strategy in preclinical studies to prevent tumor relapse after chemotherapy.

5A / 3

Novel mechanisms controlling KRAS oncogenic output: impact on tumour fitness and cancer vulnerabilities

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The MAPK pathway is a key actor downstream of virtually all known driver oncogenes in lung adenocarcinoma (LUAD). Indeed, we have previously shown that the signal intensity of the MAPK pathway downstream of KRAS is a critical factor dictating the nature of the cancer-initiating cell and controlling LUAD progression in vivo. Furthermore, accumulating evidences suggest that KRAS clusters at the membrane are an essential requirement for the activation of MAPK signalling and to ensure an optimal oncogenic activity. We are combining genetic approaches and cellular models to address these two interrelated aspects of KRAS biology: the intensity and duration of downstream signalling and the role of KRAS clusters at the plasma membrane. I will discuss their biological implications, the key role of this signalling plasticity in the resistance to KRAS specific inhibitors as well as how these features could identify novel therapeutic approaches.

5A / 4
PI3K signaling and tumoral metabolism in pancreatic cancer

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Pancreatic cancer (PDAC) patients have a low survival rate; chemotherapy does not cure. In 80% of the cases, KRAS mutation is present. PI3K pathway is a KRAS downstream target, that regulates glycolysis and insulin response [1]. Targeting PI3Kα and γ isoforms reduces PDAC tumor progression [2]. However, the importance of PI3K isoforms in tumoral metabolism regulation in PDAC is unknown. This knowledge is critical as the modulation of PDAC tumoral metabolism represents a promising therapeutic strategy.

Using transcriptomic databases of human pancreatic tumors, we searched for specific metabolic signatures associated with the level of expression of PI3Kα and PI3Kγ. We analyzed the metabolic flexibility after long-term treatment with PI3K inhibitors (Vehicle, BYL-719 (PI3Kα selective inhibitor), IPI-549 (PI3Kγ selective inhibitor) or the combination BYL-719 and IPI-549) in vitro (cell lines) or in vivo (xenograft of human tumor cells Capan-1 in female nude mice). Conversely, we analysed the same parameters in pancreatic cancer lines stably overexpressing PI3Ks. The metabolic parameters of tumor cells studied are: mitochondrial ROS, mitochondrial mass, mitochondrial membrane potential, viability and ATP production by pancreatic tumor cells in the presence of mitochondrial and / or glycolysis inhibitors. Finally, sensitivity to gemcitabine, the standard treatment for pancreatic cancer, was tested alone or in combination with metabolic pathway inhibitors (Phenformin, CPI-613 or 2-DG). In particular, we used CPI-613, a lipoic acid analogue, that blocks the activity of α-KG (α-ketoglutarate dehydrogenase) and PDH (pyruvate dehydrogenase).

Patients with high expression of PI3Kα and PI3Kγ (αHigh; γHigh) in the pancreatic tumor were associated with the basal subtype and a low probability of long-term survival; on the other hand, patients with low tumor expression of PI3Kα and PI3Kγ (αLow; γLow) exhibited characteristics of the classic pancreatic cancer subtype and had an encouraging probability of long-term survival. These tumors (αLow; γLow) expressed genes involved in glyco-oxidative metabolism while tumors (αHigh; γHigh) did not present an obvious metabolic signature. Tumors (αLow; γLow) were associated with the expression of genes involved in the assembly of cytosolic iron-sulfur aggregates and of genes involved in the assembly of mitochondrial complexes. Inhibition of PI3Kα and γ in vivo increased the level of tumor ROS (reactive oxygen species) without impacting the mitochondrial mass and the mitochondrial membrane potential. Tumor cells treated with the BYL-719 and IPI-549 combination were more sensitive to the combination Gemcitabine + metabolism modulators (CPI-613 or 2-DG) than the tumor cells treated with vehicle. Modulating the activity of PI3K changed metabolic dependencies (orientation towards a glyco-oxidative metabolism) and sensitized cells to its targeting. We are now exploring the underlying molecular mechanisms and validating whether the overexpression of PI3K mimics the effect of inhibitors.

Inhibition of PI3Ks may be a way to force pancreatic cancer tumor cells into a metabolism that makes them sensitive to combinations of chemotherapy drugs and metabolic inhibitors. These inhibitors act by increasing mitochondrial ROS leading to cell death of pancreatic cancer cells.

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Sortilin exhibits tumor suppressor-like activity by limiting EGFR function

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Lung cancer is the leading cause of cancer deaths worldwide and remains one of the most incurable. Tyrosine kinase receptors, such as the epidermal growth factor receptor (EGFR), are often aberrantly activated and drive tumor growth. Monotherapy with tyrosine kinase inhibitors to deactivate EGFR has shown initial efficacy, but their benefits tend to decline over time. EGFR acts as a transcriptional factor promoting the expression of co-oncogenic drivers, which, in turn, interact with canonical EGFR mutations to induce therapeutic relapse. This study reports that sortilin, a crucial regulator of cytoplasmic EGFR, attenuates its transducing function. Genome-wide chromatin binding revealed that sortilin interacts with gene regulatory elements occupied by EGFR. These results suggest a model, in which sortilin exhibits potential tumor suppressor-like activity by concurrently binding to regulatory elements of cMYC. Sortilin expression in lung adenocarcinoma may be predictive of the efficacy of anti-EGFR strategies.

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Characterization of a novel monoclonal antibody targeting tumor-associated macrophages

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Tumor-associated macrophages (TAM) belong to the major cell population of tumor microenvironment (TME), supporting tumor development and resistance to therapy. Targeting of TAM remains challenging, owing to their remarkable plasticity and lack of exclusive markers. To address these problems, Team 9 of CRCT developed and patented the monoclonal antibody called 6-25, using Nurse-like cells (NLC) as a model of TAM. NLCs are a type of TAM found in chronic lymphocytic leukemia (CLL), which are important for the survival of cancer B-CLL cells and can be easily generated in vitro. The 6-25 antibody has been characterized to specifically bind to various human TAM. Studies showed that naked 6-25 antibody was not toxic toward NLC or other cells present in PBMC from CLL patients and healthy donors, but could be efficiently internalized by NLC. These findings led to development of antibody-drug conjugate (ADC) version of the 6-25, that proved to selectively deplete NLC and M2 macrophages in vitro. Research on 6-25 target showed that it is a specific marker of M2 macrophages and foremost of protective NLC. Using combination of immunoprecipitation-quantitative mass spectrometry approaches and RNAseq analyses the target of 6-25 antibody was recently identified. These results will allow to further study the role and mechanism of expression of this molecule in protumoral TAM. Beside, these findings will be essential to further develop the project and eventually to use the 6-25 antibody in anti-cancer therapies, as an agent specifically targeting protumoral TAM.

Session 5B – Genome dynamics and cancer

5B / 1

Back-up pathways that support BRCA1 deficient cells

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Inhibition of poly ADP ribose polymerases (PARP) has provided a new paradigm for cancer treatment, by targeting Homologous Recombination (HR) DNA repair-deficient cancers. Cells lacking canonical HR genes are sensitive to targeting several other pathways and these might offer better or complementary treatment approaches.

In this presentation, an overview of some of the targetable alternatives will be discussed and the conference will be updated on the current understanding of how these mechanisms interact with BRCA-deficiency and each other.

5B / 2
BRCA1 prevents R-loop-associated centromeric instability

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Centromeres are defined by chromatin containing the histone H3 variant CENP-A assembled onto repetitive alpha-satellite sequences, which are actively transcribed throughout the cell cycle. Centromeres play an essential role in chromosome inheritance and genome stability through coordinating kinetochores assembly during mitosis. Structural and functional alterations of the centromeres cause aneuploidy and chromosome aberrations which can induce cell death. In human cells, the tumor suppressor BRCA1 associates with centromeric chromatin in the absence of exogenous damage. While we previously reported that BRCA1 contributes to proper centromere homeostasis, the mechanism underlying its centromeric function and recruitment was not fully understood. We discovered that, while BRCA1 is recruited to the centromeric chromatin by R-loops, it also functions there to limit the accumulation of these structures at centromeric alpha-satellite repeats. The accumulation of R-loops at centromeres in BRCA1 deficient cells results in an impaired localization of CENP-A, higher transcription of centromeric RNA, increased centromeres breakage and formation of acentric micronuclei. BRCA1 deficiency triggers a RAD52-dependent hyper-recombination process between centromeric satellite repeats, associated with centromere instability and missegregation. Altogether, our findings provide molecular insights into how BRCA1 maintains centromere stability and identity.

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IgH Locus Suicide Recombination (LSR) in Chronic Lymphocytic Leukemia (CLL): Prognosis Indicator ?

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Chronic Lymphocytic Leukemia (CLL) is an indolent hemopathy B malignancy common in the adult and incurable in which the monoclonal expansion of mature B-lymphocytes results in chronic lymphocytosis. The oncogenic processes of CLL are not exactly defined. The B cells involved are characterized by the expression of CD5 and CD23 markers on their surface and of a BCR (B Cell Receptor) consisting in the vast majority of cases of IgM expressed at low level. The uncontrolled expansion of a B cell at the origin of the malignant clone would be consecutive to the stimulation of the cell via its BCR by an antigen (Ag) and the cell signaling mediated by the BCR seems to be involved. Accumulation of DNA damage, aberrations in DNA damage response signaling, error-prone repair of DNA damage, and associated chromosomal instability are common in CLL (Popp, 2019).

B Lymphocytes are prime targets of physiological DNA damage such as DNA Double Strand Breaks (DSBs) introduced by immunoglobulin (Ig) gene rearrangements: Locus Suicide Recombination 'LSR' and Class Switch Recombination 'CSR'. LSR and CSR are genetic rearrangements of the IgH locus in activated B-cells. CSR is a mechanism that diversify Ig isotype from IgM to IgG, IgA and IgE in contrast LSR deletes constant IgH genes and results in loss of BCR and conduct to B-cell death (Peron, 2012). LSR is a physiological event but its function in B-lymphocyte homeostasis is not yet been elucidate. In this study, we used genomic DNA from PBMC of patients with CLL; we surprisingly detected LSR recombination junctions. Analysis of LSR junction number leads us to differentiate two subsets of CLL patients compared to samples from healthy donors. A group with a high number of LSR junctions named "LSR High" (the mean levels of 731 LSR junctions / 1.10 ^ 6 cells) and a group with low LSR junctions number "LSR Low" (116 LSR / 1.10 ^ 6 cells), against 290 LSR / 1.10 ^ 6 cells junctions for PMBCs of control subjects.

The clinical and biological data were integrated into the analysis and we observed that the two groups of patients differ by Treatment Free Survival TFS after diagnosis, the Binet staging for the classification of patients and the mutational status of Variable segment (VH) of the patient. The group of patients with high number of LSR junctions has features associated with an unfavorable prognosis. (Shorter TFS, non-mutated VH segment).
Furthermore, the repair joints at the LSR junctions for the group of patients with Low LSR junctions exhibit an atypical structure suggesting an abnormality in DNA DSB repair.
Our results suggest that the LSR junction count may be a prognosis marker in CLL while the structural abnormality of the LSR joints may reflect the altered response to DNA DSB. This is why we are continuing this work by evaluating DNA markers for DSBs and we are implementing the analysis of DSB repair in CLL patients by discriminating between the "LSR High" and "LSR Low" subset.

The evolution of CLL varies widely from patient to other, and our work seems to identify the LSR as a new prognosis factor.
Our project aims to interpret this new area of research in LLC and opens up very new perspectives for taking in charge and prognosis of patients. Our results provide new insights on the LSR molecular process and function.

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Control of Homology directed repair of DNA double strand breaks by the KDM8 histone demethylase

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The screening of a siRNA library identified KDM8 depletion as altering gammaH2AX staining after exposure to ionizing radiation. KDM8 depletion leads to a decrease in gH2AX 1h and 6h post ionizing radiation exposure. KDM8 is a histone demethylase first characterized for its effect on H3K36me2 but its activity is still a matter of debate and is now more assigned as a hydroxylase. Here, we show that KDM8 is quickly recruited to laser-induced DNA damage. Its expression is influencing DSB repair by homologous recombination (HR) as its depletion increases HR while its overexpression decreases HR. The effect of KDM8 depletion inducing increased HR is balanced by a concomitant decrease in NHEJ. This effect was independent of its demethylase activity and not related to cell cycle alteration. In addition, we observed by FRET a direct interaction between KDM8 and Rad51, an essential actor of the homologous recombination pathway, after DNA damage induction. This interaction was detected only after DNA damage induction. These results identify KDM8 as playing important role in DNA repair involving homologous recombination.

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New strategies to target DNA methylation: from the discovery of novel DNMT inhibitors to the identification of novel epigenetic targets

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DNA methylation, catalysed by DNA methyltransferases (DNMTs), is one of the most studied epigenetic modifications that regulate gene expression. The methylation pattern of several tumour suppressor genes (TSGs) is altered in cancer, leading to their silencing and contributing to cell proliferation and tumour progression.[1] The FDA approval of nucleoside DNMT inhibitors (DNMTi, 5-azacytidine and 5-aza-2'-deoxycytidine) for the treatment of haematological cancers validates the use of DNMTs as anticancer targets. However, as nucleoside analogues, their high toxicity and chemical instability greatly limit their clinical use.[2] Therefore, many efforts were done to identify non-nucleoside DNMTi, but to date, none entered clinical trials. This highlights the urgent need to develop novel strategies to target DNA methylation. Firstly, our group reported potent non-nucleoside flavanone DNMTi by optimisation of chemical scaffolds identified by high-throughput screening.[3] This work resulted in the development of 3-bromo-3-nitro flavanone, which inhibits human DNMT3A-c in a fluorescence-based *in vitro* assay and enhances luciferase reporter gene expression in KG-1 cell lines.[4] Besides, we also identified a novel chemical scaffold with higher potency and chemical stability. These results are promising towards the development of better anticancer therapies. Secondly, in order to identify new targets involved in DNA methylation, we employed flavonoid DNMTi to synthesise chemical probes to be used in Affinity-Based Protein Profiling (ABPP).[5] To apply this chemical biology strategy, we linked our DNMTi to a photoactivable crosslinker to trap DNMT partners directly in living cells. After purification, trapped proteins were identified by SDS-PAGE and quantitative proteomic analysis. The research on drug and probe development carried out in our group paves the way to identify novel strategies to affect DNA methylation in cancers. This will not only improve the clinical translation of DNMTi, but also help researchers to discover promising targets to defeat cancer.

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Network approaches to dissect the epigenome-phenotype connection in immune cells

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Recent technological advances have allowed us to map chromatin conformation and uncover the spatial organization of the genome inside the nucleus. These experiments have revealed the complexities of genome folding, characterized by the presence of loops and domains at different scales which can change across development and in different cell types. There is strong evidence for a relationship between the topological properties of the chromatin contacts and cellular phenotype, the details of which are being actively investigated.

An increasingly popular representation of chromatin is given by networks, in which genomic fragments are the nodes and connections represent experimentally observed spatial proximity of two genomically distant regions. This formalism has allowed us to consider a variety of chromatin features in association with the 3D structure, from expression to genes' evolutionary age. Using tools that we have generated to facilitate the global statistical analysis of these integrated 3D epigenomes[1], we have started investigating how nuclear chromatin organization can be related to gene regulation[2], replication[3], malignancy [4] phenotypic variability and plasticity.

We are particularly interested in understanding how epigenomic characteristics of immune cells are shaped through differentiation and in the presence of different stimuli. Our current efforts are directed at exploring the connection between immune cells' 3D epigenome and their behaviour in diseases including cancer, through global systems properties revealed by network theory approaches.

Similar to what we have observed in protein-protein interaction networks, external conditions could shape chromatin 3D organization with interesting implications on phenotypic heterogeneity.

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[2] Pancaldi et al. 2016
[3] Jodkowska et al. 2019 BioRxiv
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Session 5C – Patients-partenaire-formateur-co-chercheur : des savoirs expérientiels légitimes ?

5C / 1
Savoirs expérientiels et partenariat patient : une alliance indispensable

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Selon la formule consacrée, « le professionnel de santé est l'expert de la maladie tandis que le patient est l'expert de la vie avec la maladie ». Si l'on admet que le patient est (en partie) ignorant de sa maladie et que cette connaissance peut lui être transmise par le(s) professionnel(s) de santé, on envisage plus difficilement que le(s) professionnel(s) de santé soient (en partie) ignorants de ce que la maladie, la douleur, le handicap induit comme changement dans la vie quotidienne de leurs patients.
Dans sa définition la plus large, les savoirs expérientiels désignent ce que les patients apprennent du fait de vivre avec un handicap, une maladie ou une douleur chronique. Ces savoirs issus de l'expérience de la maladie sont par conséquent un élément indispensable du coapprentissage entre patient et professionnel de santé qui caractérise le partenariat-patient. Dans mon exposé, je proposerai quelques balises définitionnelles du concept de « savoir expérientiel » et de ce qu'il est susceptible d'induire comme changement dans la relation de soin.

5C / 2

VIH/Sida et Empowerment : « Quels enseignements pour la mobilisation des patients en cancérologie ? »

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Un des termes largement usités en complément de celui d'autonomie du patient est le mot anglo-saxon d'empowerment. Il est important d'identifier l'existence d'une grande diversité d'appréhension de ce concept (Jouet - 2014). Il fonde un modèle exposant les capacités d'un sujet à faire des choix éclairés pour lui-même, mais affirme également l'établissement d'un autre rapport relationnel entre soignant et soigné. Il est souvent fait référence à la lutte contre le VIH-sida pour valoriser cette démarche comme le symbole de la réussite d'une émancipation. Mais cette logique est-elle applicable en toute circonstance, et notamment à celui de la cancérologie dans le cadre de la mobilisation des patients ?

L'expérience que notre société a de la lutte contre le VIH-Sida, les modèles qu'elle a produits, la manière dont elle a obligé à repenser le soin, est due aux inconnus auxquels les parties-prenantes devaient faire face (Tourette-Turgis - 2015), les mettant de fait, sur un pied d'égalité. Une dynamique collective autour de la mort engendrant « une valeur symbolique protectrice » (Routy - 2011) renouant avec une appréhension collective de la santé qui n'existait plus depuis les grandes épidémies. Elle devenait alors l'affaire de groupes plus que d'individus, s'exprimant dans l'espace public et pas uniquement dans le colloque singulier (Adam et Herzlich - 2013). C'est ce qui l'a inscrite de manière particulière dans la mobilisation des patients. Mais est-ce l'unique raison qui a été le moteur de cette dynamique ?

Il est possible que plusieurs caractéristiques identitaires spécifiques aux communautés touchées et construites antérieurement du fait de leur interaction avec leurs environnements aient prédisposé les sujets à cette mobilisation, que ce soit de manière individuelle ou collective (Pollak - 1993). Si l'on prend l'exemple de la communauté homosexuelle, la résultante de leur construction identitaire en tant que patient s'enracine dans une quête d'identité plus profonde et bien antérieure. Les sujets ont mis en œuvre des processus et des stratégies pour se protéger de l'extérieur ce qui les a soudés et préparés à leur émancipation future. De plus, ne se mobilisant pas spécifiquement pour cette lutte, mais déjà mobilisé autour de leur identité sociale, les profils professionnels des acteurs engagés leur ont permis de maitriser les codes des espaces professionnels à investir pour défendre leurs propres intérêts.

Le concept de séropositivité produit par l'épidémie du VIH-sida a favorisé une nouvelle approche du sujet malade, modifié les relations dans le couple soigné/soignant, ouvert la voie à de postures nouvelles (Bardot - 2002). Mais avec l'avènement des trithérapies et une gestion médicale de plus en plus personnalisée ainsi que les progrès de la société sur les questions de genre, cette communauté d'intérêts perdure-t-elle encore aujourd'hui et peut-on dire que les logiques d'empowerment dans le domaine du VIH-sida sont toujours d'actualité ?

5C / 3

Le cancer du sein : une maladie chronique pas (tout à fait) comme les autres ?

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LISA - l'institut du sein d'Aquitaine

Dans cette communication, je présenterai un ensemble de réflexions issues de mon expérience et de mes échanges avec de nombreux patients atteints de cancer principalement.

Il s'agira tout d'abord de tenter de réfléchir sur les caractéristiques de la, ou plutôt des, pathologie(s) cancéreuse(s). Ces caractéristiques des cancers, au regard notamment du VIH/sida qui a joué un rôle important dans la mise en place des premiers partenariats entre patients et monde médical, déterminent peut-être la façon dont peut se déplier le partenariat en santé en cancérologie.

Session 6A – Research in Melanoma

6A / 1

Combining TNF inhibitors to anti-PD-1 and anti-CTLA-4 for the treatment of advanced melanoma patients

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Immune checkpoint blockers (ICB) targeting PD-1 and/or CTLA-4 have become the gold standard treatments for metastatic melanoma patients with up to 36% of them achieving 5-year progression free survival[1]. However, a high proportion of patients still resist or relapse following treatment initiation highlighting the need for improved therapeutical strategies.

We and others have shown that tumour necrosis factor alpha (TNF) can impede anti-tumour responses through multiple mechanisms, including the inhibition of anti-tumour responses[2, 3] and the induction of epithelial-to-mesenchymal transition/de-differentiation of tumour cells[4]. Hence, we demonstrated that blocking TNF promotes the efficacy of ICB in mouse melanoma models5. Building upon our pre-clinical work, we started in 2018 a phase 1b clinical trial (TICIMEL, NCT03293784), which aims at evaluating the safety and efficacy of combining anti-TNF (infliximab [inflix] or certolizumab [certo]) to anti-PD-1 (nivolumab [nivo]) and anti-CTLA-4 (ipilimumab [ipi]) to treat advanced melanoma patients. Results from the first phase of this trial show that both tritherapies are safe with a higher incidence of treatment- related adverse event events in the ipi/nivo/certo cohort as compared to the ipi/nivo/inflix one. This is correlated to promising sign of efficacy in patients enrolled in the certolizumab cohort[5]. When assessing the impact of treatments on systemic immune responses, we observed an increase in the maturation and proliferation of systemic T cells in patients from both cohorts. This was accompanied by a decrease in the proportions of systemic myeloid-derived suppressor cells.

Overall, these results suggest that anti-TNF may promote anti-melanoma immune responses in patients, a phenomenon we are further investigating in the second part of TICIMEL. Whether the nature of the TNF blocking agents used in this combination therapy leads to a differential modulation of the systemic and tumor immune landscapes in advanced melanoma patients is being investigated.

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6A / 2
Real time in vivo observation of the human skin microvascularization

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The ability of our microvascularization to locally adapt to metabolic needs is known to be impaired in many diseases such as skin cancers, diabetes, hypertension, Alzheimer's disease, and even more recently SARS-COV-2 [1]. However, at that time, we do not have any system able to observe and characterize in vivo the general state of our microvascularization. To solve this purpose, we design a new system devoted to real-time high-resolution imaging of the transcutaneous microvascularization. The imaging process utilizes optical interferences known as speckle, which dynamic is driven by moving red blood cells when illuminated with laser light. A specific polarimetric filtering process prevents the contribution of surface scattering and thus favors the detection of multi-scattered photons from the deeper layers of the skin. We then have access to brand new images that reveal the human skin microvasculature with an unprecedented optical penetration of few millimeters and a spatial resolution better than 100 microns for a full-field view of 12 cm2. Video imaging can serve real-time applications and a reliable microvascularization activity can be evaluated in 1 second, enabling numerous clinical applications requiring quantification. We present here this new imaging system that we call a Transcutaneous MicroVasculoscope (TMV).
The TMV is light (500g) and compact, of 22 cm long, composed of a handle and a circular head that is placed in weak contact with the patient's skin in order to strongly minimize the relative movement between the system and the patient during acquisition. Laser illumination is performed in the near-infrared domain. The microvascularization image is generated in near real-time from a succession of raw interference images. We define the Microvascular Activity Index (MAI) of each pixel as $1/\tau v$, where τv is the decorrelation time induced by multiple scattering into red blood cells. Thus, this later index increases with increasing movement of erythrocytes.
Examples of scans will be presented first on different healthy parts of the body and then on various pathological and inflammatory states of the skin, including skin cancers (melanoma, carcinoma). Skin tumors like melanoma and carcinoma are known to be abnormally vascularized [2, 3] and thus the TMV is expected to enhance the diagnosis of the dermatologist.
We underline that as with all dynamical speckle imaging techniques, our signal, generated by optical interference, is naturally extremely sensitive to sub-micro movements and is expected to be promising for detection of cancers in their earliest stages by imaging the early neoangiogenesis as we have already observed for the B16 melanoma on mice.
The TMV is a simple device, eye-safe, handy, non-invasive, and works without any contrast agent. We expect numerous clinical applications concerning the pathologies involving microvascularization, such as the monitoring and quantification of inflammatory and degenerative skin diseases, including the detection and follow-up of skin cancers. Applications concerning immediate post-surgery follow-up of flaps and grafts, and evaluation of the vitality of organs before transplantation are also underway.

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6A / 3

Vitiligo under PD-1 inhibitors for melanoma patients: what's new and how we can go further ?

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Background: Clinical factors associated with vitiligo in patients receiving anti-PD-1 remains unknown.

Objective: to better characterize the occurrence of vitiligo in patients receiving anti-PD-1.

Methods: Single-center ambispective cohort study including patients with melanoma treated with anti-PD-1. Progression-free survival, overall survival and objective tumor response were compared between patients with or without vitiligo using Kaplan-Meier curves and the log rank test. Demographic and clinical factors associated with vitiligo were evaluated by multivariate logistic regression.

Results: Of the 457 patients included in the study, 85 developed vitiligo. Vitiligo occurring in patients receiving anti-PD-1 therapies are mainly located on sites of chronic sun-exposure. The presence of vitiligo was associated with a significant improvement in overall survival and progression-free survival ($p<0.001$). In the multivariate logistic regression analyses, male patients showed an independent increased risk of developing vitiligo (OR 1.66). In contrast, the presence of pulmonary metastases was found to be an independent factor associated with a reduced risk of developing vitiligo (OR 0.50). Leukotrichia and/or a halo phenomenon around cutaneous metastases are distinct clinical factors in patients receiving anti-PD-1 inhibitors associated with the absence or a low rate of mortality, respectively.

Conclusion: Vitiligo in patients receiving anti-PD-1 for advanced melanoma is associated with better outcome. Other clinical factors such as leukotrichia and halo phenomenon around cutaneous metastasis as well as a gender effect will need further investigation.

6A / 4
Rational concepts for tumor & immune cell therapy combinations

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We use function-based, genome-wide experimental strategies to develop rational combinatorial cancer treatment, targeting both cancer and immune cells. By screening for novel therapeutic targets and predictive biomarkers, we aim to achieve more durable clinical responses for patients. On the one hand, we are increasing our understanding of how cancer cells rewire their signaling networks, to expose and exploit new pharmacologically tractable tumor susceptibilities, also in the context of immunotherapy. On the other hand, we are manipulating various cell types from the patient's own immune system to boost their specific cytotoxicity towards tumor cells. With these function-based approaches, we develop new rational combinatorial therapies, which simultaneously eliminate the patients' tumors and harness their immune system.

Session 6B – Le patient-partenaire en institution

6B / 1

Patiente Partenaire au SEIN d'une équipe d'oncologie : un dispositif innovant

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La survenue d'un cancer - événement à la fois « irruptif et disruptif » - entraine une perte des repères qu'ils soient personnels, familiaux, sociaux et/ou professionnels. Une femme sur 8 sera impactée au cours de sa vie par un cancer du sein. Celui-ci demeure la première cause de mortalité par cancer chez la femme et ce malgré une augmentation significative de la survie des patientes. Un accompagnement purement médical ne saurait donc suffire pour trouver des stratégies d'adaptation nécessaires permettant de faire face à tous les changements imposés par la maladie. A Bordeaux, L'Institut du Sein d'Aquitaine - LISA - a été créé en 2017, par un collectif de professionnels de santé afin de faciliter, grâce à l'action coordonnée de tous les praticiens, le parcours de soins des personnes. En 2018, la structure a intégré une Patiente Partenaire (PP) formée à l'expertise d'usage afin d'accompagner :

- d'une part les patientes désireuses d'échanger avec une personne ayant eu elle-même un parcours de soins,
- d'autre part les professionnels de santé pour leur offrir un point de vue inédit et penser ensemble les pratiques en santé.

Il s'agit d'améliorer le parcours de soins, personnaliser la prise en soins afin de préserver la qualité de vie de chaque patiente.

Deux processus contextualisent le recours à la pair-aidance de LISA : l'évolution de la place des usagers dans le système de santé et la reconnaissance des nouveaux métiers à travers la valorisation et la légitimation des savoirs expérientiels. C'est à partir de son expérience de la traversée de la maladie et de la compréhension de son propre processus de rétablissement que la Patiente Partenaire aide ses pairs à surmonter les obstacles et à identifier ce qui les aide à se rétablir.

En mai 2020, 260 patientes accompagnées au cours des mois précédents par la PP ont été identifiées. Un questionnaire leur a été proposé. Cette ressource a été utilisée afin de définir le Partenariat Patient à la manière de LISA et déplier ce que l'inclusion d'une PP dans une équipe de soins apporte aux patientes.
Les résultats montrent que La PP apparait comme une personne qui sécurise et soutient pendant cette situation d'adversité qu'est la maladie. Si les informations apportées par la PP sont souvent les mêmes que celles des professionnels, leur perception diffère signant une perspective inédite, ce qui contribue à une meilleure compréhension du parcours de soins. Enfin la PP participe à l'encapacitation de la patiente qui développe ainsi son pouvoir d'agir pour elle en redonnant un sens à sa vie au travers de ses expériences et en l'incitant à élaborer un projet de vie et à l'adapter au changement.

6B / 2

Intérêts et bénéfices de l'accompagnement par une patiente-partenaire pour des femmes diagnostiquées d'un cancer du sein.

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Introduction: L'accompagnement par une patiente-partenaire (PP) désigne l'approche selon laquelle des personnes ayant vécu un cancer (par exemple) sont formées et en mesure de fournir des informations, partager leur expérience, encourager et aider d'autres patients diagnostiqués d'un cancer. Dans la perspective de la théorie de la comparaison sociale, les interactions entre ces personnes sont généralement bénéfiques dans la mesure où elles favorisent une meilleure qualité de vie et de faibles niveaux de détresse émotionnelle chez les femmes diagnostiquées d'un cancer du sein. Une récente revue de la littérature indique toutefois que les études évaluant l'intérêt de l'accompagnement PP sont encore peu nombreuses et de faibles niveaux de preuve (Hu et al., 2019). Par ailleurs, des investigations relatives aux processus d'adaptation à la maladie (coping), au contrôle perçu ou encore au soutien perçu sont peu nombreuses voire inexistantes à notre connaissance. En outre, des données relatives au vécu de l'accompagnement auprès de femmes diagnostiquées d'un cancer du sein, sont manquantes.

Objectif: l'association LISA a mis en place un accompagnement PP, au sein de la Clinique Tivoli, pour des femmes diagnostiquées d'un cancer du sein. Un premier objectif consiste à évaluer l'impact de cet accompagnement sur la qualité de vie, la détresse émotionnelle des patientes, les stratégies de coping, le contrôle perçu et le soutien social perçu. Un second objectif vise à recueillir et analyser le vécu (satisfaction, bienfaits, points négatifs) des patientes suite à cet accompagnement.

Méthode: un protocole mixte de recherche, validé par le comité d'éthique de l'Université de Nantes (IRB : IORG0011023 - dossier n°22042021), a été mis en place. Une première étude transversale en ligne (Limesurvey) concernait les femmes diagnostiquées d'un premier cancer du sein, ayant plus de 18 ans au diagnostic, ayant commencé (ou terminé) leur prise en charge hospitalière, étant en mesure de répondre à un questionnaire en ligne en langue Française, en accord pour participer à l'étude. Les caractéristiques sociodémographiques, professionnelles, médicales, ainsi que la qualité de vie (QLQC30), la détresse émotionnelle (HADS), les stratégies de coping (WCC), le contrôle perçu (CLCS) et le soutien social perçu (QSSSC) ont été recueillis auprès de deux groupes de patientes (accompagnement PP ; pas d'accompagnement PP). Des analyses comparatives, stratifiées selon le délai depuis le diagnostic (1ère année, 2nde année, 3 à 5 ans après le diagnostic) seront réalisées à l'aide du logiciel R. À partir de la liste de participantes ayant participé à l'étude transversale, 24 patientes ont été tirées au sort (12 dans le premier groupe et 12 dans le second groupe) pour participer à une étude qualitative visant à mieux comprendre le vécu de la maladie avec ou sans accompagnement par une patiente-partenaire, tout en recueillant l'intérêt, pour chacune d'entre elles, d'un tel accompagnement tout au long du parcours de soin. Les entretiens ont été retranscrits. Le codage et une analyse de contenu ont été réalisés à l'aide du logiciel QDA Miner 6.

Résultats préliminaires: le questionnaire a été adressé à 1286 femmes, membres de l'association LISA. En somme, 376 réponses sont exploitables pour les analyses et 23 femmes ont participé aux entretiens qualitatifs. Les analyses sont en cours.

Discussion / Conclusion: Les résultats seront discutés. Nous nous attendons à ce que les patientes ayant bénéficié d'un accompagnement PP aient une meilleure qualité de vie, de plus faibles niveaux de détresse émotionnelle, de plus hauts niveaux de soutien perçu et de contrôle perçu ainsi qu'une meilleure capacité d'adaptation à la maladie, en comparaison avec les femmes n'en ayant pas bénéficié. De plus, nous nous attendons à ce que les patientes ayant bénéficié d'un accompagnement PP témoignent d'un meilleur vécu du parcours de soin en comparaison avec celles n'en ayant pas bénéficié.

6B / 3

Une expérimentation innovante : le salariat de patient partenaire en cancérologie

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Considérer le citoyen comme un acteur de sa santé et renforcer sa place au sein du système de santé est une des cinq orientations stratégiques du projet régional de santé (PRS) de l'Agence Régionale de Santé Nouvelle-Aquitaine. Ce PRS, paru en 2018, déploie une stratégie pour 5 ans autour de multiples objectifs et axes de travail :

- Informer mieux les usagers sur les droits et libertés : accès aux informations de santé, consentement libre et éclairé, liberté d'aller et venir, respect des croyances, ...
- Informer mieux les usagers sur les possibilités d'accompagnement qui s'offrent à eux et les décisions qui concernent leur santé
- Recueillir la parole des usagers sur leurs souhaits dans la manière d'être soignés et accompagnés et de prendre en compte cette parole
- Intégrer les usagers au cœur du système de santé que ce soit des usagers formateurs dans les programmes de formation, des usagers experts dans les programmes d'éducation thérapeutique, des usagers dans la gouvernance ou le fonctionnement des établissements, des usagers dans les équipes de soins pour mettre en œuvre, au long cours, un partenariat réussi patient / professionnel.

La notion de « Patient partenaire » n'est pas nouvelle. Elle prend sa source dans les différentes lois de modernisation de notre système de santé et l'émergence progressive des notions de droits des patients et de démocratie sanitaire (loi n°2002-303 du 4 mars 2002 relative aux droits des malades et à la qualité du système de santé, loi n° 2009-879 du 21 juillet 2009 portant réforme de l'hôpital et relative aux patients, à la santé et aux territoires et, plus récemment, loi n° 2016-41 du 26 janvier 2016 de modernisation de notre système de santé, ainsi que leurs différents décrets d'application).

En inscrivant l'éducation thérapeutique du patient dans le code de la santé publique, la loi « Hôpital, patient, santé et territoire » (HSPT) de 2009 pose la première pierre du patient partenaire.

En juin 2020, l'ARS NA a donc lancé un appel à projet visant à améliorer la qualité de la prise en charge et de l'accompagnement des patients atteints de cancer en leur proposant une ressource complémentaire au système de soin classique : le soutien de patients partenaires professionnalisés au sein des équipes de soins.

Par le lancement de cet appel à projet, l'ARS Nouvelle-Aquitaine souhaite poursuivre son engagement à reconnaître et valoriser les savoirs expérientiels dans le champ des maladies chroniques et plus particulièrement dans celui de la cancérologie.

7 établissements de santé et 11 patients partenaires de la Nouvelle-Aquitaine ont été retenus pour faire partie de cette expérimentation : le CHU de Limoges, l'Institut du sein de Charente Maritime, le CHU de Bordeaux, l'Institut Bergonié, le CH d'Arcachon, la Clinique Tivoli, la polyclinique Bordeaux Nord.

Session 7A– Poor prognosis Cancers: Brain tumors

7A / 1

Glioblastoma Metabolic Symbiosis: When Lactate Takes The Lead.

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Glioblastoma (GBM) is a common and devastating brain tumor, associated with a low median survival, despite standard therapeutic management. Among its major features, GBMs are highly angiogenic and exhibit paradoxically an elevated glycolysis. Most of differentiated cells convert glucose into pyruvate that enters into the Krebs cycle to maximize energy production in the presence of oxygen. For cancer cells, glucose uptake and catabolism are increased regardless of oxygen level. However, their energy needs are important - mainly for rapid growth - that it requires a much faster production flow. It is at this step that lactate dehydrogenases (LDH) are involved: LDHA converts efficiently pyruvate into lactate and generates NAD+ to maintain glycolysis. Thus, the lactate formed is exported into the extracellular compartment inducing an unfavorable acidification of the microenvironment. Moreover, LDHB, another LDH isoform, metabolizes lactate into pyruvate for generating energy in mitochondria. Though LDHA has already been studied in many cancers including GBM, the simultaneous role of LDH enzymes have not yet been investigated in GBM development.

Hypoxia-driven LDHA expression and lactate production increased cell invasion. Infusing 13C-lactate in starved cells rescued TCA cycle. Then, we showed that, under hypoxia, double Crispr-Cas9 LDHA/B cell growth and invasion was dramatically decreased in comparison to control cells, mainly caused by high apoptosis. Furthermore, double impairment of LDHA and B significantly reduced tumor growth and cell invasion, and induces a massive increase in mouse survival. Tracing experiments with 13C-Glucose coupled with RNA sequencing revealed how metabolism adapts to these constraints, by modifying electron transport chain subunit expressions and then cellular respiration, or by increasing lipid droplet formation.

Considered for a long time as a metabolic waste, lactate is shown here to play a critical role in GBM cell symbiosis. This study highlighted GBM adaptability through the LDH isoforms and their involvement in GBM development.

7A / 2
Glioblastoma, radiotherapy and 5-AminoLevulinic Acid: a dead end?

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Introduction

Radiosensitization of glioblastoma treatment is a major ambition to increase survival of this incurable cancer. The metabolite 5 aminolevulinic acid (5-ALA) is normally metabolized by the heme biosynthesis pathway into the intermediate fluorescent metabolite protoporhyrin IX (PPIX). Exogenous administration of 5-ALA leads to differential PPIX accumulation in glioblastoma compared to normal brain tissue, which can help R0 glioblastoma resections in clinical routine. Although PPIX accumulation was reported to radiosensitize cancer cells in different preclinical models, this potentiality in glioblastoma has remained unexplored

Materials and methods

The patient-derived tumor cell line, P3, self-assembling as spheroids, was used to explore PPIX accumulation after 5-ALA exposition by spectroscopy and flow cytometry. Cell death and spheroid growth were studied with or without 5-ALA, in combination with increasing doses of radiotherapy. An orthotopic brain tumor model was developed with P3-Luciferase spheroids. We evaluated tumor growth and tumor burden of encephalic multifractionated radiation doses. PPIX kinetic of accumulation in tumor and healthy brain cells was determined after intra peritoneal injection of 5-ALA. Finally, 5-ALA radiosensitization was explored in the spheroid-PDX model.

Results

PPIX maximal accumulation occurs between 4h after exposition to 5-ALA of P3 spheroid, and clears off almost completely at 24h. As expected, radiation therapy increased significantly sphere mortality at 7 and 14 days at 10Gy, and a slowed growth at 4Gy and 10Gy. Unexpectedly, 5-ALA treatment did not major mortality-induced radiotherapy. The survival of mice with orthotopic transplantations of PDX-spheroids was better with a radiation dose of 5 x 3Gy, three times a week [73-83 days] as compared to 5 x 2Gy [48-62], 3 x 2Gy [41-47], and no radiotherapy [15-24]. The survival was correlated to tumor growth measured by bioluminescence. PPIX accumulated more in orthotopic P3 tumors than in the healthy brain cells the first 4 hours after 5-ALA injection. Tumor growth and survivals were similar in mice irradiated at 5x2Gy regardless of 5-ALA treatment (RT group [53-67], RT+5-ALA group [40-74], HR=1.57, p=0.24).

Conclusion

Our results show for the first time than in a preclinical tumor model relevant to human glioblastoma with a treatment schedule paralleling clinical routine, 5-ALA administration, although leading to important accumulation of PPIX, does not potentiate radiotherapy. Such approach is important to evaluate in clinically-relevant models the validity of well-founded therapeutic improvement. However, PPIX and other porphyrin intermediates present photo excitability potential amenable to dynamic photoradiotherapy.

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7A / 3

Targeting glioblastoma with in vitro amplified Vδ2neg γδ T cells: preclinical study

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Background. Glioblastomas (GBM) are the most common adult primary brain tumors, highly infiltrative and aggressive, with a dismal patient prognosis despite standard-of-care treatments (Stupp protocol). They are characterized by an important inter and intra-tumoral heterogeneity but few coding mutations, and a strongly immunosuppressive immune infiltrate. Because of these hallmarks, GBM have been so far resistant to most of immunotherapy strategies based on inhibitory checkpoint inhibitors and vaccination. Alternatively, cell therapies based on *ex vivo*amplified tumor-infiltrating αβ T cells, CMV-specific αβ T cells, and CAR T cells, have shown promising results at the preclinical stage, but limited efficacy in patients, notably because of loss of targeted antigens and tumor escape. γδ T cells represent another relevant population for therapy, as they are HLA- and coding mutation-independent, recognize metabolites and stress signals associated with cellular transformation, and are a positive prognostic factor for multiple cancers including brain tumors. They are composed of two main subsets with distinct physiologies: Vγ9Vδ2 T cells and Vδ2^{neg} γδ T cells. Although the Vγ9Vδ2 subtype has shown promising results against GBM in preclinical studies, a Vδ2^{neg} γδ T cell-based therapeutic approach remains to be investigated.

Methods. Matched blood and tumor samples were obtained from GBM patients at the CHU de Bordeaux. Alternatively, blood samples were obtained from healthy donors. On the one hand, Vδ2^{neg} γδ T cells were specifically amplified *in vitro* from healthy donors and GBM patients' peripheral blood mononuclear cells, using a combination of an anti-CD3 agonist and cytokines (IL-4, IL-21, IL-1β, IFNγ, IL-15), as previously described. On the other hand, tumor samples were processed to generate a biobank of patient-derived GBM cancer-stem cells (CSCs) cultured in monolayers and as spheroids. The cytotoxic potential of amplified Vδ2^{neg} γδ T cells against GBM targets was subsequently assessed by a combination of flow cytometry and live-imaging analyses (Incucyte technology). Finally, to decipher the contributions of the γδTCR versus Natural Killer (NK) cell receptors in GBM killing, NK cell receptors and respective ligands expressions were assessed by flow cytometry followed by CRISPR/Cas9-mediated knockout of candidate receptors at the surface of amplified Vδ2^{neg} γδ T cells and functional analyses.

Results. We observed a reproducible Vδ2^{neg} γδ T cell expansion from both healthy donors and GBM patients. Amplified cells efficiently killed several laboratory GBM cell lines but also GBM patient-derived CSCs cultured in monolayers. In 3D cocultures, amplified Vδ2^{neg} γδ T cells were also cytotoxic, but required IL-15-mediated stimulation to completely eliminate GBM spheroids from all patients. Amplified cells expressed the NK receptors NKG2D, DNAM-1, NKp44 and NKp30, whereas only DNAM-1 ligands (PVR, Nectin-2) and NKG2D ligands (ULBP2/5/6, MICA/B) were expressed by target GBM cells. In 2D functional assays, amplified Vδ2^{neg} γδ T cells killed target cells independently of their γδTCR, while NKG2D was involved.

Perspectives. These results provide a first *in vitro* proof-of-concept for the use of amplified Vδ2^{neg} γδ T cells, in combination with IL-15 treatment, to target and eliminate GBM tumors. They remain to be completed by testing their efficiency in a preclinical mouse model of patient-derived xenografts. In this model, we will assess the efficacy of adoptively transferred Vδ2^{neg} γδ T cells, alone or in combination with the IL-15 superagonist N-803.

7A / 4

Understanding the immune microenvironment to improve immunotherapy for pediatric brain tumors.

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Medulloblastoma (MB) is the most common malignant brain tumor in children. Recent molecular analysis has defined four main subgroups of MB, exhibiting different histology, expression profiles and prognosis. Standard therapies have a limited effect on group 3 of MB. Despite multimodal therapy, including surgical resection, craniospinal irradiation and aggressive chemotherapy, approximately 30% of patients remain incurable, due to the acquisition of resistance properties. Survivors suffer from severe long-term side effects from these therapies, such as neurological disorder or secondary cancer. The development of more effective and specific therapeutic strategies that can increase the efficacy of current treatment without additional toxicity is of a high priority. The cytotoxic effect of those therapies on tumor cells are well documented in the literature, but little is still known on their effects on immune tumor micro-environment (TME). To improve outcomes, combination treatments with cancer immunotherapy agents may be necessary. In the past decade, immunotherapy is emerging as a powerful approach to treating cancer, but MB is viewed as an immunologically "cold" tumor, due to its low mutational burden and poor immune infiltration; and therefore, unlikely to benefit from immunotherapy. However, recent studies from our group and others suggest that a better understanding of the communication between tumor cells and their immune TME may allow more effective immunotherapies to be developed. We aim to decipher the changes induced by standard therapeutic regimen on immune TME, and to identify approaches to enhance anti-tumoral adaptive immunity at MB tumor site. The identification of biomarkers, a better characterization of the interactions of the tumor cells and its immune TME, associated to an increase of cytotoxic T-cells will lead to development of more effective therapies. The following research plan is described in the context of MB but would be of interest for other types of primary or metastatic brain tumors for future directions.

7A / 5

Insight on the tumoral cell diversity, their formation and proliferation in IDH1-mutant diffuse low brain gliomas: a key role for Notch1 signaling

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Gliomas affecting adults are devastating brain tumors. They are classically divided as high grade (glioblastomas) and diffuse low grade gliomas which have different mutations and prognosis. Recent single cell RNA seq and previous immunohistology studies have revealed that both high- and low-grade gliomas contain a diversity of tumoral cells with different phenotype and properties. Diffuse grade II gliomas (oligodendrogliomas and astrocytomas) are slow-growing brain tumors that progress into high-grade gliomas. A majority of these tumors have a mutation in the IDH1 (isocitrate dehydrogenase) gene. These tumors present an intratumoral cell heterogeneity, and no reliable markers are available to distinguish the different cell subtypes. In addition, the molecular mechanisms underlying the formation of this cell diversity is also ill-defined. A better description of this cellular heterogeneity and its formation would certainly help define innovative therapeutic strategies.

To study cellular heterogeneity and active pathway in IDH1-mutant diffuse low grade gliomas (oligodendrogliomas and astrocytomas tumors), we used immunofluorescences on cryosections of freshly-resected IDH1-mutant gliomas. We found that SOX9 and OLIG1 transcription factors, which specifically label astrocytes and oligodendrocytes in the normal brain, identified the presence of two largely nonoverlapping tumoral populations in IDH1-mutant oligodendrogliomas and astrocytomas. Astrocyte-like SOX9+ cells additionally stained for APOE, CRYAB, ID4, KCNN3, while oligodendrocyte-like OLIG1+ cells stained for ASCL1, EGFR, IDH1, PDGFRA, PTPRZ1, SOX4, and SOX8. GPR17, an oligodendrocytic marker, was expressed by both cells. These two subpopulations appear to have distinct BMP, NOTCH1, and MAPK active pathways as stainings for BMP4, HEY1, HEY2, p-SMAD1/5 and p-ERK were higher in SOX9+ cells.

Notch1 is a highly-conserved pathway controlling cell differentiation and proliferation during development and in several pathological situations. We used primary cultures and a new cell line to explore the influence of NOTCH1 activation/inhibition and BMP treatment on the IDH1-mutant glioma cell phenotype. This revealed that NOTCH1 globally reduced oligodendrocytic markers and IDH1 expression while upregulating APOE, CRYAB, HEY1/2, and an electrophysiologically-active Ca2+-activated apamin-sensitive K+ channel (KCNN3/SK3). This was accompanied by a reduction in proliferation. Similar effects of NOTCH1 activation were observed in non tumoral human oligodendrocytic cells, which additionally induced strong SOX9 expression. BMP treatment reduced OLIG1/2 expression and strongly upregulated CRYAB and NOGGIN, a negative regulator of BMP.

The presence of astrocyte-like SOX9+ and oligodendrocyte-like OLIG1+ cells in grade II IDH1-mutant gliomas raises new questions about their role in the pathology. This phenotypic interconversion mediated by Notch1 pathway may play a role in treatment resistant and tumor relapse.

Session 7B – Extracellular matrix & cancer

7B / 1

Extracellular Matrix Mechanical Properties and Regulation of the Intestinal Stem Cells: When Mechanics Control Fate

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Intestinal stem cells (ISC) are crucial players in colon epithelium physiology. The accurate control of their auto-renewal, proliferation and differentiation capacities provides a constant flow of regeneration, maintaining the epithelial intestinal barrier integrity. Under stress conditions, colon epithelium homeostasis in disrupted, evolving towards pathologies such as inflammatory bowel diseases or colorectal cancer.

A specific environment, namely the ISC niche constituted by the surrounding mesenchymal stem cells, the factors they secrete and the extracellular matrix (ECM), tightly controls ISC homeostasis. Colon ECM exerts physical constraint on the enclosed stem cells through peculiar topography, stiffness and deformability.

However, little is known on the molecular and cellular events involved in ECM regulation of the ISC phenotype and fate. To address this question, combining accurately reproduced colon ECM mechanical parameters to primary ISC cultures such as organoids is an appropriated approach.

Here, I'll discuss colon ECM physical properties at physiological and pathological states and their bioengineered in vitro reproduction applications to ISC studies.

7B / 2

The bacterial genotoxin, cytolethal distending toxin, modulates cell differentiation and elicits epithelial to mesenchymal transition

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We are frequently exposed to bacterial genotoxins of the gut microbiota, such as cytolethal distending toxin (CDT) and colibactin. The CDT genotoxin is prevalent in many clinically relevant mucosal pathogens. CDT is active as a heterotrimeric complex of which CdtB subunit is the active subunit that displays dual DNase and phosphatase activities. CDT triggers potent DNA damage in host cells that are predisposing factors in the development of cancers. CDT from Helicobacter hepaticus, a mouse pathogen, was shown to be directly involved in the development of murine hepatocarcinoma, demonstrating, for the first time, the role of CDT in cancer development. Preliminary studies have shown that CDT induces a certain phenotype reminiscent of epithelial to mesenchymal transition (EMT), a process by which cells lose their epithelial traits in favor of mesenchymal characteristics conducive to cell motility.

In the present study, we investigated the different steps of EMT process in response to CDT/CdtB. Whole genome microarray-based identification of differentially expressed genes was performed in vitro on intestinal epithelial cells while following the ectopic expression of the active CdtB subunit of H.hepaticus CDT. Microarray data showed a CdtB-dependent regulation of transcripts involved in EMT. The key transcriptional regulators of EMT, SNAIL1 and ZEB1, were upregulated both at the RNA and protein level in response to CdtB. EMT markers, Vimentin and Fibronectin, were also upregulated. CdtB also induced the disassembly of tight junctions, adherens junctions and desmosomes, as well as a decrease in cellular adherence. As expected, CdtB promoted a profound remodeling of the actin cytoskeleton and formation of cellular protrusions such as lamellipodia. Additionally, CdtB activated the expression and activity of some matrix metalloproteases and increased cell motility. This study demonstrated that CDT, via its CdtB subunit, elicits EMT, supporting the idea that infection with bacterial genotoxin-producing bacteria can promote malignant transformation.

7B / 3

Electric fields as powerful tools to transiently modulate cutaneous extracellular matrix

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PURPOSE

Some clinical applications of electroporation such as gene electrotransfer (GET) or antitumor drug delivery (electrochemotherapy, ECT) have shown that electrical field applied presents a beneficial effect on wound healing. However, no relevant studies were led to better understand the impact of electric field on human cutaneous cells and extracellular remodelling. In the presented study we focused on extracellular matrix (ECM) remodelling following electroporation in a self-assembled in vitro human skin substitute.

METHODS

Self-assembly approach was used to produce human dermal substitutes rich in endogenous ECM. In this approach, cells produce themselves their own ECM, meaning that no exogenous scaffold is used to promote the 3D organization. Electric parameters applied in ECT and GET conditions are the ones classically used in the literature. Composition and organization of ECM were analyzed by optical and electronic microscopy, Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared spectroscopy (IRTF-ATR). Genes modulation was analyzed by transcriptomic performed 4h post-electroporation using Clariom S Affymetrix array. ELISA assays were used to quantify several growth factors (PDGF, TGFβ) or matrix-related proteins (collagens, MMPs, TIMP) expression over the time. Collagens were chemically quantified in whole tissue by hydroxyproline assay. Metalloproteinases MMPs activities were also quantified over the time post-electroporation.

RESULTS

Electronic microscopy and IRTF-ATR allowed to visualize a polarization of the dermal substitute which is rich in cells on the top (in contact with cell culture medium) and in collagens on the bottom (in contact with petri dish). We demonstrated that both ECT (microsecond) and GET (millisecond) pulsed electric fields induced 1) a rapid modulation (4h after electrostimulation) of mRNA's genes composing the matrisome, particularly a down-regulation of pro-collagens and ECM maturation's enzymes such as transglutaminase TG2 and LOX-like; 2) a transient decrease in pro-collagens production and hydroxyproline tissue content within a week after electrostimulation; 3) a long-lasting ROS-dependent over-activation of MMPs for at least 48h and 4) a down-regulation of TGF-β.

CONCLUSION

We finely characterized ECM contained in human dermal skin substitute through cutting edge methodological approaches (microscopies, IRTF-ATR and DSC). We demonstrated that electroporation induced a transient collagen 's degradation, through decrease of collagens secretion and maturation in parallel with the increase of MMPs activity dependant on ROS generated by the cells. These observations underpin that pulsed electric fields, a technology already approved for clinical use combined with anti-cancer agents, are particularly promising to provide local and effective treatment of abnormal ECM. This local degradation of ECM could improve drug delivery efficiency at tumor site for example.

7B / 4

Using nanoparticle tracking analysis (NTA) for appreciation of up and down extracellular vesicle secretion

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Extracellular vesicles consist in heterogenous small vesicles, bounded by portions of plasma membrane, and secreted by most eukaryotic cells, being healthy or pathological. They are classically divided in microvesicles, exosomes and apoptotic bodies; the classification being mainly based on biogenesis, size and composition of the vesicle. Due to the variety of origin, the size range is comprised between 30 and 1000 nm, exosomes being known to be the smallest (30-150 nm) (Kurian TK, 2021). The content of these vesicles is both the reflect of the donor cell's unsorted composition and a result of selection, in addition to non-specific exosomal constitutive proteins. So, they have functions in cell signaling, modifying their surrounded cells' behavior. This capacity is particularly important in tumor development (Mashouri BMC, 2019). The RAB family, especially RAB27A and RAB27B, has been described to be crucial for exosome secretion (Van Niel et al 2018 ; Auger et al ; Brunel et al, 2021). Both isoforms are responsible for the docking and fusion of MVBs on plasma membrane, the last step before exosome release. When working with such EVs, or more strictly with exosomes, one of the difficulties relies on the reproducibility and standardization of techniques used for their characterization (editorial, JEV 2020).

In the present study, our initial aim was to evaluate effects of exosome secretion inhibition in two tumoral models, glioblastoma and colorectal cancer. To reach this goal, we performed transfection of two representative cell lines, respectively U87-MG and HCT116, with 3 different shRNA targeting RAB27a. EVs were isolated following an adaptation of Théry et al.'s protocol (Théry et al., 2006) using differential ultracentrifugation's. The final pelleted and washed EVs are then resuspended either in PBS for Nanotracking Analysis (NTA), or in cell lysis buffer for Western Blot analysis. As an alternative, the cell culture supernatants were also analyzed after only the 300 x g and 2 000 x g centrifugations (named crude supernatant). EVs quantification was evaluated using NanoSight NS300® (Malvern Panalytical Ltd, UK) with specific parameters according to the manufacturer's user manual (NanoSight NS300 User Manual, MAN0541-01-EN-00, 2017).

Whereas tanscriptomic and proteic analysis confirmed RAB27a down-regulation, we did not detect any EVs quantitative variation with NTA analysis, neither after EVs purification nor in crude supernatant. In a second step, we aimed to reduce EVs secretion either by transient transfection with siRNA RAB27A or pharmacological inhibitors (nexinhib20 or indomethacin). Same lack of quantitative variation was observed with the 3 different approaches.

In light of these data, we wondered if NTA allows monitoring of EVs secretion's enhancement. To this purpose, we either treated both cell lines with 0.5 µM rotenone (Wu et al., 2015, Neuroscience) or we cultured them in hypoxia (1% O2) for 48h vs normoxia (20% O2). The EVs secretion in crude supernatants was significantly enhanced in rotenone-treated pLKO (control: scramble shRNA) cells for both HCT116 and U87-MG, without modification of the mean size. Concerning hypoxic conditions, the NTA analysis showed that both cell lines produced significantly more EVs after 48h of 1% O2 culture. Such an enhancement of EVs secretion started to appear straight after 24h of hypoxia even if it reached significant values only for U87.

Our work emphasized the weakness of using only one characterization method to assess EVs secretion. Western blotting is ongoing in order to evaluate the EVs content, which could explain a variation in the nature of EVs rather than in the quantity when inhibiting the exosome secretion.

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Tumor cells-released tracks promote cell migration on type I collagen

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Metastasis is the leading cause of cancer-related deaths. During this process, tumor cells acquire invasive and migratory capacities in order to invade the surrounding tissues. To this, the tumor microenvironment is modified to facilitate cancer cells proliferation and dissemination. Multiple mechanisms are involved in this evolution, including cell-cell communications through the tumor microenvironment and the extracellular matrix modifications. Indeed, extracellular vesicles such as exosomes or migrasomes are already known to induce protumor features such as migration, promoting tumor development and metastasis formation.

Here we describe a new type of extracellular vesicles (referred as tracks) specifically released by cancer cells along type I collagen fibers during cell migration. We could characterize these tracks, their structure as well as their composition in term of proteins and nucleic acids, and could show that they are different from classical extracellular vesicles known so far. These tracks are characterized by a discoidin domain receptor 1 (DDR1) staining. Moreover, these tracks are very stable structures and can be internalized by neighboring cells. After internalization, they can modify the differentiation status of cells able to internalize these tracks.

These data suggest that these collagen-associated tracks have a role in cell-cell communication and participate in the remodeling of the tumor microenvironment. Even if their function needs to be fully elucidated, their protein and RNA compositions could suggest that these tracks could help to promote cell proliferation and invasion.

These tracks seem to be a new player in the tumor invasion process and could provide a better understanding underlying this process.

Session 7C – Le patient formateur

7C / 1
L'expérience de Bobigny

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Deux programmes visant principalement à réduire les injustices épistémiques composent l'expérience de Bobigny qui se rapporte à des enseignements par et avec des patients dans des formations initiales en santé. Celle-ci repose sur trois fondements : (1) un fondement démocratique : les patients sont recrutés par des patients et ils sont des collègues comme les autres ; (2) un fondement épistémique : les patients sont recrutés pour leurs savoirs et (3) un fondement pragmatiste : les apprentissages se font sous forme d'enquêtes basées sur l'expérience des étudiants et des patients et les programmes sont révisés au fur et à mesure des évaluations, en vue de leur optimisation. Dans le cadre du premier programme (PEP13), les évaluations conduites ont montré que la « perspective patient » s'enseigne d'autant plus qu'elle est stabilisée car élaborée collectivement et que son appropriation s'évalue notamment via la réduction des injustices épistémiques herméneutiques et des stigmatisations discursives. Quant au second programme (EXPAME), dans lequel sont mobilisés les savoirs expérientiels explicites de patients, il produit des savoirs incorporés conduisant à des apprentissages transformationnels diminuant les injustices testimoniales, sous réserve qu'ils soient mobilisés dans de petits groupes d'étudiants réunis en inter-professionnalité et que les apprentissages soient basés sur des enquêtes expérientielles qui alternent une pédagogie des moments de réussite (Galvani, 2020) et une pédagogie par l'erreur (Giordan, 2012).

7C / 2

Du témoignage bénévole à la co-construction d'une séquence pédagogique rémunérée

Sandra ALLAIRE DE LA FUENTE

Patient formatrice
Présidente de l'association : Le repaire de Kikou.

En 2019, je viens de terminer les traitements liés à mon deuxième cancer. La coordinatrice de la Ligue contre le cancer me propose de venir témoigner auprès d'étudiants en oncologie. L'idée est de leur présenter les soins de support dont j'ai bénéficié, en quoi cette prise en charge a été bénéfique et quel soutien les associations peuvent représenter pour eux aussi. Je suis heureuse de cet échange, de leur intérêt, de la pertinence de leur question. Je leur présente même mon projet d'un livret pédagogique pour parler du cancer aux enfants. Moi qui sors, juste de ce parcours difficile, je ressens une certaine fierté d'être là. Par la suite, je m'inscris au DU de PP en cancérologie de Sorbonne Université. Entre autres, j'y apprends à présenter un témoignage à visée pédagogique selon la méthode de CTT, dont 5 questions :

- Comment la maladie est arrivée dans votre vie … ?
- Ce que la maladie vous a appris ?
- De quoi auriez-vous eu besoin en termes de … ?
- Qui vous a aidé(e), comment et de quelle manière précise …
- Qu'est-ce que vous aimeriez transmettre comme message maintenant ?

En parallèle la Ligue contre le cancer me propose un nouveau témoignage pour un IFSI. Là, je coconstruis la présentation avec la Cadre chargée de la formation qui me propose de me rémunérer pour les 2 heures de témoignage et d'échanges. L'idée est justement la visée pédagogique pour les étudiants. Selon la méthode j'expose mon parcours. Les questions des étudiants sont relatives à ma maladie et aux conséquences sur ma vie. Cet échange me ravit mais je ne l'analyse pas encore.

Après le DU, je poursuis par 3 Master-Class : ETP, GAP, et Patient formateur occasionnel des soignants. Dans le cadre de cette formation Patient formateur Sorbonne nous propose d'intervenir dans une école d'ergothérapie, avec des intervenants et des observateurs. Cette prestation est également rémunérée. Comme pour l'IFSI, je suis ravie de la pertinence des questions.

Ce que j'aime avec cette méthode de témoignage ce sont les questions que l'on me pose lors des échanges, ces questions que j'aurai aimé que l'on me pose quand j'étais en traitement pour une prise en charge globale de ma maladie. En leur retournant le compliment sur la pertinence de leurs questions, quelque part, je leur donne l'autorisation de faire de même avec les patients. Ça c'est ce qui m'anime aujourd'hui.

Récemment, au fur et à mesure de nouvelles rencontres, notamment au comité de pilotage du CHU de Bordeaux, je me vois confier de nouvelles missions, dont la prochaine est de coconstruire une séquence pédagogie en ETP pour les IBODE. Là, je serais rémunérée pour un temps entre 4 et 6 heures.

Voilà, comment d'un témoignage bénévole, aujourd'hui, je crée des séquences pédagogiques à destinées des futurs soignants pour lesquelles je suis rémunérée.

7C / 3

Du paternalisme médical au partenariat en santé : accompagner le changement auprès des établissements de formation des professionnels de santé

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La participation des patients à la formation des professionnels de santé constitue une rupture que l'on peut qualifier de paradigmatique, non seulement au regard de ce qui a cours dans ce champ particulier que, de façon plus large, au regard de ce que l'on peut observer dans l'ensemble des formations préparant aux métiers adressés à autrui. L'objet de cette contribution est d'envisager cette mobilisation de patients partenaires formateurs à la fois comme un changement effectif et comme un opérateur potentiel de changement. Sur le premier axe, il s'agit de considérer que l'introduction de ce partenaire singulier conduit à repenser les logiques profondes qui prévalent concernant l'ingénierie de formation. Le deuxième axe se propose quant à lui d'élargir le périmètre de la réflexion et de considérer qu'en agissant sur la formation on agit aussi sur le métier et sur son devenir. C'est l'hypothèse selon laquelle l'introduction de patients partenaires formateurs contribue à la promotion du partenariat en santé que nous nous proposons d'explorer. Nous nous appuierons pour ce faire :

- sur une recherche-intervention commanditée par l'Agence Régionale de Santé Occitanie visant à promouvoir le partenariat en santé et sur le fonctionnement et les productions de l'un des groupes de travail dédié à la formation des professionnels de santé et des patients.
- sur l'expérimentation déployée au sein des Institut de Formation en Santé de Bordeaux afin d'implémenter le partenariat en santé.

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Lancement d'un dispositif de formation par les patients au sein de la ligue contre le cancer de Gironde | intervention à distance

Guy KANTOR et patients témoins de la ligue contre le cancer

Institut Bergonié

Session 8A – Poor prognosis Cancers: Pancreatic cancer

8A / 1

Learning from biology: The challenge of pancreatic cancer prevention

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The progress of the knowledge on pancreatic cancer genetics and biology in the last two decades has been formidable. However, our understanding of its etiology is still limited impairing the definition of high-risk populations. We will discuss the following points: Which are the true preneoplastic lesions? What is their genetic make-up? What is their risk of progression? Are we making good use of mice to understand these processes in humans? Can we prevent their progression to cancer? Can we make use of this information to identify high-risk individuals? Which are the challenges to integrate all the data to reach personalized pancreatic cancer prevention?

8A / 2

Discovery of Soluble Pancreatic Cancer Biomarkers Using Innovative Clinical Proteomics and Statistical Learning.

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Pancreatic ductal adenocarcinoma (PDAC) late diagnosis is primarily attributed to its asymptomatic progression combined with absence of any reliable screening markers. This leads to one of the deadliest cancer with a 5-years survival rate less than 10%[1]. Diagnosis is provided by endoscopy-guided fine-needle biopsy (EGFNB) only, which is invasive, risky and with a poor level of negative predictive value (NPV). Nevertheless, EGFNB remains the gold standard for diagnosing PDAC and enabling the right treatment for the patients.

In this proof-of-concept study we developed a novel proteomic approach which recovers the soluble proteins in the EGFNB that remains a rich source of potential biomarkers[2]. Proteomic analysis of the soluble proteins led to over 2500 identifications, which were subjected to subsequent statistical analysis. To build the subsequent protein signature score (PSS), we used several resampling methods[3-6] at different steps of the analysis and an algorithm derived from microarray analysis techniques[7,8].

We followed 58 patients that underwent pancreatic EGFNB, of which 43 were diagnosed as PDAC while 15 had non-cancerous lesions. The PSS achieved 0.917 and 0.853 of sensitivity and specificity rates respectively. We then linked the PSS with clinical data to provide a decision algorithm achieving 100% of positive predictive value and 92.3% of NPV.

Due to their soluble nature, the newly discovered protein biomarkers bare the potential to be detected in the patient serum. This will enable the development of non-invasive blood-sample based assays to a larger patient cohort, leading to the hope of promoting a population-based screening test, allowing for quicker management at an earlier stage.

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Translatome-based classification reveals a dual metabolic dependency of a new tumor subtype of pancreatic cancer.

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Molecular profiling of Pancreatic Ductal Adenocarcinoma (PDA) transcriptomes identifies two main prognostic subtypes (basal-like and classical), but does not enable personalized first-line treatment. Although mRNA translation is highly dysregulated in both PDA cancer cells and their microenvironment, there is a paucity in transcriptome-wide studies of mRNA translation.

To assess whether mRNA translation could provide a distinct perspective on PDA, we used a collection of twenty-seven pancreatic Patient-Derived Xenografts (PDX) and studied their transcriptome-wide variation of mRNA translation (translatome). An unsupervised bioinformatics analysis of translation efficiencies across tumors revealed a subtype exhibiting a low protein synthesis rate in combination with activated translation of mRNAs encoding effectors of the integrated stress response (ISR), including the transcription factor ATF4. Functional characterization of the "ISR-activated" human cancer cells revealed high drug resistance, low autophagic capacity, and importantly, metabolic impairments in the serine synthesis and transsulfuration pathways.

Therefore, the drug-resistant cancer cell phenotype showing auxotrophy to both serine and cysteine may be amenable to targeted therapy.

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Targeting cancer cells with basal-like, mesenchymal phenotype with oncolytic virus to inhibit the growth of pancreatic cancer.

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Pancreatic cancer (PDAC) is soon to become the second cause of death by cancer in the western world and remains one of the most aggressive of all cancers due to the lack of efficient treatment or diagnostic markers. Recently, molecular investigations revealed two main tumor phenotypes that stratify patients with PDAC. While gene expression classifications proved prognostic value, PDAC molecular subtyping is yet to inform precision medicine strategies. Oncolytic virotherapies are fastly emerging as credible anticancer agents for the treatment of numerous cancer entities. Oncolytic viruses have already shown great safety during clinical trials. Among them, the fibrotropic minute virus of mice prototype (MVMp) shows promise, but its oncolytic potential has not been explored in PDAC models. We report here that MVMp specifically targets and kills primary pancreatic cancer cells with a mesenchymal, basal-like profile, both from mouse or patient origin, in vitro and in vivo, when cells with more classical plhenotype were left unarmed. Molecular investigations indicate that RhoC is critical for MVMp infection of cancer cells. Systemic MVMp injection recruits and synergizes with the immune system to provokes tumor growth inhibition in orthotopic syngeneic models of PDAC with basal-like, mesenchymal phenotype. Agzain, tumor cells with classical phenotype were moderately impacted by oncolytic treatment. Collectively, we demonstrate herein for the first time that MVMp is specific and oncolytic in PDAC tumors with mesenchymal, basal-like profile, paving the way for precision medicine opportunities for the most aggressive form of PDAC tumors.

8A / 5

Towards the identification of Cancer-Associated Fibroblast signaling underlying pancreatic cancer aggressiveness

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Pancreatic ductal adenocarcinoma (PDAC) presents an exuberant stroma (80% of the tumor mass). In this stroma, Cancer-Associated Fibroblasts (CAFs) are the most abundant cells. CAFs secrete large quantities of extracellular matrix and soluble proteins that promote cancer cell aggressivity. We discovered two pharmacological approaches to inhibit CAF pro-metastatic effects.

First, we targeted protein synthesis, which is specifically high in CAFs as compared to normal pancreatic stellate cells, through activation of the G protein coupled somatostatin receptor sst1. We showed synergistic anti-metastatic effects when associating the sst1 agonist (SOM230, Novartis) to chemotherapy (gemcitabine), in the KPC (Pdx-1-Cre ; LSL-KrasG12D/+ ; LSL-Trp53R172H/+) mouse PDAC model, involving reduction of both tumor cell aggressiveness and ECM deposit, in correlation with decreased tumor recruitment of M2 macrophages and reduced tumor and plasmatic CSF-1 concentrations.

Second, we explored the benefit of Focal-Adhesion Kinase (FAK) therapeutic targeting in CAFs, since fibrosis and pancreatic tumor stiffening mainly involving CAF production of ECM may require FAK activity. Our data show that FAK activity is increased in CAFs as compared to normal pancreatic stellate cells, in correlation with a worse prognostic in PDAC patients (n=120). FAK inhibition within CAFs results in a drastic decrease of tumor cell metastasis in vivo as well as of ECM protein expression and deposition in vitro.

Those results support the idea that protein synthesis and FAK activity within CAFs are key and druggable players in PDAC metastatic progression.

Session 8B – Le patient co-chercheur

8B / 1

Implication des patients en recherche : retours d'expériences et réflexions sur différentes modalités

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Site de Recherche Intégrée sur le Cancer (SIRIC) - Bordeaux

BRIO, le Site de Recherche Intégrée sur le Cancer (SIRIC) de Bordeaux, est labellisé pour 5 ans par l'Institut National du Cancer en réponse à un appel d'offre compétitif visant à promouvoir des programmes de recherche translationnelle et intégrée sur le cancer. Au-delà de ses missions d'accompagnement scientifique et de communication, BRIO souhaite donner une place plus importante aux patients dans le processus de recherche afin de ne plus seulement faire POUR, mais aussi AVEC les patients, ex-patients et aidants.

Cette implication se manifeste notamment par la création, dès 2016, d'un collectif : ASPERON & Co (Associations et Patients Engagés pour la Recherche en Oncologie & Communauté professionnelle) qui réunit (ex-)patients, proches, bénévoles d'associations, et professionnels de la santé et de la recherche. Tous les ans, les membres du collectif se penchent sur une thématique en lien avec la recherche sur le cancer, partagent savoirs scientifiques et expérientiels, et produisent, avec l'équipe de BRIO, une soirée (ou des contenus vidéos) à destination du grand public et des ressources associées.

En parallèle de ces actions, BRIO ambitionne d'impliquer des patients sur d'autres aspects que celui de la vulgarisation scientifique. Avec la création d'un groupe de patients-partenaires pour accompagner le suivi d'un projet de recherche en épidémiologie et d'un comité de patients et aidants pour préparer la relabellisation du SIRIC, BRIO teste différentes modalités et tente de démontrer les intérêts que peuvent représenter, à la fois pour les patients et les professionnels de la recherche, l'implication de patients dans de tels dispositifs :
- Le projet de patients-partenaires du suivi de l'étude REALYSA (étude nationale en vie réelle sur les lymphomes) s'intéresse à l'impact de la création d'un groupe de patients-partenaires sur le suivi de cette étude par les participants, durant les 9 années prévues. Ce groupe de patients réfléchira notamment à la communication autour de l'étude et la création d'une communauté REALYSA.
- La mise en place d'un comité de patients et aidants, afin de faire participer au processus d'élaboration du dossier de relabellisation SIRIC, quelques patients et aidants sélectionnés : de la rédaction de l'appel à projets pour inviter les chercheurs à déposer leur projet de recherche à l'accompagnement des projets retenus en termes de lien recherche-société, y compris sur l'implication des patients.

Au travers de ces initiatives qui se déroulent actuellement, BRIO expérimente différentes modalités de partenariat entre la recherche et les patients et s'interroge sur les leviers et les freins à l'implication de patients en recherche. L'équipe de BRIO partage et confronte régulièrement ses expériences avec d'autres acteurs de l'engagement, à la fois dans le domaine de la recherche mais aussi du soin et de la formation. Cette session est l'occasion de présenter les modalités et les leçons apprises de ces expériences, et d'échanger sur les réflexions qu'elles ouvrent sur l'implication des patients en recherche.

8B / 2

Construire un appel à projets avec et pour les patients : retour d'expérience du Cancéropôle CLARA

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En janvier 2021, le Cancéropôle Lyon Auvergne-Rhône-Alpes (CLARA) a ouvert un appel à projets intitulé « L'expérience patient au cœur des projets de recherche en biologie médicale, en technologies pour la santé et en sciences humaines et sociales ». La particularité de cet AÀP était double puisqu'il s'agissait, d'une part, de soutenir des projets de recherche intégrant un ou des patients comme partenaires de recherche, mais aussi, d'autre part, de co-construire cet AÀP avec les patients eux-mêmes.

Cette initiative s'inscrit dans la ligne des recommandations émises par la HAS, en particulier celle intitulée Soutenir et encourager l'engagement des usagers dans les secteurs social, médico-social et sanitaire, publiée en juillet 2020, qui vise notamment à « promouvoir l'engagement des personnes concernées dans les programmes de recherche ou d'évaluation et dans la conception de solutions innovantes en santé ou en accompagnement social et médico-social ».

Cette communication vise à partager l'expérience récente, et nouvelle pour le Cancéropôle CLARA, de co-construction de ce dispositif, avec les « premiers concernés ». Elle entend présenter la façon dont, concrètement, les expertises de ces acteurs peuvent être intégrées à toutes les étapes de la construction d'un dispositif de financement de la recherche, de l'écriture du cahier des charges au suivi des projets, en passant par la conception de la grille d'évaluation et la définition des modalités de sélection des projets. Elle montrera que toutes les disciplines scientifiques ont pu être mobilisées, et synthétisera les difficultés, enjeux et réussites de cette initiative. Elle visera aussi à appréhender son esprit de collaboration concrétisé avec le département universitaire des patients Grenoble Alpes créé en décembre 2020.

8B / 3

Recherche profane et autothérapie. Quelques pistes sur le concept de recherche translationnelle centrée patient

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Bien que la théorie des mutations somatiques (TMS) soit actuellement le paradigme dominant dans la recherche en cancérologie, il existe des théories alternatives, largement moins répandues, mais qui, sous plusieurs rapports, viennent questionner la TMS. L'une d'entre elle fait du métabolisme - et plus spécifiquement de l'effet Warburg - un élément clé de la cancérogenèse. Par la voie de l'analyse scientométrique, il est possible de montrer que quatre des publications du biologiste allemand Otto Warburg, prix Nobel de physiologie ou médecine « pour sa découverte de la nature et du mode opératoire de l'enzyme respiratoire », présentent un profil citationnel typique des phénomènes de reconnaissance tardive. Peu cités entre les années 1950 et 2000, ces travaux, publiés pour deux d'entre eux dans Science, voient leurs scores de citation s'envoler. Il semble que ce regain d'intérêt ne soit pas lié à la traduction des savoirs produits par les approches métaboliques du cancer en traitements reconnus. Pourtant, des patients - dont il est difficile d'estimer le nombre - pratiquent des formes d'autothérapie non médicamenteuse basés sur des travaux qui s'inscrivent dans l'approche métabolique. Ce constat m'a convaincu de participer au développement d'un projet de recherche axé sur la piste métabolique, qui présente la particularité de s'appuyer sur l'observation de ces pratiques d'automédication.

Cette posture exige d'abord une meilleure connaissance de la façon dont les patients qui se traitent appréhendent leurs pathologies cancéreuses et les thérapies existantes. Elle exige par ailleurs que l'on réfléchisse à la façon dont il est possible d'articuler une épistémologie de cette connaissance profane avec une épistémologie des sciences du vivant et de la médecine, de voir comment les résultats qu'obtiennent les patients en autothérapie pourrait aboutir à des questionnements biologiques et médicaux susceptibles de déboucher sur une meilleure compréhension des cancers et sur des traitements. Cette réflexion épistémologique doit en outre se doubler d'une interrogation sur les modalités possibles de la collaboration entre biologistes, médecins et patients qui ne partagent pas le même espace social d'ancrage. C'est en d'autres termes la formulation de quelques pistes d'investigation qui est visée dans cette communication autour de ce que l'on pourrait appeler une recherche translationnelle centrée patient puisque les malades seraient à la fois à l'origine et à l'aboutissement du mouvement de translation des savoirs.

Posters – Axis 1 “Cell signaling and Therapeutic Targets”

P101
Loss of Rnd3/RhoE favors entosis in hepatocellular carcinoma cells

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Entosis is a form of cell-in-cell structures (CICs), it is defined by the invasion of one or more viable cells into another of the same type. The formation of entotic cells is extensively studied *in vitro* after matrix detachment or under glucose-starvated condition. The entotic cell engulfment is controlled by the epithelial adherens junctions and the proteins of RhoA/ROCK pathway. The new clinico-histopathological studies suggest that entosis might be associated with an aggressive phenotype and poor prognosis in many cancers. However, entosis has never been studied in hepatocellular carcinoma (HCC).

Using various approaches, the aim of our study was to characterize the entosis mechanism in HCC. To do so, we searched for entosis inducers in HCC cell lines, we characterized the different stages of the process and we addressed the molecular regulators of this phenomenon.
Our results demonstrate an increase in the number of entotic events in HCC cells when cultured in suspension state and after nutrient-deprivation. We further used electron, confocal and time-lapse microscopy to describe the stages and the fate of entotic cells. Moreover, we found that entosis is induced after Rnd3 silencing in HCC cells. Rnd3 protein, belonging to the Rho GTPase family, is a negative regulator of RhoA/ROCK pathway. We demonstrated that entosis induced by Rnd3 inhibition is dependent on the presence of RhoA and the protein kinase ROCK. We further developed a global approach in order to identify specific markers of entotic cells. A proteomic analysis on the dissected HCC entotic cells allowed us to identify new markers of entotic cells. These markers are currently used to help the detection of entotic cells in human HCC tissues.

In conclusion, we found that HCC cells are able to perform entosis. The relationship between the expression of Rnd3 and the entosis mechanism is crucial, as our published data identified Rnd3 as a tumor suppressor in HCC. Altogether, these data suggest the involvement of entosis in liver tumor progression and highlight a new perspective for the entosis analysis in medicine research as a novel therapeutic target.

P102

Unravelling the role of early dissemination in colorectal cancer

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Introduction:
Over 8 million people die from cancer every year almost invariably due to metastasis but despite more than 100 years of research, tumor dissemination remains poorly described.
In the classical model of metastasis, tumor cell dissemination occurs at late stages of tumor development. However, some studies performed in different types of cancer such as in melanoma, breast and pancreatic cancers; suggest that rare tumor cells spread to distant sites much earlier than previously believed. Despite colorectal cancer's CRC high incidence and mortality, worldwide, early tumour dissemination has not yet been studied in this cancer.

Our aims were first to demonstrate that early tumour dissemination process occurs in CRC using a genetically engineered mouse model, to assess the roleof early disseminated cells eDTC in distant organs as well as to validate data on patient blood samples.

Material and methods:
We have generated an inducible mouse model that enables us to lineage trace dissemination at the very early stages of tumoral development thanks to the expression of tdTomato and deletion of APC gene specifically in the intestinal epithelium. eDTCs were searched in the liver, the main organ that is prone to get colonized by metastatic CRC cells, using tissue clearing, intravital live imaging, and immunolabelling. The impact of eDTCs in the liver was assessed using CyTOF/Hyperion and validated with immunostainings and confirmed with in vivo using adapted assays.

Results and discussion:
In the liver of these mice, we first demonstrate the presence of eDTCs along with a strong infiltration of macrophages and cancer-associated fibroblast CAFs suggesting a microenvironmental remodelling. Concomitant with the theory of seed and soil, this liver remodelling has a strong impact on metastatic colonization where we have demonstrated that it enhances the welcoming of future waves of metastatic cells harboring powerful mutations.

Conclusion:
In conclusion, this project functionally validates for the first time the existence of an early dissemination process in CRC in mice and proposes a causal role of these cells in an early pre-metastatic niche preparation. Further studies to deeply characterise the liver remodelling are ongoing in order to identify potential actionable targets to prevent this early pre-metastatic niche preparation.

P103

Mutated ß-catenin regulates extracellular vesicles machinery in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the most common primary liver tumor in adults and represents the sixth most common cancer in the world and the fourth leading cause of cancer-related death. HCC is a pathology with a poor prognosis: the diagnosis remains challenging because of its difficulty to be detected early in disease's progression and due to the lack of effective therapies. Since 2008, Sorafenib (multi-kinase inhibitor) has been the first-line reference for the treatment of HCC. More recently, several clinical trials based on immunotherapy have shown their effectiveness on overall survival. Thus, the combination Atezolizumab (anti-PDL1 antibody) with Bevacizumab (anti-VEGF antibody) is now the first-line treatment for advanced HCC. Despite this therapeutic advance, clinical data suggest that immunotherapy could be less effective in patients with β-catenin-mutated HCC. These tumors are characterized by an environment devoid of immune infiltrates, leading to resistant-immunotherapy tumors. However, how the β-catenin oncogene promotes this immune escape and how tumor cells trigger immunosuppressive cascades is not yet fully understood. Our project focuses on the involvement of β-catenin signalling in tumor cells/immune cells communication through extracellular vesicles (EVs).

Using a transcriptomic analysis performed in HepG2 cells, an alteration of the EVs machinery upon knock-down of mutated β-catenin has been identified. In the same model, we also found a defect in the secretion of EVs when β-catenin is mutated. We further identified two target genes of the EVs machinery whose expression is dependant on β-catenin signalling. These results were confirmed in two other cell lines, and in HCC human samples mutated for β-catenin. Thus, these results suggest that β-catenin mutations may inhibit EVs formation and/or secretion in liver tumor cells. As EVs and their contents (chemokines, mRNAs, miRNAs…) are essential factors for intercellular communication, we now hypothesized that this decrease in the production of EVs could lead to defective recruitment of leukocytes, making these tumors poor in immune infiltrates and resistant to immunotherapy.

Our results provide new knowledge on the impact of β-catenin mutations on the tumor microenvironment and may allow the development, from liquid biopsies, of a new tool for stratifying patients with HCC for the response to immunotherapies.

P104

Characterization of a novel monoclonal antibody targeting tumor-associated macrophages

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Tumor-associated macrophages (TAM) belong to the major cell population of tumor microenvironment (TME), supporting tumor development and resistance to therapy. Targeting of TAM remains challenging, owing to their remarkable plasticity and lack of exclusive markers. To address these problems, Team 9 of CRCT developed and patented the monoclonal antibody called 6-25, using Nurse-like cells (NLC) as a model of TAM. NLCs are a type of TAM found in chronic lymphocytic leukemia (CLL), which are important for the survival of cancer B-CLL cells and can be easily generated in vitro. The 6-25 antibody has been characterized to specifically bind to various human TAM. Studies showed that naked 6-25 antibody was not toxic toward NLC or other cells present in PBMC from CLL patients and healthy donors, but could be efficiently internalized by NLC. These findings led to development of antibody-drug conjugate (ADC) version of the 6-25, that proved to selectively deplete NLC and M2 macrophages in vitro. Research on 6-25 target showed that it is a specific marker of M2 macrophages and foremost of protective NLC. Using combination of immunoprecipitation-quantitative mass spectrometry approaches and RNAseq analyses the target of 6-25 antibody was recently identified. These results will allow to further study the role and mechanism of expression of this molecule in protumoral TAM. Beside, these findings will be essential to further develop the project and eventually to use the 6-25 antibody in anti-cancer therapies, as an agent specifically targeting protumoral TAM.

P105
Remobilize Leukemic Stem Cells under TKI in CML

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BCR-ABL is a chimeric oncogene generated by a reciprocal translocation between chromosomes 9 and 22 (Philadelphia chromosome) that leads to the fusion of the BCR gene with the ABL gene, inducing the oncoprotein BCR-ABL. This translocation is found in most patients with Chronic Myelogenous Leukaemia (CML). The constitutively activated oncoprotein BCR-ABL induces multiple signalling abnormalities, leading to enhanced proliferation, inhibited apoptosis and stimulated migration in hematopoietic stem cells. BCR-ABL tyrosine kinase inhibitors (TKI) are effective in inducing remissions, and prolonging survival of CML patients. However, TKI treatment fails to eliminate quiescent leukemic stem cells (LSC) in hematopoietic niche. The aim of this study was remobilize quiescent leukaemia stem cells through BCR-ABL signalling pathway.

We used Ba/F3 cells stably transfected by $p210^{BCR-ABL}$. We observed Ba/F3p210 cell motility by time-lapse microscopy (JuliStage) under TKI, SOCE (Store Operated Calcium Entry) inhibitor SKF96365 and EGF (Epidermal Growth Factor) treatment. We also studied calcium entries through SOCE, Rho GTPase activation state and co-immunoprecipitation experiments allowing characterization of BCR-ABL and channel complexes in the different conditions. Finally, we used leukaemia stem cells from CML patients (C34+ LSC) to study proliferation/quiescence state in these conditions.

Previously, we showed that RhoA-GTPases activation and BCR-ABL-binding was controlled by p210BCR-ABL DH/PH domain and Rac1/Cdc42-GTPases activation was Vav-dependent after phosphorylation by BCR-ABL.

We show here that TKI induced immobilization of Ba/F3p210 cells, caused by inactivation of Vav/Rac and ROCK/ADF/MLC signalling pathway, although RhoA was still under activated form in this condition. Surprisingly, SKF-96365 (SOCE inhibitor) treatment in addition to TKI triggered amoeboid movements without displacement. Cell motility was fully restored by EGF (Epidermal Growth Factor) in addition to TKI and SKF96365. We show that the RhoA and Rac1 signalling pathways are restored in these conditions. On CD34+ LSC, we measured the proliferation after sorting (CD34+/CD38-/CD49f+). TKI (4 days) inhibits the proliferation of a part of the CD34+ LSC. TKI and SKF induced a slight increase of non-proliferating CD34+ cells but increased apoptosis/necrosis of CD34+ LSC. We also show that the non-proliferating cells express more SOX2 than proliferating cells.

Together, our results highlight a link between BCR-ABL, SOC channels and Rho GTPases in BCR-ABL cell lines and in CML patients. Activation mechanisms of Rac1 during TKI/SKF96365/EGF treatment remain to be explained. Mechanisms of TKI and SKF96365 treatment on proliferation seem to be related to SOX2-regulator proteins.

P106
A mouse model of Monoclonal Light Chain Amyloidosis

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Background: Monoclonal Light Chain Amyloidosis (AL) is a paraneoplasic disease due to the production by a plasma cell clone of an abnormal immunoglobulin (Ig) light chain (LC) whose aggregation in fibrils (so called amyloid fibrils) and deposition in tissues eventually lead to end-organ dysfunction. Although mostly associated with small and otherwise asymptomatic proliferations (MGUS or Monoclonal Gammopathy of Undetermined Significance), targeting the plasma cell clone using therapies for multiple myeloma is mandatory to stop the evolution of the disease. However, deposited fibrils can last for long in organs leading to frequent patient death. Despite being the most frequent type of amyloidosis, research on AL amyloidosis suffers from the lack of reliable animal models that could allow a better understanding of the disease and the design of new therapeutic strategies. In the present study, we aimed at reproducing AL amyloidosis in a mouse model.

Methods: We developed an original transgenic approach using an insertion of a human pathogenic LC gene in the endogenous mouse kappa locus, such as the LC is produced by the naturally Ig producing B and plasma cells. Then, to avoid the association of human LCs with endogenous murine HCs, we backcrossed this strain with the DH-LMP2A mice, characterized by a high number of plasma cells devoid of endogenous HC. This strategy mimics an MGUS with the production of an excess of monoclonal pathogenic LC, proved efficient to reproduce in mice several monoclonal gammopathies of clinical significance (MGCS) and confirmed the efficiency of plasma cell targeted treatments not only to stop the evolution of the disease but also to recover organ functions.

Results: Despite strong LC production, mice did not naturally develop AL amyloidosis. In vitro, the full length LC was resistant to amyloid formation at physiological conditions but the variable domain (IGLV6) showed high propensity to form fibrils. A single injection of amyloid fibrils and/or seeds, obtained from the variable domain (VL) of the human LC gene, led to amyloid deposits starting at 1 month post-injection, especially in the heart, spleen, liver and, to a lesser extent, in the kidney. We confirmed that the deposits contain the full-length human LCs, which elongate VL fibrils in vivo.

Conclusions: This is, to our knowledge, the first transgenic mouse model of AL amyloidosis closely reproducing human lesions, especially in heart. Further studies are needed to better understand the early biochemical events leading to AL amyloidosis in vivo, but this model already shows that a partial degradation of the LC is likely required to initiate amyloid fibrils and that once seeded, the full length LC can elongate these fibrils. This mouse model opens new perspectives to better understand the toxicity of amyloid LC, their involvements in different biological processes and organ dysfunction. Combined therapies aiming at actively removing amyloid fibrils in organs together with plasma cell depletion will be evaluated for the first time thanks to this model.

P107

Keeping balance: studying phosphatases as novel RAS-MAPK pathway regulators in lung adenocarcinoma

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In lung adenocarcinoma (LUAD), virtually all the genetic alterations driving tumor progression are directly linked to the RAS-MAPK pathway. Most of these modifications tend to upregulate global activity of the pathway in order to confer a proliferative advantage to the tumor cells.

However, in our previous results we have shown that an excess of the MAPK pathway signaling is detrimental for the cell. Through mechanisms that are far from being fully decoded, the tumor must undergo a process of selection for a set of both positive and negative regulators to keep balance.

We have used a previously described transcriptional signature composed of 6 genes to determine MAPK activity levels in KRAS mutant tumors from TCGA patient's gene expression data. Paradoxically, high MAPK tumors have better prognosis compared to those with lower intensity of pathway, suggesting again that tumors that select for moderate levels of MAPK activation are more aggressive whereas highly active lesions appear associated to stress phenotypes.

In these subgroups, we focused our attention on phosphatases expression, as they are proteins that will have an important role in regulating this kinase cascade and they have been already implicated in the MAPK network in the literature. When performing the analysis, we identified that the expression of several genes coding for phosphatases and their regulatory subunits were differentially expressed in the high and low MAPK tumor subgroups. For example, we detected PPP2R3A and PPP2R2C, two independent regulatory subunits of PP2A, to be differentially expressed in high and low MAPK tumors, respectively. We will present our strategy to study their role in the pathway regulation.

We also have a particular interest in one of the dual specify phosphatases that was originally described as part of the signature, DUSP4. This particular phosphatase forms a negative feedback loop with the ERK proteins, and its expression is dependent on MAPK activation. However, in the KRAS-mutant patients, DUSP4 transcriptional levels actually anti-correlate with pathway activation. Taking a closer look into the genomic data, we identified that the DUSP4 locus is very susceptible to copy number variations (CNVs). Low MAPK patients, with poor survival, show increased copy number gains of the DUSP4 locus. In contrast, high MAPK patients, with better prognosis, showed increased copy number losses of this particular locus since early stages of the disease. In this case, our hypothesis is that permanently loosing DUSP4, a negative regulator of the pathway, has a deleterious effect for advanced tumors as they suffer from sustained MAPK activity.

In order to elucidate how DUSP4 deletion affects KRAS dependent tumor progression, we are using an inducible mutant KRAS mouse strain ($KRas^{LSLG12V}$) We will present how this system can be used to model copy number losses of this phosphatase by infecting the mice with a viral system combining both the expression of Cre recombinase, allowing KRAS activation, and a CRISPR/Cas9 mediated knock-out of DUSP4. This strategy will allow us to study how DUSP4 loss affects tumor initiation and progression in a KRAS driven context.

P108

Involvement of Reptin in invadosome formation and tumor invasion

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Metastasis formation is the main cause of cancer related death. These metastases are the consequence of tumor invasion that is the ability of cancer cells to colonize new tissue. To do so, cells must migrate across anatomical barriers, notably by degrading the extracellular matrix (ECM). This ability is conferred by invadosomes, which are membrane protrusions composed of F-actin structures associated with MMPs activity.

In a previous study, we used an approach combining laser microdissection and mass spectrometry analysis to define the invadosome proteome in the NiH3T3-SrcY527F cell model. These cells overexpress a constitutively active form of Src protein promoting invadosome formation. This approach revealed that Reptin (RUVBL2) is 6 times enriched in invadosomes in comparison with the total cell lysate. Reptin is a AAA^+-ATPase involved in different cellular functions including DNA repair, replication and molecular co-chaperoning complexes. Reptin is a member of the R2TP complex, which is required for the assembly and conformation of many protein complexes.
We demonstrated that Reptin, as well as the other members of the R2TP complex (Pontin, RPAP3 and PiH1D1), colocalize with invadosomes. By a siRNA approach we have shown that Reptin depletion significantly decrease the cells ability to form invadosomes and to degrade the ECM. Moreover, in Reptin depleted cells, we noticed a recover of the wildtype phenotype characterized by the presence of stress fibers and the absence of invadosomes. That point reflects a loss of SrcY527F activity suggesting a molecular link between Src and Reptin. We confirmed this hypothesis showing that Reptin depletion significantly decreases the phosphorylation state of SrcY527F on Tyr419 without affecting its total expression level.

Our aim now is to identify the molecular mechanism involved in the modulation of Reptin-dependent Src activity. We want to identify kinase or phosphatase responsible for this regulation. For this purpose, we will use exploratory proteomic approaches to investigate Reptin depletion impact on the whole cell proteome and phosphoproteome to highlight Reptin partners in invadosomes. Taken together these results will allow us to better understand the mechanism of invadosome formation mediated by Reptin and the R2TP complex. Globally, this work will allow a better comprehension of mechanisms involved in the process of tumor invasion.

P109

Use of mesenchymal stem cells derived nanoghosts to prevent resistance to tyrosine kinase inhibitor in lung cancers

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EGFR tyrosine kinase inhibitors, such as Osimertinib, are effective therapies for treating EGFR-mutated non-small cell lung cancer (NSCLC) but patients systematically relapse. This may be due to a small population of drug-tolerant cells (DTCs) acquiring mutations of resistance. The team has shown the importance of the RHOB GTPase in the DTC phenotype. No specific inhibitor of RhoB has been found so far but we have shown the efficacy of Tat-C3 (C3 exoenzyme from Clostridium botulinum, inhibitor of RHOA, RHOB and RHOC GTPases, coupled to a permeant peptide, tat) to prevent the development of resistance in our EGFR-mutated NSCLC cell lines. As Tat-C3 cannot be used clinically due to its possible systemic toxicity, we propose to use therapeutic vectors derived from bone marrow mesenchymal stem cells (BM-MSC), the nanoghosts (NG), to deliver C3-exoenzyme to NSCLC cells. NGs have been shown to preserve the tropism of BM-MSC for tumour cells while eliminating their pro-oncogenic properties. Using boyden chamber assay, flow cytometry and different microscopy and molecular biology approaches, we evaluated the relevance of using nanoghosts as a therapeutic vector in our cell lines by assessing their uptake as well as the efficacy of the C3 encapsulated in the nanoghosts. Almost all the cells internalized NGs very quickly. We also demonstrated the efficacy of NGs loaded with C3 in inhibiting RHOB.

P110
PAMR1 as a New Biomarker of Colorectal Cancer

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According to statistics from the Global Cancer Observatory 2020, Colorectal cancer (CRC) ranks the third place in terms of incidence and the second in terms of mortality among cancers worldwide. There are no specific and sensitive diagnostic methods/biomarkers for early detection of CRC. Despite the different treatments, high risks of re-occurrence are associated with advanced and metastatic CRC stages. All together necessitates understanding the molecular mechanisms behind CRC occurrence thus allowing early diagnosis, more targeted treatments, and high chances of recovery.

We focused our attention on PAMR1 (Peptidase Domain Containing Associated with Muscle Regeneration 1), which was frequently inactivated by promoter hypermethylation in breast cancer tissues and considered as a putative tumor suppressor. PAMR1 is a multi-domain protein with a cubilin domain (CUB domain) and EGF-like domain, similar to those found in SCUBE2, which inhibits breast-cancer cell migration and invasion. We thus wondered if PAMR1 could exert a similar role in CRC.

RNASeq data, available in Firebrowse database, showed that PAMR1 was significantly downregulated in CRC (626 tissue samples) compared to normal colorectal tissue (51 tissue samples). In addition, our analysis showed that this downregulation, found in the four CRC stages, appeared as early as the first stage. Thus, the question that arises concerns the role of PAMR1 as an early biomarker of CRC. Consistent with bioinformatic analyses, the expression of PAMR1 protein was found to be lower in CRC tissue samples than in normal ones (CRB collaboration), whatever the stage analyzed by western blot. Since the protein was not detected in the secretome of our three CRC cell lines (HCT116, HT29, and SW620) due to its low expression, a RT-qPCR was performed and confirmed very low RNA levels (according to Ct values). We still do not know if it is due to an epigenetic inactivation of PAMR1 as in breast cancer.

Due to the very low endogenous expression of PAMR1 in CRC, two experimental approaches were carried out to understand the role of this protein in CRC, namely exogenous treatments of CRC cell lines with recombinant PAMR1 or a stable overexpression of untagged PAMR1 in these cells. Using recombinant PAMR1 produced by stably transfected CHO cell lines and added to culture medium, a reduced cell proliferation was observed, particularly for HT-29 exhibiting the lowest endogenous PAMR1 expression. Future studies concern PAMR1 overexpression in HT-29 cell line and study of its impact on cell proliferation, migration and invasion.

Moving to a next level, our investigation will be directed toward the molecular understanding of activity of PAMR1. PAMR1 is one of target proteins of the Protein O-Fucosyltransferase 1 (POFUT1), which was observed to be overexpressed in CRC and considered as a promising new biomarker of CRC. POFUT1 is responsible for O-fucosylation of PAMR1's unique EGF like domain, that could modulate protein-protein interactions. The presence of the EGF-like domain and/or its O-fucosylation could thus affect PAMR1 function. To answer this question, recombinant PAMR1 without EGF-like or mutated on its O-fucosylation site could be used for exogenous treatments and compared to wild-type PAMR1. Finally, we will investigate the potential effect of PAMR1 overexpression on signaling pathways involved in proliferation such as the canonical Wnt/ beta-catenin signaling pathway.

Our ultimate goal is to know if PAMR1 has a tumor suppressive role in CRC or not. If so, PAMR1 would be counted as a novel strategy for early diagnosis or even improved treatments of CRC.

P111

Calcium homeostasome remodeling associated with melanoma phenotype plasticity: What role in melanoma progression and therapy resistance?

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Malignant transformation of melanocytes causes melanoma, the most aggressive form of skin cancer. Despite recent advances in treatments, patients who present with a metastatic disease still have a very poor prognosis. Approximately 60% of patients with cutaneous melanomas have driver-mutations in the BRAF gene. leading to uncontrolled proliferation and apoptosis resistance. This has guided the clinical development of single drug targeted therapies (i.e., BRAF inhibitors, BRAFi), and more recently of BRAFi+MEKi combinatorial therapies. These MAPK pathway-targeting approaches have shown clear clinical benefits on patient survival. Yet, a significant portion of patients initially do not respond. As for the others, these treatments seem to work for a limited time only due to melanoma tendency to develop mechanisms of acquired drug resistance. There is therefore an urgent necessity to address this situation by developing new strategies to predict, prevent and/or overcome therapeutic failure.

Recent studies have suggested that Ca2+ signalling plasticity may contribute to adaptive resistance to targeted therapies. Calcium act as an essential second messenger in a plethora of vital cellular functions. Intracellular Ca2+ homeostasis can be controlled by hundreds of proteins, only a subset of which being expressed at any given time in one cell type/state. Pathophysiological remodelling of the "Ca2+-signalling toolkit" expressed by a cell includes critical changes in several actors and pathways. In the specific case of cancers, this defines state specific molecular/calcium signatures influencing the phenotype of the tumour cells and clinical outcome. These signatures represent therefore promising diagnostic and prognostic biomarkers in cancers. Information regarding plasticity in ion channels expression could also reveals interesting drug targets.

By switching from a proliferative to an invasive state, melanoma cells can acquire resistance to targeted therapeutic. Using functional approaches, we have shown that melanoma drug-induced phenotype plasticity is associated with changes in the calcium signalling modes of the melanoma tumor cells. Indeed, serum-induced calcium responses are amplified in the MITFlow/AxlHigh pharmaco-resistant and invasive cells compared to the MITFHigh/Axllow proliferative ones. Yet, despite being consistent with growth factor receptors overexpression/activation in the resistant cells, this is in contrast with the observed impaired Store-operated Calcium Entry (SOCE) capacities of these cells. Indeed, SOCE is the classical calcium influx pathway recruited downstream growth factor receptors activation suggesting that the cells change the way they mobilize calcium downstream identical plasma membrane receptors. Mechanistically, SOCE inhibition in the pharmacoresistant cells seems to occur through Wnt5A/PKC-induced Orai1 inhibitory phosphorylation. We are currently investigating the functional importance of this Calcium signalling plasticity.

To draw a global picture of this Ca2+ remodeling we used a RNA-seq based gene expression approach to exhaustively analysis the calcium-signaling toolkit expressed in each state. This strategy revealed the overexpression of two drug-actionable calcium channel targets in the resistant/invasive cells. Pharmacological inhibition of these channels impaired survival and can reverse MAPKi resistance in these cells. Hence these channel inhibitors might have clinical benefit by restricting and/or counteracting the resistance to MAPKi-based therapies.

P112

Fascin-1, a target of ß-catenin, regulates epithelial-mesenchymal plasticity of tumor hepatocytes

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β-catenin (β-cat) is an effector protein of Wnt pathway playing a structural and transcriptional role. When the Wnt pathway is OFF, β-cat is either at cell junctions contributing for cell adhesion or degraded in the proteasome once phosphorylated by GSK3ß. When Wnt pathway is ON, β-cat translocates to the nucleus and plays a transcriptional role important for cell survival. The CTNNB1 gene encoding β-cat is mutated in cancers, and especially in Hepatoblastoma (HB) where up to 80% of tumors carry the CTNNB1 mutation. The mutation is often a deletion of exon 3 and monoallelic, leaving a WT allele of β-cat. The interplay between the WT and mutated form and their contribution to HB tumors has never been explored.

Here, we aim to study the role of the mutated and wild-type forms of β-cat in HB cell lines. Using a siRNA strategy, we create a model targeting each form of ß-cat and allowing to dissociate the two functions of β-cat. This model serves to explore the cell phenotype by analyzing the proliferation, differentiation status and target gene profiles.

Our results revealed an antagonist role of WT and mutated ß-catenin on hepatocyte differentiation, attested by alteration of hepatocyte markers expression and bile canaliculi formation. We characterized Fascin-1 as a target of ß-catenin involved in tumor cell differentiation. Using ß-cat mouse models, we found that Fascin-1 is highly expressed in mesenchymal undifferentiated tumors. We further found that Fascin-1 is a specific marker of the embryonal component in human HBs. Using fascin specific inhibitors, we are currently addressing whether Fascin-1 could be a potential target in HB.

In conclusion, our work demonstrated that Fascin-1 expression is linked to loss of differentiation and polarity of hepatocytes. We present Fascin-1 as a new player in the epithelial-mesenchymal plasticity of tumor cells associated to ß-catenin pathway alteration in the liver and as a new potential target in HB.

P113

Identification of specific targets to control macrophage infiltration into tumors: use of a CRISPR/Cas9 screen on conditionally immortalized Hoxb8 macrophages

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Macrophages are innate immune cells capable of infiltrating all body tissues. In the case of solid cancers, macrophages are massively recruited in the tumor. Their abundance is a factor of poor prognosis as they promote tumor growth, neo-angiogenesis, chemoresistance and the development of metastases. Therefore, the control of macrophage infiltration within tumors is a therapeutic challenge.

Earlier studies revealed that macrophages can adapt their migratory mode to the physical properties of the extracellular matrix. As such, they use mesenchymal migration to infiltrate dense tumors. In sharp contrast, they use the ameboid migration mode to infiltrate soft or inflamed tissues. To date, there are no specific inhibitors of mesenchymal migration. Here, we propose to identify new effectors of macrophage mesenchymal migration. We devised a pan-genomic CRISPR/Cas9 screen to functionally assess the contribution of each individual genes in macrophages and assess their capacity to migrate in 3D Matrigel. To do so, we engineered Cas9-expressing myeloid progenitor cells that were conditionally immortalized with Hoxb8 and transduced with a lentiviral library to deliver guide RNAs. Pooled knocked out cells are separated according to their capacity to migrate and the genes involved are identified by next generation sequencing. Our approach will now allow us to identify candidate genes that are specifically involved in macrophage mesenchymal migration. This strategy should uncover new pharmacological targets to specifically block the recruitment of tumor-associated macrophages while preserving healthy macrophage subsets to maintain an effective immune response.

P114

Alterations in ER depletion and Store-Operated Calcium entry permit GNAQ/11 mutated Uveal Melanoma survival

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Uveal melanoma (UM) is the most common primary tumor of the eye in adults. Despite being rare, it can cause serious vision problems and, in 50% of the cases, liver metastases compromise patient's survival.

UMs are initiated by driver mutations in the G protein-coupled receptor (GPCR)/GNAQ-11/Phospholipase C Beta 4 (PLCB4) signal transduction pathway. 90% of UMs bear gain-of-function mutations in GNAQ/11 encoding α subunits of the GPCR associated Gα/11 proteins while a small proportion are due to driver mutations in the Cysteinyl Leukotriene Receptor 2 (CYSLTR2) or the PLCB4.All these mutually exclusive oncogenic mutations have the same functional output, resulting in constitutive activation of PLCβ which hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP2) in diacylglycerol (DAG) and inositol triphosphate (IP3).

So far, UM research only addressed DAG-induced pro-oncogenic effects and identified its essential role in stimulating the MAPK pathway by recruiting PKC and RasGRP3.
However, nothing is known on the role of the second arm of the deregulated PLC signaling, the IP3/calcium pathway. Yet, calcium controls several vital functions and any unregulated calcium elevations is cytotoxic. Hence, it is not known how UM cells cope with sustained IP3 production to escape calcium overload-induced cell death and whether the deregulated IP3/calcium pathway promote UM tumor progression.

Our results show the existence of a regulation mechanism preventing IP3-induced ER calcium depletion and the associated calcium entry, likely to be caused by the loss of responsive IP3 receptors. Furthermore, by modulating the oncogenic GNAQ/11 pathway, we have discovered a negative feedback loop involving PKC and the calcium channel Orai1 leading to impaired store-operated calcium entry. Our results also show that drugs increasing these entries induced cell death. Altogether our data indicate UM cells remodel calcium pathway to prevent ER stress and maintain their survival. Our current efforts are now focused on further identifying the molecular mechanisms underlying this remodeling and to test drugs modulating this pathway as potential new therapeutic strategies for this eye life-threatening disease.

P115

Farnesyltransferase inhibitors prevent relapse to EGFR tyrosine kinase inhibitors in Non-Small Cell Lung Cancer

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Drug-tolerance is one of the major non-genetic mechanism that drives resistance to targeted therapies. Deciphering the vulnerabilities of drug-tolerant cells (DTC) thus represents an attractive strategy to prevent relapse in patients. Here, we provide a step-by-step characterization of the molecular and phenotypic events that allow epidermal growth factor receptor (EGFR) mutant non-small-cell lung cancer (NSCLC) to enter and exit from a drug-tolerant state in response to EGFR tyrosine kinase inhibitors (TKi). We have found that DTC display a highly reversible and unconventional pseudo-senescent and contractile phenotype. This phenotype is highly dependent on activation of several farnesylated proteins including RHOB and RND3. Combination with tipifarnib, a clinically approved farnesyltransferase inhibitor (FTi), prevents relapse to EGFR-TKi in vitro. Our results strongly support the use of FTi in combination with EGFR targeted therapies to prevent relapse in EGFR-mutated lung cancer patients.

P116

Tumor cells-released tracks promote cell migration on type I collagen

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Metastasis is the leading cause of cancer-related deaths. During this process, tumor cells acquire invasive and migratory capacities in order to invade the surrounding tissues. To this, the tumor microenvironment is modified to facilitate cancer cells proliferation and dissemination. Multiple mechanisms are involved in this evolution, including cell-cell communications through the tumor microenvironment and the extracellular matrix modifications. Indeed, extracellular vesicles such as exosomes or migrasomes are already known to induce protumor features such as migration, promoting tumor development and metastasis formation.

Here we describe a new type of extracellular vesicles (referred as tracks) specifically released by cancer cells along type I collagen fibers during cell migration. We could characterize these tracks, their structure as well as their composition in term of proteins and nucleic acids, and could show that they are different from classical extracellular vesicles known so far. These tracks are characterized by a discoidin domain receptor 1 (DDR1) staining. Moreover, these tracks are very stable structures and can be internalized by neighboring cells. After internalization, they can modify the differentiation status of cells able to internalize these tracks.

These data suggest that these collagen-associated tracks have a role in cell-cell communication and participate in the remodeling of the tumor microenvironment. Even if their function needs to be fully elucidated, their protein and RNA compositions could suggest that these tracks could help to promote cell proliferation and invasion.

These tracks seem to be a new player in the tumor invasion process and could provide a better understanding underlying this process.

P117

Role of collagens and their receptors in the development of Wilms tumors

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Wilms tumor (WT) or nephroblastoma is the third solid tumor in children. It develops from the embryonic cells that form the kidney. Histological analysis of these tumors shows the presence of 3 different cellular contingents (epithelial, stromal, blastemal) and allowed classification into 3 risk groups (low, intermediate and high risk). High risk tumors have a majority of blastemal cells or anaplastic cells derived from one of the 3 cellular contingents but having large nuclei. In Europe, despite the use of effective treatments, 15% of intermediate-risk WTs and 30% of high-risk WTs will recur. The 5-year survival rate then drops from 90% to 60%. In addition, treatments are responsible for side effects, such as heart and kidney dysfunctions or, eventually, development of secondary cancers.

Tumor stroma is made up of cells of various types and the extracellular matrix (ECM). This ECM interacts with receptors present on the surface of tumor cells and, thus, participates in tumor development. The major components of ECM are collagens which bind to receptors such as integrins or discoidin domain receptors (DDR1 and DDR2). DDR1 expression is deregulated in many cancers, promoting or inhibiting tumor development, depending on the cancer. In renal cell carcinoma (RCC), DDR1 exerts an anti-tumoral effect, its overexpression inhibiting tumor growth and cell migration *in vitro* and tumor development *in vivo*.

Genomic and transcriptomic studies of WTs have highlight numerous genomic and chromosomal alterations present in these tumors (WT1, CTNNB1 etc.). Nevertheless, no proteomic study of each cellular contingent has been published. Molecular analysis of these tumors is difficult due to the lack of cells in culture, but very recently, a 3D culture technique, in the form of tumor spheroids, has enabled very long-term cell culture of blastemal and epithelial cells.

A preliminary proteomic analysis, carried out in the laboratory from the tumor contingents, revealed in high-risk blastemal tumors, a profound ECM remodeling, particularly of collagens, as well as a modification of the intracellular signaling linked to integrins and to other ECM receptors. The objective of the project is to analyze the role of DDR1 in the development of WT. The laboratory hypothesizes that, as in RCC, DDR1 induces an anti-tumor effect in WT. For that, WT-CLS1 cells overexpressing different levels of DDR1 or inactivated for DDR1 expression were obtained. Overexpression of DDR1 in WT-CLS1 cells induces an anti-tumoral effect by reducing the growth, migration and development of spheroids in the presence of collagen I. Inactivation of the receptor will also decrease cell migration but has no impact on growth or development of spheroids.

In conclusion, during the development of WT, tumoral cells must finely regulate collagen and DDR1 expressions, necessary for maximum cell growth, migration and invasion.

P118
Proline Metabolism in Triple Negative Breast Cancer

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Introduction and objectives: Cancer cells modify their metabolism to grow and survive in restrictive environment. Whereas the use of glutamine by cancer cells is well documented, the role of other amino-acids, including Proline, are less established. Emerging from former studies of the team on E4F1-p53-dependent metabolic controls, my project is to explore the role in cancer of the mitochondrial Aldehyde dehydrogenase ALDH4A1, an enzyme that triggers Proline to Glutamate/α-ketoglutarate conversion. As a p53 target gene, its expression is frequently down in tumors such as Triple Negative Breast Cancers (TNBC). The aim of my thesis is to decipher the impact of ALDH4A1 on TNBC cells, to further explain why its expression is lost in the early steps of the tumorigenesis of these cells.

Method: To achieve this goal, I modulated ALDH4A1 expression in the TNBC cell line SUM159 and in transformed human mammary epithelial cells (HMEC) and assessed their ability to grow and survive in attached (2D) and unattached (3D) conditions.

Results: My data shows that Up and Down-regulation of ALDH4A1 in SUM159 or transformed HMECs modify their capacity to grow and survive (anoikis) in 3D conditions, and to form colonies in agar or large spheroids, suggesting it has tumor suppressive properties. Differential RNASeq and PCR/Western validations on TNBC cells re-expressing ALDH4A1 unexpectedly revealed that Hif-dependent responses to 3D growth and Hypoxia are strongly altered in these cells.
Conversely, endogenous ALDH4A1 level is strongly downregulated in hypoxic conditions, further suggesting this enzyme (p53-dependent Proline catabolism) is a key and hitherto unidentified player of the response to hypoxic environments, as observed at the center of solid tumors.

Discussion/conclusion: My current hypothesis is that ALDH4A1 reexpression reduced Hif1α protein levels by increasing α-Ketoglutarate levels which is a cosubstrate of PHDs, a family of enzymes that negatively regulate it. This could in turn reduce the ability of the cells to answer to hypoxic stress induced by cell aggregation due to 3D growth, leading to a reduced cell proliferation and cell death.
In conclusion, ALDH4A1 reexpression in TNBC cells reduced their ability to grow in Soft Agar and induced Anoikis and its impact on Hif Signalling could explain why its expression is lost in the early stages of tumorigenesis.

P119

Role of p53 Gain-of-Function mutations in of hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the main primary liver cancer, with poor prognosis and rising incidence. Genetic alterations of the tumor suppressor gene TP53 are among the most frequent events in HCC (40%). In contrast to other tumor suppressor genes, p53 inactivation occurs mainly through missense mutations, which alter the p53 wild type function and exert a dominant negative (DN) effect on the wild type protein. Moreover, it has been proposed that the oncogenic mutations confer novel activities to the p53 protein, defining a Gain-of-Function (GOF) phenotype. Despite many reports supporting the mutant p53 GoF phenotype, the issue remains controversial, probably because the GoF phenotype is likely to be both mutant and the cancer type-specific. HCC present a specific spectrum of TP53 mutations, and the question of GoF acquisition in this tissue has not been thoroughly investigated.

The goal of my PhD project is to investigate the functional consequences of p53 point mutations on hepatic carcinogenesis. We have selected eight p53 mutants of interest (V157F, R159P, R175H, R248W, R248Q, R249S, R273H, R273C) to study both their dominant negative and gain of function phenotypes. We use a mouse model to trigger tumorigenesis via stable transfection of hepatocytes in vivo by hydrodynamic gene delivery (HGD). Expression of each p53 mutant, or genetic inactivation of p53 as control, is combined with a strong oncogenic event (RasG12D mutation or c-Myc overexpression). Tumors typically arise in 3 to 6 weeks, and are analysed in terms of growth, aggressiveness (vascular invasion, intra- and extra-hepatic metastases), anatomopathology and stromal composition. To get further insight into mechanistic aspects of mutant p53 activities, we generated tumor-derived cell lines from tumors of distinct genetic combinations. Our results highlight striking differences in phenotypes of tumors harboring p53 mutants commonly present in human HCC.

P120

FAK kinase activity in cancer-associated fibroblasts impacts PDAC vascularization

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Introduction

Chemotherapy reaches the tumor through the vasculature, making the blood vessel quantity and quality a key regulator of the chemotherapy efficacy. Importantly, cancers such aspancreatic ductal adenocarcinoma (PDAC) are very poorly vascularized (collapsed and permeable vessels). PDAC, one of the most aggressive cancers is characterized by a strong desmoplastic reaction (accumulation of extracellular matrix (ECM) proteins due to the fibroblasts activation into cancer-associated fibroblasts (CAF) (Belhabib et al. Cancers 2021)), that participates to the generation of the aberrant and leaky vasculature. We hypothesize that regulating the desmoplastic reaction could promote vessel "normalization" and subsequently enhance chemotherapy penetration within the tumor.

We have recently identified that inhibiting the protein tyrosin kinase FAK (Focal adhesion kinase) in CAFs drastically decreases PDAC spontaneous metastases (Zaghdoudi et al. EMM 2020) supporting the idea of the implication of blood vessels. We postulate that the inactivation of FAK in CAFs may induce a crosstalk with vasculature cells (endothelial cells or pericytes) leading to the normalization of the vasculature.

Results

In order to address that question, we developed two PDAC immunocompetent mouse model in which CAFs express an active or inactive FAK: 1- orthotopic and syngeneic mouse model of co-grafted FAK-WT or FAK-KD (kinase Dead) fibroblasts plus pancreatic tumor cells (KPC) and 2- immunocompetent inducible pancreatic fibroblast specific inactivation of FAK mice model.

By performing a Matrisome (mass spectrometry analysis leading to ECM protein quantification) on mice tumors from model 1, we show that FAK inactivation specifically in CAFs dramatically modifies the ECM composition: important decrease of "pro-tumoral ECM protein" level associated with a strong increase of basement membrane (BM) protein level. Immunohistochemical analysis of the BM protein localization reveals an accumulation of Collagen IV specifically around the blood vessels. As vessel BM regulates their stability and permeability, we evaluated the impact of fibroblastic FAK inactivation on vascular permeability by performing a «Miles Assay» in vivo. This test consists in the injection of a dye into the mice tail vein and analyzing its leak into different organs (an accumulation of the dye into an organ indicates that the vasculature is abnormally permeable). We show that fibroblastic FAK inactivation decreases vascular permeability in PDAC tumors and in lungs (metastasis organ) revealing a vessel «normalization». The next step was to identify the involved mechanisms: we hypothesized that CAFs, dependently of their FAK activation status, could secrete soluble factors impacting BM protein production/secretion by endothelial cells or pericytes. Thus, pericytes or endothelial cells were incubated with conditioned medium produced by CAFs pretreated or not with FAK inhibitor (FAKi), and BM protein level were analyzed by immunofluorescence and western blot. Preliminary results support the idea that fibroblastic FAK activity is a key regulator of the establishment of a crosstalk between CAFs and vasculature cells involved in the pericyte-induced BM protein generation and deposition around vessels.

Conclusion

Altogether, we show that FAK inactivation in CAFs inhibits spontaneous metastasis (Zaghdoudi et al EMM 2020) and «normalizes» blood vessels through a mechanism involving a CAF/pericyte crosstalk and a pericyte-induced BM protein production. Our work supports the idea that a co-treatment composed of FAK inhibitor and chemotherapy should enhance chemotherapy delivery within the tumor (as the vasculature should be normalized), thus, being a treatment beneficial for PDAC patients.

P121

New insights into the regulation of p190RhoGAP from the study of cancer-associated mutations

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A tight spatio-temporal regulation of Rho GTPases is required to achieve proper cell migration. The GTPase-activating protein, p190RhoGAP (p190A), the main negative regulator of RhoA, localizes to membrane protrusions such as lamellipodia and invadopodia. *ARHGAP35*, the gene encoding p190A, was found mutated in 15% of endometrial tumors and 2% of global cancers.

In order to get insight into the impact of cancer-associated mutations on p190A, we performed a structure/function analysis of the protein. This approach led to the identification of a sequence sufficient to ensure proper targeting of p190A to lamellipodia. A construct of p190A deleted of the identified "protrusion localization sequence" (p190AΔPLS) cannot target to these actin-based structures. We further pointed out cancer-associated mutations in PLS (S866F and Δ865-870), that alter p190A subcellular localization. In addition, we identified S866F and Δ865-870 mutations as gain of function mutations, favoring tumor cell migration (Binamé et *al*. JCB. 2016).

The present work focuses on the molecular and the functional characterization of these mutations. We found that alteration of the PLS (p190AΔPLS construct or p190A mutants) increased the RhoGAP activity of the protein. This result is in favor of an auto-inhibitory folding of the molecule, involving the PLS and masking the GAP domain. Co-immunoprecipitation experiments demonstrate that PLS is able to interact with the C-terminal part of the protein that contains the GAP domain. We demonstrated that this interaction is lost if PLS harbors S866F and Δ865-870 mutations, given a molecular explanation to the gain of function mutations. Indeed, our work suggests that p190A exist in two forms in the cell, an inactive conformation with a masked GAP domain and an active conformation allowing p190A GAP function towards RhoA.

Altogether, our data unveil a new mechanism of regulation of p190A. Acting on this new regulatory mechanism may become of interest to counteract the impact of cancer-associated mutation in tumor cells.

P122

Assessment of immune inhibitory checkpoints expressed by EBV latency III B cells and impact of fucoidans on the PD-L1/PD-1 axis

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The Epstein Barr virus (EBV) infects more than 95% of the world population and persists latently in the body. It has the ability to immortalize B cells*in vivo* and *in vitro* (Lymphoblastoid Cell Lines, LCLs) and is associated with many lymphomas. EBV latency III B cells (proliferation program) express all of the virus latency genes that activate survival and proliferation signals. For immunocompetent individuals, the host/virus balance allows the immune system to control proliferating cells. In immune deficiency case, this immune balance is disrupted, latency III B cells escape to the control of the immune system, and lymphomas may emerge.

Based on published laboratory work, EBV latency III B cells mimic regulatory B cells (Bregs). They secrete immunosuppressive cytokines (IL-10, TGF-β, IL-35), and the expression of the LMP-1 (Latent Membrane Protein 1) viral protein enhances the expression of the immuno-inhibitor PD-L1 (Programmed Death-Ligand 1). Co-culture of EBV latency III B cells and T cells in an autologous model show that PD-L1 binds to PD-1 on the surface of CD4 T cells, resulting in the expansion of anergic regulatory T cells (Tregs). Tregs, through the secretion of TGF-β, inhibit the proliferation of CD4 and CD8 effectors T cells. This may contribute to their escape from the anti-tumoral immune T-cell response and the emergence of B lymphomas (Auclair et al., J Immunol, 2019).

T and NK cells play a major role in the surveillance of EBV latency III B cells and immune checkpoints, other than PD-L1/PD-1, are likely to be involved in the inhibition of the anti-tumoral immune response. Results of others and us show an overexpression of i/HLA class I molecules (ligands for NKG2A/B and KIRs), ii/ CD80 and CD86 (ligands for CTLA4) and iii/ PD-L1 and PD-L2 (ligands for PD-1). We also observed intracellular overexpression of galectins 3 and 9 (LAG3 and TIM3 receptors, respectively) which could be secreted. CD112 and CD155 (ligands for PVRIG, TIGIT and TACTILE) and Ceacam-1 (ligand for TIM3) are not expressed, therefore these inhibitory axis will not be involved.

At the same time, we considered the restoration of the anti-tumoral response by targeting the PD-L1/PD-1 immune checkpoint with fucoidans. These sulfated polysaccharides extracted from brown seaweed are of recent interest in oncology. When used as adjuvant, they exhibit anti-tumor effects, immunomodulatory properties and reduction in chemotherapy side effects. Otherwise, they can decrease the expression of PD-L1 in some solid tumor cell lines (breast and fibrosarcoma).
Different fractions of fucoidans (*Fucus vesiculosus* seaweed) were obtained as part of a collaboration with the UMR CNRS 7266 LIENSs -La Rochelle University. Our preliminary results on LCLs emphasized a decrease in proliferation, an increase in apoptosis and a decrease in PD-L1 expression at the transcriptional and at the protein level (on the cell surface of apoptotic as well as of intact cells). The underlying molecular mechanisms remain to be studied. To assess fucoidans contribution to the restoration of the immune response and the interest of their use as an adjuvant for immunotherapies targeting PD-L1/PD1, we want to use the autologous co-culture model of EBV latency III B cells and T cells already developed. We expect that a co-treatment with fucoidans and anti PD-L1 blocking antibodies will be more effective than each treatment alone.
Our project aims to study the functional role of the immune inhibitory checkpoints of EBV latency III B cells in order to identify new therapeutic targets. Otherwise, we wish to clarify the role of fucoidans as an approach in their interaction with conventional anti-tumor treatments.

P123

RNA-based therapeutics for the treatment of B lymphoid and plasma cell diseases

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RNA-based therapeutic strategies are currently expanding and cover a wide range of applications. In the future, these RNA-based approaches will allow rapid generation of drugs for the treatment of many diseases, including personalized medicines. Indeed, these drugs are cost-effective, relatively simple to manufacture and can target previously undruggable pathways. Recent advances allowing the incorporation of chemical modifications during the synthesis of these RNA drugs have significantly improved their tissue uptake and intracellular delivery. Our team has been working for several years on the development of RNA-based strategies using antisense oligonucleotides (ASO) for the treatment of B-lineage cell diseases, including lymphoproliferative disorders such as B lymphomas, multiple myeloma and monoclonal gammopathies. We developped antisense strategies targeting immunoglobulin (Ig) transcripts or oncogenes involved in the process of tumorigenesis (Refs 1-3; patents: WO2017089359, WO2020245182, EP20306003, EP21305513).

Here, we analyzed the impact of ASO targeting Ig transcripts to lessen either Ig secretion as antibody or membrane Ig expression as B-cell receptor (BCR). This antisense approach is based on the use of ASO to drive alternative polyadenylation (APA) of Ig heavy chain transcripts, by targeting either the secreted or the membrane polyA site (PAS). This process of APA occurs naturally during plasma cell differentiation to allow the transition from BCR expression to antibody secretion.

We demonstrated that treatment with morpholino ASO targeting the secreted IgE PAS drastically decreased IgE secretion and inversely increased membrane-IgE mRNA expression. In addition, ASO treatment induced apoptosis of IgE-expressing U266 myeloma cells, and RNA-seq revealed attenuation of their plasma cell phenotype. Remarkably, systemic administration of ASO coupled to Pip6a as an arginine-rich cell-penetrating peptide decreased IgE secretion in vivo (4).

Current works are ongoing to decrease BCR expression and signaling using ASO targeting the membrane IgM, IgA or IgG PAS. Preliminary data indicate that ASO are excellent tools to decipher the role of BCR signaling during plasma cell differentiation. In addition, decreasing BCR signaling should be useful for the treatment of B-cell cancers with a BCR-signaling addiction such as chronic lymphoid leukemia (CLL), follicular lymphoma (FL) or diffuse large B-cell lymphoma of activated phenotype (ABC-DLBCL). Future directions will help to decipher whether ASO treatment should reinforce the effects of Ibrutinib (BTK inhibitor targeting BCR signaling) on lymphoma cells.

Altogether, this ASO-mediated APA strategy could be an effective way to decrease BCR signaling in B-lymphoid malignancies or the secretion of pathogenic antibodies.

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P124

Leukaemia Inhibitory Factor signalling for targeting Cancer Stem Cells in gastric adenocarcinoma

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Cancer stem cells (CSCs) chemo-resistance mechanisms contribute to tumour maintenance and dissemination. Though rare, not all CSCs are involved in tumour metastasis. Some CSCs, Metastasis initiating cells (MICs), possess exacerbated epithelial-to-mesenchymal transition (EMT) and invasive characteristics facilitating tumour primary site evasion and metastasis initiation. Hippo pathway involvement in gastric tumorigenic and invasive properties has recently been established and Leukaemia Inhibitory Factor Receptor (LIFR) and its ligand LIF were found to inhibit gastric CSC tumorigenicity through Hippo kinases LATS1/2 activation.

Since LIF impact on gastric MIC invasive properties has never been investigated, this study evaluated LIF effect on MIC invasive properties in GC cell lines and patient-derived xenograft (PDX) cells. RTqPCR and immunofluorescence were used to decipher LIF treatment effect on EMT markers expression as well as on invasive phenotype of GC cells. LIF effect on invasion capacity of GC cells and CSC were evaluated by using 2D and 3D-collagen invasion assays. XMU-MP-1 was used as Hippo kinase inhibitor to evaluate Hippo pathway participation to observed effects.

LIF treatment decreased GC cell lines and PDX cells invasion capacity in vitro. In addition, decrease in EMT process, linked to the decrease in invasion was observed. LIF anti-invasive effect was reversed by Hippo kinase inhibition, highlighting the role of the Hippo pathway in LIF-dependent effects in GC cells.

In conclusion, this study first displays Hippo kinases- dependent LIF anti-invasive properties in GC cells. LIF treatment could *in fine* constitute a new CSCs-targeting strategy to help decrease relapse cases and bad prognosis in GC.

P125

Autophagy alteration and its role in promotion of migration by epithelial-mesenchymal transition in early-stage colorectal cancer

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Colorectal Cancer (CRC) is one of the major public health stakes, especially due to its late detection and its therapeutic management. Indeed, Cancer Stem Cells (CSCs) are able to self-renew and give birth to new tumor cells indefinitely as they are multipotent. CSCs are also responsible for resistance to chemotherapeutic treatments that only target highly cycling cells, as they are known to follow a quite long and nearly quiescent cell cycle which is promoted by a high basal autophagy level. Autophagy is a recycling process involved in cell homeostasis as it helps degrading misfolded proteins and damaged organelles, whereas it was also shown to be involved in cancer development by promoting cell survival. Tumor cells are also able to communicate with each other by secreting Extracellular Vesicles (EVs) that carry genetic information and proteins, thus promoting tumor aggressiveness and invasion of their environment. As both processes are known for being involved in the promotion of cancer survival and progression, we studied the impact of altered autophagy on the secretion of EVs and cancer cells' aggressiveness.

In vitro cell models were developed by using two CRC cell lines called HCT116 and Colo-205 that representing different mutational status and stages.

Modulations in autophagy were operated by using shRNAs targeting ATG5, and a scramble shRNA (pLKO) as control. Cell models were validated by analysis of their transcriptomic and proteomic expression profiles. Chloroquine (CQ) was also used as a positive control for autophagic flux inhibition following a 3h treatment at a concentration of 25µM.

RNA-seq was achieved on our cell models through a collaboration with Beggs Lab in Birmingham (UK). Functional analyses of our cell models were achieved by evaluating their migration ability using scratch assays and Boyden chamber assays, as well as in vivo Zebrafish xenografts with the help of the XenoFISH platform in Bordeaux. Analysis of the secretion of EVs was assessed by Nanoparticle Tracking Analysis (NTA) using the Nanosight NS300 (Malvern Panalytical).

The inhibition of ATG5 was revealed by RT-qPCR and Western Blot assays in both HCT116 and Colo-205 cell lines. Autophagy alteration was confirmed by analysis of the expression of the currently used marker LC3-II and compared with the effects of CQ on the autophagic flux. Autophagy is thus altered in shATG5 cell models but not fully inhibited, which explains the differences observed between shATG5 cells and CQ-treated cells.

HCT116 shATG5 showed an increased migration compared to control, while it is the opposite for Colo-205 shATG5: this difference can be explained by the different stages each cell line represents. BrdU assays showed no significant variation in any cell line.

Following these results regarding migration, we wondered about epithelial-mesenchymal transition (EMT) and analysed the expression of the different isoforms of E-cadherin in both cell lines by Western Blot assays, as well as EMT-associated transcriptional factors SNAI1/2 and RUNX2 by RT-qPCR. HCT116 shATG5 showed a decreased expression of E-cadherin compared to control and especially for 80 kDa and 120 kDa (total) isoforms, whereas cleaved forms (50 kDa and 30 kDa) expressions seemed to be increased, consistent with the EMT process. The expression of E-cadherin regulator Rab11 was also increased in HCT116 shATG5. In comparison, Colo-205 shATG5 demonstrated a steady expression of E-cadherin isoforms.

The treatment of HCT116 pLKO cells with shATG5-derived EVs did not show any significant variation in cell migration, whereas HCT116 pLKO treated with EVs-depleted shATG5-derived supernatant showed similar increase in migration than HCT116 shATG5 cells, which was different for Colo-205.

These results suggest that autophagy alteration in early-stage CRC (HCT116 cell line) is associated with increased migration and that a yet-unknown-factor is secreted in supernatants to increase neighbouring cells' aggressiveness.

P126

How upregulated flotillins promote exosome secretion and perturb intercellular adhesion

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Tumor cell invasion and metastasis formation are the major cause of death in patients with cancer. Flotillins are upregulated in many cancer types (carcinomas and sarcomas), and this is associated with poor prognosis and the acquisition of invasive cell properties. The team previously demonstrated that flotillin upregulation is sufficient to promote tumor cell invasion. We showed that high flotillin levels increases the secretion of exosomes, a particular subtype of extracellular vesicles (EV) promoting cancer cell invasiveness, metastasis and a pro-tumorigenic micro-environment. We aim to identify the molecular mechanisms by which upregulated flotillins promote exosome secretion. At the functional level we analyze the potential role of these exosomes in cell-cell adhesion perturbations. These exosomes are enriched in N-cadherin and we hypothesize that they could perturb cadherin-mediated cell-cell junction stability. This project will have a strong impact in the cancer cell biology field and will also open new therapeutic strategies for aggressive flotillin-positive tumors.

P127

Exploring the function of PKCtheta at the centrosome in aggressive breast cancer cells

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The centrosome is an organelle that mainly works as the main microtube organizing center (MTOC) of the cells. When centrosome organizes the network of microtubules (MT), it takes part in many essential cellular processes, such as cell shape, polarity, migration and division. Centrosome is composed of mother and daughter centrioles and is surrounded by a pericentriolar matrix (PCM)., The mother centriole is different from the daughter centriole by the presence of two projection structures, the distal and subdistal appendages. During the processes of the microtubule nucleation, microtubules always prefer anchoring to the subdistal appendages of mother centriole. An in-depth study of MT nucleation has been undertaken, but the structure and function of the subdistal appendages are still poorly understood. The serine/threonine kinase PKCtheta has been found to be highly expressed in a very metastatic subtype of breast cancer and it is also involved in the control of migration and invasion of these aggressive cancer cells as well as their metastatic activity. We also uncovered that PKCtheta is also able to control the proliferation ability of these cancer cells. More precisely, the complete loss of PKCtheta drives these cancer cells into p53-independent senescence. The controls of both cell migration and cell division by PKCtheta suggest that this kinase could be a critical factor for the integration of these two cellular behaviors during cancer progression.

Recently, we surprisingly found that, in addition to the localization to cytoplasm and nascent adhesions, PKCtheta is also localized at centrosomes during interphase and mitosis, thereby PKCtheta could be regarded as a bridging link between cell migration and division. Consistently, PKCtheta loss also leads to some serious defects related to the centrosome in aggressive breast cancer cells: the MT network that is normally organized by the centrosome into a radial array loses its centrosomal anchorage, and the distance between centrioles is abnormally increased indicating an issue with centriole proximity. We have also discovered that PKCtheta is highly enriched in one of the two centrioles, which suggests its function possibly present in either the mother or daughter centriole. By using a biomarker specific to the mother centriole (Ninein), we were able to detect the association of PKCtheta mainly with the mother centriole. As Ninein is a component of the subdistal appendages that anchor the MT, the enrichment of PKCtheta at the mother centriole could have a link with the abnormal phenotype observed on MT organization upon the PKCtheta loss.

Lately, we have found that PKCtheta binding to centrosome is MT-independent, and thus we explored the interaction between centrosomal proteins and PKCtheta. Our co-immunoprecipitation experiments showed that PKCtheta only interacted with CEP170, a subdistal appendage component, while no interaction was detected with Ninein (subdistal appendages), Cep164 (distal appendages), Rootletin (proximal region) and γ-tubulin (PCM) in breast cancer cells. Besides, when depleting PKCtheta in breast cancer cells, we found that the MT was no longer anchored to the centrosome by using immunofluorescence method. In the next steps, we will explore the positioning of PKCtheta within the centrosome structure and study its link with the MT network and the centriole proximity.

Altogether, our data strengthen the emerging oncogenic function of PKCtheta and further support strategies targeting PKCtheta as treatment for the aggressive subtype of breast cancer.

P128

Eukaryote translation initiation factor 3 subunit H promotes invadosome formation and matrix remodeling upon Src induction

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Introduction

Metastasis formation is the first cause of death in patients with cancer. Targeting the ability of tumoral cells to leave their primary site of growth and move into different tissue compartments represents an important therapeutic target in cancer. Progression of tumoral cells is linked to microenvironment remodeling and extracellular matrix (ECM) degradation. ECM degradation by tumoral cells is partly due to cellular structures called invadosomes. Invadosomes are dynamic structures composed by F-actin and metalloproteinases which have a key role in ECM degradation. With an innovative method that combines laser microdissection and mass spectrometry analysis developed by our lab, we previously showed that invadosomes were enriched with translational machinery and proteins involved in translation such as eukaryotic elongation and initiation factors. The eukaryotic initiation factors 3 subunit H (eIF3H) has been identified and is enriched in invadosomes compared to whole cells.

Objective

We hypothesized that eIF3H has a determinant role in invadosome development (from initiation to maturation) and ECM degradation leading to tumoral progression.

Results

We showed an increase of eIF3H expression in cellular model of invadosomes (3T3-Src) compared to their control (3T3-WT). This increase is prevented by PP2 treatment (Src inhibition) suggesting that eIF3H expression is Src-dependant in 3T3-Src cells. The inhibition of eIF3H by siRNA strategy limited invadosome formation and ECM degradation by 3T3-Src cells. We also assessed the potential signaling could link eIF3H and invadosome: inhibition of mTOR pathways (rapamycin treatment) reduces eIF3H expression associated with a decrease of invadosome formation and ECM degradation.

Conclusion

These results revealed that the translational factor eIF3H, through mTOR signaling, has a key role in invadosome formation and activity on ECM degradation suggesting his potential involvement in tumoral progression.

P129

Dysregulations of ceramide metabolism promote cell dedifferentiation and alter TNF-dependent cell migration in melanoma

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Cutaneous melanoma is a deadly skin cancer the aggressiveness of which is directly linked to its metastatic potency. In the last decade, the development of immunotherapy targeting immune checkpoints revolutionized the care for patients with metastatic melanoma. However, almost half of patients treated with immunotherapy targeting CTLA-4 and PD-1 are non-responders or relapse within the first two years after treatment induction[1]. My research team recently identified that TNF (Tumor Necrosis Factor) and ceramide metabolism alterations in melanoma cells contribute to melanoma progression and resistance to immunotherapy [2,3,4,5]. In this context, the interconnections between this major pro-inflammatory cytokine and this lipid metabolism are unknown. TNF is known to promote the dedifferentiation of melanoma cells[6],a mechanism that gives tumor cells the ability to metastasize and resist immunotherapy. Our project aims at studying the molecular mechanisms that control the dedifferentiation of melanoma cells induced by TNF and to identify original targets involved in tumor cell aggressiveness and resistance to immunotherapy. By various approaches (RNA Seq,PCR array, RT-qPCR, Biochemistry of proteins), we showed that the dedifferentiation of melanoma cells induced by TNF is associated with a decreased expression of the acid ceramidase (AC), an enzyme that breaks down ceramide into sphingosine. Interestingly, AC knockdown triggers similar dedifferentiation profile as observed with TNF treatment. One functional consequence of melanoma cell dedifferentiation is an increase in migration abilities. Overexpression of AC in melanoma cells inhibits TNF-induced migration as evaluated in Boyden chamber. Overall, these results suggest that TNF-dependent decrease of AC may participate to melanoma metastatic spread. Whether AC also plays a direct role in TNF-induced resistance to immunotherapy is still being investigated.

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P130

Acidic microenvironment promotes Pancreatic Ductal Adenocarcinoma cells selection inducing more aggressive cancer cells: role of Store-Operated Ca2+ signals

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Pancreatic ductal adenocarcinoma (PDAC) is characterized by a poor prognosis, due to late diagnosis and chemoresistance, and by a particular acidic microenvironment, which could play a key role in promoting its progression and aggressiveness. Aberrant Ca^{2+}signals are known to be involved in cancer and PDAC context, Ca^{2+}-permeable and pHe-sensitive ion channels play an important role, by sensing microenvironmental cues and transducing signals to activate intracellular downstream pathways involved in PDAC progression, modeling PDAC microenvironment and acting themselves as drivers of its aggressiveness.

Our hypothesis is that acidic microenvironment and Ca^{2+} signaling could work in synergy to induce and/or to select the most aggressive PDAC cancer cells phenotypes. Then, the objectives are the identification of acidic pH-inducible Ca^{2+} pathways leading to one or more aggressive cell phenotypes in PDAC cancer cell lines and the identification of Ca^{2+}-permeable channels (and partners) which are regulated by pHe and are required for cancer cell progression.

Data was obtained using PANC-1 cells and different cell models: control cells grown in pHe 7.4, PANC-1 exposed for 4 days to pHe 6.6, to study early low pHe selection stages, and PANC-1 pHe-selected cells, exposed for 1 month to pHe 6.6 prior recovery to pHe 7.4 for 2 weeks. The role of low pHe in PDAC hallmarks was studied in terms of viability by MTS assay and trypan blue exclusion assay, adhesion, migration by time-lapse videomicroscopy and invasion by transwell system, while its effect on Ca2+-permeable channels expression was studied by qPCR and on intracellular Ca^{2+} signals, specifically Store Operated Calcium Entry (SOCE) and Ca^{2+} oscillations, by Ca^{2+} imaging using Fura-2. The contribute of SOCE in Ca^{2+} oscillations and PDAC processes were assessed using Synta66, a selective ORAI1 inhibitor.

During the first days of selection (4 days pHe 6.6), pHe acts as a stressor factor, decreasing cells' proliferation, adhesion and invasion abilities, while after 1-month exposure to acidic pHe and recover to physiological pHe, PANC-1 cells show a more aggressive phenotype in terms of proliferation, adhesion, migration and invasion respect to control cells. Concerning Ca^{2+}signals, 4 days pHe 6.6 cells are characterized by decreased FBS-induced Ca^{2+} oscillations' frequency (below 3.5 mHz) respect to control cells (16-33 mHz), while pHe-selected cells show a recover of fast Ca^{2+} oscillations, with overexpression of ORAI1, a SOC channel. Selective inhibition of ORAI1 with Synta66 resulted in Ca^{2+} oscillations' initiation and maintenance, demonstrating their SOCE dependency. These data correlate with SOCE, as a decrease in SOCE is observed during early stages of selection and by an increase in pHe -selected cells.

Ca^{2+} oscillations' frequency and SOCE data might correlate also with ORA1 expression, as there is a tendency in its downregulation during early pHe selection phases, while it is overexpressed in pHe-selected model. ORAI1-mediated Ca^{2+}entry might be involved also in the activation of signaling cascades that lead to increased invasion of only pHe -selected cells, as Synta66 treatment led to no difference in control cells' invasive abilites. Preliminary data allowed to identify Src kinase as a potential downstream target involved in PANC-1 cells' invasion.

Low pHe exposition decreases SOCE and slows Ca^{2+}oscillations, promoting apoptosis of weaker cancer cells, selecting more aggressive cancer cell phenotypes; in turn higher Ca^{2+} entry by upregulation of SOC channels and faster Ca^{2+} oscillations after 1-month pHe- induced selection and recovery to physiological pHe may trigger Ca^{2+}-dependent signaling pathways involved in PDAC progression.

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Insight on the tumoral cell diversity, their formation and proliferation in IDH1-mutant diffuse low brain gliomas: a key role for Notch1 signaling

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Gliomas affecting adults are devastating brain tumors. They are classically divided as high grade (glioblastomas) and diffuse low grade gliomas which have different mutations and prognosis. Recent single cell RNA seq and previous immunohistology studies have revealed that both high- and low-grade gliomas contain a diversity of tumoral cells with different phenotype and properties. Diffuse grade II gliomas (oligodendrogliomas and astrocytomas) are slow-growing brain tumors that progress into high-grade gliomas. A majority of these tumors have a mutation in the IDH1 (isocitrate dehydrogenase) gene. These tumors present an intratumoral cell heterogeneity, and no reliable markers are available to distinguish the different cell subtypes. In addition, the molecular mechanisms underlying the formation of this cell diversity is also ill-defined. A better description of this cellular heterogeneity and its formation would certainly help define innovative therapeutic strategies.

To study cellular heterogeneity and active pathway in IDH1-mutant diffuse low grade gliomas (oligodendrogliomas and astrocytomas tumors), we used immunofluorescences on cryosections of freshly-resected IDH1-mutant gliomas. We found that SOX9 and OLIG1 transcription factors, which specifically label astrocytes and oligodendrocytes in the normal brain, identified the presence of two largely nonoverlapping tumoral populations in IDH1-mutant oligodendrogliomas and astrocytomas. Astrocyte-like SOX9+ cells additionally stained for APOE, CRYAB, ID4, KCNN3, while oligodendrocyte-like OLIG1+ cells stained for ASCL1, EGFR, IDH1, PDGFRA, PTPRZ1, SOX4, and SOX8. GPR17, an oligodendrocytic marker, was expressed by both cells. These two subpopulations appear to have distinct BMP, NOTCH1, and MAPK active pathways as stainings for BMP4, HEY1, HEY2, p-SMAD1/5 and p-ERK were higher in SOX9+ cells.

Notch1 is a highly-conserved pathway controlling cell differentiation and proliferation during development and in several pathological situations. We used primary cultures and a new cell line to explore the influence of NOTCH1 activation/inhibition and BMP treatment on the IDH1-mutant glioma cell phenotype. This revealed that NOTCH1 globally reduced oligodendrocytic markers and IDH1 expression while upregulating APOE, CRYAB, HEY1/2, and an electrophysiologically-active Ca2+-activated apamin-sensitive K+ channel (KCNN3/SK3). This was accompanied by a reduction in proliferation. Similar effects of NOTCH1 activation were observed in non tumoral human oligodendrocytic cells, which additionally induced strong SOX9 expression. BMP treatment reduced OLIG1/2 expression and strongly upregulated CRYAB and NOGGIN, a negative regulator of BMP.

The presence of astrocyte-like SOX9+ and oligodendrocyte-like OLIG1+ cells in grade II IDH1-mutant gliomas raises new questions about their role in the pathology. This phenotypic interconversion mediated by Notch1 pathway may play a role in treatment resistant and tumor relapse.

P132
JMV7048, First-in-class PROTAC degrader of PXR

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Colorectal cancer is the second deadliest cancer in the world and tumor recurrence have been reported in 30 to 50 % of patients. Therapeutic failure is now explained by the existence of a subpopulation of cancer cells called cancer stem cells (CSCs). Notably, these CSCs over-express the transcription factor PXR, controling the expression of a large gene network involved in drug metabolism and detoxification, including ALDH1A1 and CYP3A4. In a xenograft model, we previously shown that PXR knockdown by shRNA inhibits tumor relapse of patient derived cells after chemotherapy treatment. To date there is no clinically relevant PXR antagonist. PROteolysis TArgeting Chimeras (PROTACs) have become a promising and appealing technology for modulating a protein of interest (POI) by degradation, including undruggable targets. PROTACs are hetero bifunctional molecules that connect a POI ligand to an E3 ubiquitin ligase recruiting ligand with an optimal linker. They give multiple advantages such as a long-lasting effect, because it requires a de novo protein synthesis, and a catalytically mode of action due to their successful dissociation after promoting polyubiquitination of the POI. Our first innovative demonstration consists in turning a high PXR affinity agonist (6m, Kd< 1nM, JMV6845) into a PXR PROTAC. By TR-FRET competitive and gene reporter assays we first identified a 6m-linker scaffold able to bind to the ligand binding domain (LBD) (kd50= 18.36nM) and activates PXR. X-ray crystallography studies reveal that the core of the molecule resides within the ligand binding cavity while the linker tail emerges from the LBD surface. Based on this scaffold we synthetized and tested several PROTACs targeting a variety of E3 ubiquitin ligases (CRBN, IAP, VHL, etc.). We finally identified 3 molecules, including out lead molecule JMV7048, leading to a significant PXR protein degradation and PXR signalling pathway in colon cancer cells. The use of ubiquitin E3 or 26S proteasome inhibitors confirmed its mode of action. Finally, despite poor pharmacokinetic properties (Tmax<5 minutes and AuCt=5189 ng/mL*h), we observed that intravenous injections of JMV7048 (25mg/mg) in SCID mice reduced the PXR expression in human xenografted tumours. The final objective is to improve JMV7048 bioavailability and test this molecule as an adjuvant strategy in preclinical studies to prevent tumor relapse after chemotherapy.

P133

Identification and validation of TRPV2 as new druggable target in metastatic melanoma

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Discovery of therapeutic targets against metastasis is of primary importance since being the main cause of cancer-related death. Deregulation of calcium homeostasis has been involved in numerous cellular metastatic behaviors, although the molecular determinants supporting these processes remain often unclear. Here, we showed that the expression of the plasma membrane TRPV2 calcium channel is a prominent feature in melanoma progression and dissemination. In fact, TRPV2 activity was sufficient to confer an invasive phenotype to non-invasive melanoma cells. Conversely, the invasive and migratory potential of highly metastatic melanoma cells was abolished upon TRPV2 silencing. Finally, through a retrospective study we have shown that TRPV2 overexpression is a marker of advanced malignancy and bad prognosis in human melanoma tumor samples. Altogether, TRPV2-induced Ca2+ signaling orchestrates in vitro motility and invasiveness of melanoma cells, as well as in vivo metastatic melanoma tumors dissemination. As our study revealed this channel as a promising prognostic biomarker and therapeutic target for the treatment of advanced melanoma, we are now developing and testing new TRPV2 synthetic inhibitors for which preliminary data will be presented.

P134

Targeting M2 macrophage with novel highly selective and potent CSF1R inhibitors for the treatment of cancer

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Background and Aims:
Cancer is one of the leading causes of death worldwide. After surgery, radiotherapy and chemotherapy, immunotherapy represents the fourth major area of cancer treatment. Immune cells represent an attractive target due to their ability to modulate the microenvironment in favor of tumor growth. Macrophages are the most abundant cells of the immune system in the tumor microenvironment. In addition, cancer patients with a high rate of macrophage infiltration have a lower survival rate. The newly recruited macrophages to the tumor site will be polarized into the M2 macrophage also called "tumor-associated macrophages (TAMs)" by a series of cytokines (CSF-1, IL-10, IL-4, etc...) produced by tumor microenvironment. These polarized M2 macrophages will then promote tumor development and invasion by contributing to tumor growth, angiogenesis, epithelial-mesenchymal transition and extravasation to metastatic sites. The CSF-1 produced by cancer cells plays both a role as a chemotactic substance for the recruitment of immune cells but also promotes M2 (pro-tumoral) polarization of macrophages. BCI Pharma goal is to create a novel class of kinase inhibitors and apply its proprietary platform to develop selective and potent CSF1R inhibitors in order to target M2 macrophages in cancer.

Methods:
Effects of inhibitors on CSF1R were evaluated in luciferase reporter assay. Cell viability assays have been measured using WST1 reagent and gene expression using real-time PCR. We used a MDA-MB-231-Luciferase cell line to analyze cell growth in coculture with macrophages.

Results:
Novel CSF1R inhibitors were identified in a CSF1R-Stat5 luciferase reporter assay, compounds inhibited Stat5 activation through inhibition of CSF1R with IC50 values in the nanomolar range. We showed that CSF-1 participates in M2 polarization mechanism and CSF1R inhibitors display an anti-M2 activity without any impact on pro-inflammatory M1 macrophages. This M2 macrophages phenotype is also induced by breast cancer cells (MDA-MB-231) and this process is also inhibited by CSF1R inhibitors. In turn, M2 macrophages participate to tumor growth in vitro.

Conclusions:
BCI Pharma developed potent CSF1R inhibitors displaying nanomolar efficacy in CSF1R inhibition. Their inhibitory effects have been demonstrated in multiple in vitro assays related to TAM and the most promising candidates will be tested in a cancer pre-clinical animal model.

P135

Store-operated calcium channels control proliferation and self-renewal of cancer stem cells from glioblastoma.

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Glioblastoma is the most frequent and deadliest form of primary brain tumors. The current standard therapy is safe maximal resection followed by concurrent radiotherapy and chemotherapy. Despite multimodal treatment, glioblastoma remains a refractory malignancy with 90 % of patients experiencing tumor relapse and an average life expectancy of no more than 15 months. Glioblastoma displays extensive tumor heterogeneity and contains a small population of cells, called glioblastoma stem cells, that are endowed with the ability to regenerate the bulk of the tumor and that are highly resistant to treatment, accounting therefore for tumor relapse.

Comparison of transcriptomic signatures of stem-like- and more mature glioblastoma cells disclosed an enrichment of calcium (Ca^{2+}) signaling transcripts in glioblastoma stem cells. Being one of the major ways of Ca^{2+} influx into non excitable cells, store-operated channels (SOC) are Ca^{2+} channels of plasma membrane that open in response to the decreased Ca^{2+} concentration in the endoplasmic reticulum. SOC are primarily formed by Orai1 and TRPC1 (Transient Receptor Potential Canonical 1) proteins, and are activated by STIM1 (Stromal Interaction Molecule 1), the endoplasmic reticulum Ca^{2+} sensor. Physiologically, the SOC can be activated by various molecules in the microenvironment *via* G protein-coupled or tyrosine kinase-coupled receptors, acting through the phospholipase C (PLC) pathway, and inositol triphosphate (IP3) which allows the Ca^{2+} release from the endoplasmic reticulum. This Ca^{2+} entry through SOC not only allows refilling of Ca^{2+} stores, but also evokes a sustained Ca^{2+}signal that drives a wide range of cellular and physiological effects.

Because SOC regulate the self-renewal of adult neural stem cells that are possible cell of origin of glioblastoma stem cells, we analyzed the expression and roles of SOC in glioblastoma stem cell cultures previously derived from five different glioblastoma surgical specimen. Immunoblotting and immunocytochemistry experiments showed that glioblastoma stem cells express Orai1 and TRPC1, two core SOC proteins, and their activator STIM1. Ca^{2+} imaging demonstrated that these SOC are functional. Pharmacological inhibition of SOC-dependent Ca^{2+} entries with SKF-96365, YM-58483 or GSK-7975A, decreased proliferation, impaired the capacity to form gliomaspheres and reduced expression of the stem cell marker SOX2 in glioblastoma stem cells.

Our data showing the ability of SOC inhibitors to reduce self-renewal of glioblastoma stem cells paves the way for a strategy to target cells considered responsible for conveying resistance to treatment and tumor relapse.

P136

TRPM8-Rap1A interaction inhibits prostate cancer cell adhesion and migration

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Aim: Emerging evidence indicates that TRPM8 channel plays an important role in prostate cancer (PCa) progression, by impairing the motility of these cancer cells. TRPM8 expression in PCa was shown to be regulated by steroid hormones and receptor signaling and indeed, it results down-regulated in metastatic androgen-independent PC3 cells with a consequent reduction in PCa cell migration. Here, we reveal a new aspect of PCa motility control via direct protein-channel protein interaction with the small GTPase, Rap1A.

Methods: The functional interaction was assessed in PC3 cells by active Rap1 pull-down assays and live-cell imaging experiments. Molecular modeling analyses allowed the identification of four putative residues involved in the interaction of the two proteins. Among these residues, three were validated by GST-pull-down, co-immunoprecipitation, and PLA experiments, namely the TRPM8 E207 and Y240 and Rap1A Y32. These residues were mutated and their functional role was further studied by adhesion and migration assays on PC3 cells. Confocal analysis and Ca^{2+}-imaging experiments were also performed to assess TRPM8 subcellular localization in our cellular model.

Results: TRPM8 inhibits PCa cell migration and adhesion by intracellularly trapping Rap1A in its inactive form (Rap1 N17A), thereby preventing its activation at the plasma membrane where it normally promotes cell adhesion through the β1-integrin signaling. Residues E207 and Y240 in the sequence of TRPM8 and Y32 in that of Rap1A are critical for the interaction between the two proteins. In particular, PC3 cells overexpressing TRPM8 with the double mutations E207A Y240A have shown a reduced interaction with the inactive form of Rap1A and, concomitantly, a less prominent inhibition of cell adhesion and migration with respect to those overexpressing TRPM8 wt. Moreover, we demonstrated that in PC3 cells transfected with TRPM8 the channel is not only expressed on the plasma membrane but also in the ER, from where it may bind Rap1A in its inactive form thus preventing its translocation to the plasma membrane and consequently its activation.

Conclusion: Our data shed light on a new role for TRPM8 that goes beyond its channel function and involves direct protein-protein interaction with the small GTPase Rap1A, crucial in mediating cell migration and adhesion. This study deepens our knowledge on the mechanism through which TRPM8 would exert a protective role in metastatic PCa thus providing new insight on its possible use as a new therapeutic target in PCa treatment.

P137

Modulation of the Hippo signaling pathway in gliomas by EGFR

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Gliomas are the most frequent malignant primitive brain tumors in adults. The Hippo signaling pathway plays a key role in numerous solid tumors, but few studies have been done in gliomas. Numerous receptor tyrosine kinases, such as EGFR, present deregulations in gliomas. The aim of this work was to study a possible link between EGFR and the Hippo pathway in tumors and in glioma stem cells. An *in situ* immunohistochemistry approach by using tissue microarray on 225 samples from patient tumors and an in vitro approach by studying 4 patient-derived glioma stem cell lines were performed. The results obtained by immunohistochemistry on tissue sections of patients have shown a significant association between EGFR activation state and tumour aggressiveness. Indeed, survival analyses have shown that the EGFR expression and its activation state would be a prognostic marker in glioma patients. Moreover, a positive correlation has been shown between the activated form of EGFR, phosphorylated on tyrosine 1068, and YAP expression. Finally, the in vitro study, after the inhibition of EGFR by gefitinib, highlights a modulation of phosphorylation of serine 397 of YAP depending on the mutational status of PTEN. These preliminary results suggest a potential modulation of the Hippo signalling pathway by EGFR in gliomas, via the S397 phosphorylation of YAP through the PI3K/AKT signalling. Therefore, the mutational status of PTEN should be taken into account during a potential therapeutic strategy aiming at the Hippo signalling in gliomas.

P138

Biological and molecular characterization of cancer stem cells in brain metastases from colorectal cancer

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Brain metastases (BM) from colorectal cancer (CRC) are associated with a dreary prognosis. BM stem cells (BMSC) from breast and lung cancer have already been described, but those from CRC have not yet been identified. In this study, we identified and characterized BMSC of CRC from patients' samples.

Further characterization of BMSC population were assessed in vitro and in vivo by performing clonogenic, limiting dilution assays as well as immunofluorescence and Western-blot analysis. A chicken chorioallantoic membrane (CAM) model was conducted and finally xenograft experiments using BALB/c-nude mice were realized.

Two patient-derived cell lines (BMSC-CRC1 and BMSC-CRC2) were obtained. These cells formed metaspheres and contained tumor-initiating cells with self-renewal properties. The BMSC-CRC lines expressed stem cell surface markers such as CD44v6, CD44 and EpCAM in a serum-free media and colorectal adenocarcinoma markers such as CK19, CK20 and CDX-2 in media enriched with fetal bovine serum. The CAM model confirmed the invasive and migratory capabilities of these cells. Moreover, the phenotype and markers in the tumor mice xenograft adequately recapitulated the original patient BM.

To our knowledge, and for the first time, BMSC-CRC have been successfully characterized. These promising results lead us to believe that BMSC-CRC could be useful future therapeutic targets.

Posters – Axis 2 “Genome Dynamics and Cancer”

P201

IgH Locus Suicide Recombination (LSR) in Chronic Lymphocytic Leukemia (CLL): Prognosis Indicator?

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Chronic Lymphocytic Leukemia (CLL) is an indolent hemopathy B malignancy common in the adult and incurable in which the monoclonal expansion of mature B-lymphocytes results in chronic lymphocytosis.

The oncogenic processes of CLL are not exactly defined. The B cells involved are characterized by the expression of CD5 and CD23 markers on their surface and of a BCR (B Cell Receptor) consisting in the vast majority of cases of IgM expressed at low level. The uncontrolled expansion of a B cell at the origin of the malignant clone would be consecutive to the stimulation of the cell via its BCR by an antigen (Ag) and the cell signaling mediated by the BCR seems to be involved. Accumulation of DNA damage, aberrations in DNA damage response signaling, error-prone repair of DNA damage, and associated chromosomal instability are common in CLL (Popp, 2019).

B Lymphocytes are prime targets of physiological DNA damage such as DNA Double Strand Breaks (DSBs) introduced by immunoglobulin (Ig) gene rearrangements: Locus Suicide Recombination 'LSR' and Class Switch Recombination 'CSR'. LSR and CSR are genetic rearrangements of the IgH locus in activated B-cells. CSR is a mechanism that diversify Ig isotype from IgM to IgG, IgA and IgE in contrast LSR deletes constant IgH genes and results in loss of BCR and conduct to B-cell death (Peron, 2012). LSR is a physiological event but its function in B-lymphocyte homeostasis is not yet been elucidate. In this study, we used genomic DNA from PBMC of patients with CLL; we surprisingly detected LSR recombination junctions. Analysis of LSR junction number leads us to differentiate two subsets of CLL patients compared to samples from healthy donors. A group with a high number of LSR junctions named "LSR High" (the mean levels of 731 LSR junctions / 1.10 ^ 6 cells) and a group with low LSR junctions number "LSR Low" (116 LSR / 1.10 ^ 6 cells), against 290 LSR / 1.10 ^ 6 cells junctions for PMBCs of control subjects.

The clinical and biological data were integrated into the analysis and we observed that the two groups of patients differ by Treatment Free Survival TFS after diagnosis, the Binet staging for the classification of patients and the mutational status of Variable segment (VH) of the patient. The group of patients with high number of LSR junctions has features associated with an unfavorable prognosis (Shorter TFS, non-mutated VH segment).
Furthermore, the repair joints at the LSR junctions for the group of patients with Low LSR junctions exhibit an atypical structure suggesting an abnormality in DNA DSB repair.
Our results suggest that the LSR junction count may be a prognosis marker in CLL while the structural abnormality of the LSR joints may reflect the altered response to DNA DSB. This is why we are continuing this work by evaluating DNA markers for DSBs and we are implementing the analysis of DSB repair in CLL patients by discriminating between the "LSR High" and "LSR Low" subset.

The evolution of CLL varies widely from patient to other, and our work seems to identify the LSR as a new prognosis factor. Our project aims to interpret this new area of research in LLC and opens up very new perspectives for taking in charge and prognosis of patients. Our results provide new insights on the LSR molecular process and function.

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P202

Experimental detection of chromatin associated G4 forming sequences (G4access)

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In mammals, majority of promoters are characterized by CpG islands- large areas of high GC content and CpG dinucleotide- that largely contribute to apparent nucleosome depleted regions (NDRs). Interestingly, sequences predicted to form G-quadruplexes (G4s) secondary structures of DNA are over-enriched at the precise location of experimental NDRs upstream of promoters 1. In sillico approaches have already been used to predict over 350,000 predicted G4 (PG4) sequences all over the human genome.

G4s are single-stranded and dynamic DNA structures alternative to B-DNA. They are stabilized by K+ ions and organized around four stretches of guanine (G) that associate in planar arrangement to form stacked tetrads. Over the past decade, G4 structures, already well characterized in vitro, have emerged as genomic regions with a high regulatory potential for multiple biological processes in vivo. The sequencing of many genomes has revealed that PG4s show non-random localizations, correlating with functionally important genomic regions such as promoters, replication origins, enhancers or insulators. Many studies also propose that G4s could play an in vivo role in diverse biological processes such as transcription, replication, stabilization of RNAs, regulation of translation, telomere protections, control of alternative gene splicing. In addition, many recent studies are shedding light to G4s as good target for antitumor therapeutics.

Here we describe G4access, a novel approach to isolate and sequence G4s associated to open chromatin, based on their resistance to Micrococcal nuclease. G4access is an antibody-independent procedure that allows for high enrichment of predicted G4s (PG4s) motifs, most of which can be confirmed in vitro. Using this technique in several human and mouse cell lines, we were able to show a cell-specific enrichment that both relates to apparent nucleosome depletion and transcription at promoters. G4access allows scoring for PG4 pattern variation linked to nucleosome positioning changes that occur following treatment with a G4 ligand. It also allows in hybrid mouse ES cells to reveal an unexpected role of G4s in the context of imprinting control regions in which we propose that they will hallmark allelic active loci. Finally, we also extended our procedure to non-mammal species showing less annotated G4s in their genome and found a decreased but remaining substantial G4 enrichment. Overall, our study not only provides a novel tool for studying G4 forming sequences in cellular context but also indicates their essential role as promoter element, in chromatin opening and nucleosome positioning.

P203
Cell polarity orders the cell cycle control system

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Healthy cell proliferation requires the correct ordering of cell cycle events and the monitoring of these events via checkpoints that delay cell cycle progression when problems are encountered. Checkpoint abrogation and the ensuing loss of correct cell cycle control can result in aneuploidy, a hallmark of tumours. Despite the critical importance of correct cell cycle ordering, the underlying mechanisms are poorly understood.

Using budding yeast as a model system, we have discovered that the establishment of a polarity axis is a key event that controls the correct ordering of the cell cycle. As in all eukaryotes, polarity axis establishment in budding yeast requires the activation of the Rho GTPase Cdc42, which governs the axis along which cells grow and divide by controlling actin dynamics (1). Defects in Cdc42 signalling or actin disorganization trigger a Swe1 (Wee1)-dependent checkpoint that delays mitotic entry via Cdk1 inhibitory phosphorylation (2). Using specific mutations that perturb cell polarity by ablating the lipid association of a Cdc42-associated scaffold protein and Cdc42 GEF (bem1 cdc24 lip), we observe catastrophic cell cycle defects (3, 4).

In contrast to wild type cells in which successive waves of Cdk1 activity associated with different cyclins impart temporal order on cell cycle events (5, 6), the cell cycle in the bem1 cdc24 lip mutant is characterized by considerable G1-, S- and M- phase cyclin overlap, stemming from gradual cyclin synthesis and sluggish degradation. The biological consequences of this misregulation include the misordering of G1 and S phase events, the scrambling of spindle pole body asymmetric segregation and the accumulation of multinucleate cells. These dramatic cell cycle defects accumulate despite robust Swe1 (Wee1)-dependent inhibitory phosphorylation of Cdk1. Using genetic tools available in budding yeast, we are currently testing how cells respond and adapt to the checkpoint, which serves as a model for checkpoint adaptation. Collectively, our study illustrates an unexpected mechanism through which cell cycle ordering is controlled to ensure robust, healthy cell proliferation.

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P204

Epstein-Barr Virus Transcriptome in Angioimmunoblastic T Cell Lymphoma (AITL) compared to Other Lymphomas and Cell Lines.

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Objective: The Epstein-Barr virus (EBV) is a ubiquitous human gamma-herpesvirus that infects the majority of population. Latency is a notable feature of herpesviruses and of course of EBV that is able to maintain its genome in host cells for a prolonged period in the absence of virion production. The EBV is linked to the development of a variety of carcinomas and B or T lymphomas. Nine proteins (EBNA-1, -2, -3A, -3B, -3C, -LP and LMP-1, -2A, -2B) as well as two RNAs (EBER, Epstein - Barr virus-encoded small RNAs, and BARTs, Bam-HI A rightward) and miRNAs are expressed during latency and may play a fundamental role in the cell proliferation. Its association with several malignancies makes EBV one of the most important oncogenic viruses. EBV is associated with angioimmunoblastic T cell lymphoma (AITL), the most common form of peripheral T-cell lymphomas, in more than 80% of cases. AITL, which mostly affects the elderly, is an aggressive lymphoma with a poor prognosis. This lymphoma, although rare, is more common in Europe than in other regions of the world. Few studies have focused on the relation between the EBV and AITL and it is not clear now what role the virus plays in this pathology. However, the characteristic of EBV through transcriptome analysis is also limited. Our objective, at the time of performance technologies, was to examine the EBV transcriptome in AITL biopsies compared to other EBV-positive lymphomas and cell lines by RNA-seq.

Materials and Methods: We studied 14 AITLs and 21 other lymphoma samples and also 11 cell lines including B95-8, 4 Burkitt's lymphoma (BL), 2 NK/T lymphoma and 4 lymphoblastoid cell lines (LCLs). Total RNA was extracted and poly(A) mRNAs were selected *via* oligo(dT) beads. Libraries were prepared after EBV mRNA captured by specific probes and finally sequenced on an Illumina MiSeq. Reads were aligned against hg19, EBV1 and EBV2 and EBV reads were normalized to transcripts per million (TPM).

Results: Results showed no significant difference between AITLs and other lymphomas and AITL patients did not form a cluster. BARTs, comprising BARF0, RPMS1, and A73, were the latency transcripts the most present in AITLs, suggesting they may participate in this lymphoma with the form of lncRNAs and/or miRNAs. Thus, BARTs, already described as highly expressed in carcinoma cells, were found very expressed in AITLs, other lymphomas and LCLs unlike in cell lines. We also showed, in AITLs, a type of latency described until now in carcinomas called latency IIc. This latency includes the expression of EBNA-1, LMP-2, and BNLF2a, which blocks antigen presentation to cytotoxic T lymphocytes. BCRF1, encoding a homologous protein of human interleukin 10, vIL-10, was in addition present in AITLs. The co-expression of these two proteins, BNLF2a and BCRF1, can contribute to immune escape and survival of infected cells and tumors. In order to understand the behavior of EBV in lymphoma tissues, we compared results obtained for our patients to those obtained for cell lines and we showed that viral behavior was not specific to a type of pathology.

Conclusion: These global results suggest the involvement of EBV in AITL development like other lymphomas.

P205

The chromatin compaction mediated by the E3 ubiquitin ligase TRIP12 affects genome organization and DNA damage repair.

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TRIP12 (Thyroid Hormone Receptor Interacting 12) is a nuclear HECT (Homologous to the E6-AP Carboxyl Terminus) domain-containing E3 ubiquitin ligase. TRIP12 is overexpressed in many cancers and is associated with a poor prognosis. This protein is part of the ubiquitin associated-degradation system. Numerous E3 ubiquitin ligases are deregulated in cancers. Amongst other substrates, TRIP12 is notably known to regulate the free nuclear pool of BAF57 (Brg1 Associated Factor 57), a component of the chromatin remodelling complex SWI/SNF and ASXL1 (Additional SeX combs Like 1), a polycomb repressive deubiquitinase of H2AK119Ub.

We showed that the N-terminal region (~440 aa) of TRIP12 is an IDR (intrinsically disordered region) which is responsible for the interaction of TRIP12 to the chromatin. More interestingly, we showed by immunofluorescence that an overexpression of TRIP12-IDR fused to GFP (now called IDR-GFP) modifies the organization of the genome forming chromatin condensates in a dose-dependent manner in several cellular models. Indeed, after transient transfection, cells expressing a low level of GFP-IDR show tiny chromatin condensates while moderate IDR-GFP-expressing cells harbor distinct DNA condensates. Ultimately, high IDR-GFP-expressing cells display a maximal compaction of DNA molecules similar to chromosomes mitotic condensation. We confirmed that the IDR-GFP-mediated condensates correspond to heterochromatin foci as they colocalize with the specific heterochromatin protein HP1.

We further studied the impact of DNA condensates formation on cell growth. Only high IDR-GFP expressing cells display a lower growth rate and an alteration of cell cycle distribution. These cells accumulate in G2 phase suggesting that such DNA condensates perturbs cell division. It is well known that DNA compaction can affect DNA repair system. Interestingly, in IDR-GFP transfected cells, we observed a significant inhibition of the 53BP1 foci in response to irradiation in a dose dependent manner.

Finally, we investigate the impact of TRIP12-GFP and IDR-GFP overexpression on genome accessibility. By an ATAC-seq approach, we demonstrated that the global genome accessibility is drastically modified in the presence of IDR-GFP and to a lesser extent with TRIP12-GFP.

Altogether, our results highlight a new role for TRIP12 on chromatin compaction through its N-terminal region. However, further investigations will be necessary to better understand what are the consequences of such genomic modifications on cancer cell lines.

P206

Special A-T rich Binding protein 1 (SATB1) is a critical regulator of immunoglobulin gene expression and a potential tumor suppressor in B cells by limiting mutations in oncogenes

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Mature B cells have complex function to produce a large diversity of antibodies (soluble immunoglobulins) in order to fight diverse pathogen infections. Functional mature B cells undergo, in an antigen-dependent manner, a second round of remodelings in their immunoglobulin (Ig) genes named Class Switch Recombination (CSR) and Somatic Hypermutation (SHM). CSR offers the possibility to change immunoglobulin type while SHM increases Ig affinity for its antigen. Since leading to double strand breaks in the case of CSR and to mutations as a consequence of unfaithful DNA repair in the case of SHM; these genetic events need an accurate control to avoid illegitimate events. Indeed, alteration in such mechanisms can conduce to illegitimate recombinations or off-target mutations that can cause tumor development. For a meticulous control of such events, driven by loci accessibility and regulatory transcripts, IgH locus contains different cis-regulatory regions such as the Core-Eμ enhancer flanked by two Matrix Attachment Regions (MARsEμ) a structure strikingly conserved between species. We suspect some MARs binding protein (MARs BP) as Special A-T rich Binding protein 1 (SATB1) to be involved in some B cell remodeling events. SATB1 is able to bind some MARs regions; in this way it can modulate chromatin organization and regulate genes transcription until 50kB from this binding site. Described as essential for brain development, SATB1 is also involved in immune system since critical for T-cell maturation. This protein has also been documented as involved in lung or breast cancer tumor development; displaying either tumor suppressor or oncogenic functions. Since SATB1 expression is differentially expressed in naïve and activated B cells, we questioned the function of this factor with a conditional knock out model deleting Satb1 in B-lineage cells by using a Cd79a-cre allele. B cell development, Somatic Hypermutation and Class switch recombination were analyzed in Satb1 conditional-knock out context. Peripheral B cells devoid of SATB1 modified Ig expression pattern, depending of their subset, through a mechanism involving transcription. This MAR BP acts as an activator of Ig gene transcription in inactivated B cell, and becomes a repressor when B cell were activated. Its deletion induces an increase of SHM frequency at Ig gene loci and, more strikingly increases off-target mutation in B cell-specific oncogenes such as Bcl6 and Pim1.Our study confirmed involvement of SATB1 in physiological Ig gene transcriptional regulation and, since also involved in oncogene mutation, highlighted a critical function of this protein as a potential tumor suppressor. Deciphering precisely SATB1 gene targets in B cell lineage will potentially help to decipher global functions of this nuclear factor in tumor development.

P207

Palindromic structure of IgH 3' Regulatory Region is involved in B Cell Nuclear Organization

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B Cell development is divided into two stages: the first stage is characterized by V(D)J recombination and the second is characterized by two major events mediated by the Activation Induced Deaminase (AID) enzyme, which are somatic hypermutation (SHM) and class switch recombination (CSR). These events, while necessary for the B cell, could be dangerous for B cells due to point mutations and double strand DNA breaks induced during SHM and CSR rearrarangements and therefore can be the cause of haematological malignancies, notably because of the close proximity of oncogenes to the IgH locus during genetic events. Consequently, IgH rearrangements must be tightly controlled especially by two regulatory regions of the IgH locus in mice: the Eμ-MARs region and the 3' Regulatory Region (3'RR). The 3'RR region stands as a super-enhancer that contains four core elements (hs3a, hs1.2, hs3b and hs4). hs1.2 is flanked by inverted repeated intervening sequences (IRIS) that form a 25kb-long quasi-palindrome. Although highly divergent in different species, IRIS sequences always stand as inverted copies on both sides of hs1.2, preserving a 3'RR symmetry. Several mutant mouse models carrying partial or total deletions of the 3'RR region allowed us to elucidate its function on transcription, CSR, SHM and Ig production during B cell development. In 3'RR deficient mice, a total absence of SHM, CSR has been reported. Analysis of four complementary models carrying partial deletions of the 3'RR (PAL KO, ΔIRIS, ΔleftPAL and c3'RR), identified distinct functions for both core enhancers and the quasi-palindrome. While SHM requires both 3'RR enhancers and its palindromic architecture, enhancers alone could support CSR. More recently, in the Δ3'RR model, we have shown that both IgH loci localize further from each other, demonstrating its role in nuclear organization.

Here, we tried to decipher the role of the 3'RR palindrome in nuclear organization at nuclear, supranucleosomal and genomic scales in resting and activated B cell in mouse models carrying the deletion of palindromic region (PAL KO) or carrying only 3'RR core enhancers (c3'RR). To investigate this, we used multiple approaches, from a reference technique, 3D-FISH, to innovative molecular biology experiments such as ATAC-Seq, 3C-HTGTS, LAM-HTGTS and DeMinEr, a tool developed by our team to analyze very low levels of mutation. This set of approaches allowed us to assess IgH nuclear positioning and heterochromatin addressing, global genome accessibility, loop conformation and translocation as well as mutation rates.

First, using 3D-FISH in B cells from PAL KO mice, our results show that IgH localization within the nucleus is modified. We observed an increase in distances between both IgH loci, relocation of IgH alleles to the nuclear periphery and accrued localization of IgH loci in heterochromatin. The last point is in accordance with the decrease in global accessibility to the IgH locus observed in PAL KO B cells, by ATAC-Seq. Absence of the palindrome structure also induced a decrease in loop formation between the 3'RR and the rest of IgH locus, leading to a modification of the interactions with the 3'RR. This total nuclear reorganization lead to an increase in mutations within oncogenes and an increase in illegitimate rearrangements. Indeed, in the PAL deficient mice, we observed an increase in Bcl6 or cMyc mutation rates as well as an increase in translocation events. While quite similar to the PAL KO model, c3'RR B cells do not share all of features previously described. With all of these elements, we propose that the 3'RR palindromic structure could be involved in IgH regulation by modulating its location within the nucleus and participating in maintenance of legitimacy during late B cell genetic rearrangements, whereas the presence of only core 3'RR enhancers restores an almost normal nuclear organization.

P208

Spatial intra-tumoral heterogeneity in liver metastasis from primary colorectal tumor - a way for evolution and treatment resistance

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Cancer is a heterogeneous tissue in term of metabolic and signalling pathways pattern depending on the area and their connection with stroma and mutational states which leads to mosaicism inside the tissue is also mainly describe. It occurred in the primary tumor and once metastasis are formed, they will also evolved, and behove in a different way. This description in one of the therapeutic resistance mechanism that exists leading to bad outcome, relapse and inefficiency of therapies, notably given another complexicity level of choosing targeted therapy. In order to explore this tumor evolution amongs space and time, we had access to biopsies of patients with colorectal cancer firstly diagnosed that evolved with liver metastasis and their normal tissue (normal colon and normal liver). As a means to describe the heterogeneity inside cancer tissue, we analysed their proteomes and their transcriptomes. The aim is the find pathways regarding both transcriptomics and proteomics analysis, if they are in common, different and complementary. We used transcriptomics for gene expression findings but also for the sequences themselves and their mutational state behind to go further. One of our goal was to have access to microenvironment realm, because the cross-talking occurring between cancer cells and the stroma cells reprogrammed for the purpose of draining energy, nutriments, growth factor to the tumor is mainly needed by the tumor. However, this proceed can behave in a different manner for the metastasis since they arrived in a healthy tissue. Finally, clinical data obtained from patients, their outcome and their therapeutic assay were a track that guided us in our exploration.

P209

H2A.Z1/H2A.Z2, two isoforms of the same histone variant as critical regulators for stemness and cell lineage fate

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The involvement of the histone variant H2A.Z and its isoforms in the regulation of gene expression is an increasing exciting field considering the impact of such regulations in physio-pathology. Indeed, we and other recently showed that H2A.Z1 and H2A.Z2 isoforms exert cooperative or antagonistic transcriptional regulations on subsets of genes involved in key processes, such as proliferation, senescence or several organ functions. However, the impact of these isoforms on tissue homeostasis merits to be more thoroughly studied. In this work, we analyze the relative role or each H2A.Z isoform on parameters of the intestinal epithelial homeostasis. We demonstrated the cooperative effects of both proteins on the proliferation of stem and progenitor cells, as well as in the differentiation of the secretory lineage, but their antagonistic roles on enterocyte-related differentiation. Thus, we highlighted, for the first time, how isoforms of an histone variant specifically regulate tissue homeostasis and impact the organ function, as well as disease processes.

P210

The recognition of double strand breaks by Ku is impaired by the presence of long Guanine tracts

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In cells, DNA essentially exists in B-form. However, non-B DNA such as G-quadruplexes (G4) have been detected in the human genome (1). G4-forming sequences have been shown to be enriched at telomeres and transcription active regions and are associated with DNA-break hot spots and with somatic copy number alterations in various cancer types.

These facts suggest that the presence of G4 at double strand breaks could decrease the efficiency of DNA repair mechanisms. Two pathways can solve these double strand breaks (DSB): Homologous Recombination (HR), which can occur during the late S to G2 phases of the cell cycle, and Non-Homologous End Joining (NHEJ), which can proceed throughout the cell cycle.

Here, we question the possibility that the very first step of NHEJ, which consists in the DSB recognition by Ku, is altered by G4 present in the vicinity of double strand breaks. For that, we use the single molecule technique called high troughput Tethered Particle Motion that we developped (2). We track the motion of Ku-coated particles on double strand DNA molecules engineered with various putative G4 sequences at their free end. We show that long telomeric regions impairs the capacity of Ku to recognize DSB.

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P211

CDK8 and CDK19 control the CFTR pathway in the intestinal epithelium

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CDK8 and CDK19 form a highly conserved cyclin-dependent kinase subfamily and interact with the Mediator transcriptional coregulator complex, which is required for expression of all genes. CDK8 or CDK19 are thought to control expression of different genes, but cells lacking either kinase have only minor transcriptional alterations. Here, by a combination of conditional gene knockout and CRISPR-Cas9-mediated gene disruption, we demonstrate that CDK8 and CDK19 function redundantly to control tissue-specific gene expression. Although CDK8 is essential for early mouse development, it is dispensable both for normal intestinal homeostasis and efficient tumourigenesis. Individual knockout of Cdk8 and Cdk19 in intestinal organoids has only limited effects on gene expression, with Cdk19 compensating for loss of Cdk8. In contrast, their combined deletion, although not cell lethal, causes progressive loss of proliferative capacity. The Cystic Fibrosis Transmembrane conductance Regulator (CFTR) pathway is transcriptionally and functionally downregulated, leading to increased mucus accumulation and secretion by goblet cells. Our results suggest that Mediator kinases do not play essential roles in differentiated cells, but cooperate to regulate tissue-specific transcriptional programmes during differentiation.

Posters – Axis 3 “Translational Research, from Biology to Clinics”

P301

Platinum-based chemotherapy induce inflammation via a non-canonical STING pathway dependent on ATM activation

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Upper Tract Urothelial Cancers (UTUC) are aggressive tumours of ureter or renal pelvis. They are treated as bladder cancer with more than 50% of relapse justifying the need of new therapeutic options. For this purpose, we study therapeutic combinations to stimulate the immune system by platinum-based chemotherapy in order to potentiate the effect of an anti-PD-L1 (Durvalumab). Indeed, UTUC usually present low tumour immune infiltrate that may limit their response to immunotherapy. The aim is to compare the effects of three combinations (cisplatin + gemcitabine, carboplatin + gemcitabine and oxaliplatin + gemcitabine) and to determine the best inducer of tumoral immunogenicity.

Using UTUC cell line(UM-UC-14) we have evaluated the cytotoxicity effects of the chemotherapies combinations in 2D and 3D cell cultures. We have assessed their potential (i) to induce DNA damage using image cytometry, (ii) to induce PD-L1 expression using flow cytometry, (iii) to activate the cGAS/STING pathway using qPCR, and finally (iv) to stimulate the anti-tumor immunity by in vitro and in vivo experiments.

Our results demonstrate that all three chemotherapies combinations present synergistic effects in UM-UC-14 (UTUC cell line) spheroid cultures. These treatments also induce DNA damage pathway activation in UM-UC-14 cells demonstrated by an increase of γH2AX, phospho-ATM, phospho-CHK1 and phospho-CHK2 positive cells. We found an increase of PD-L1 membrane expression after treatment in UTUC cell line. Moreover, RNA Seq analyses indicates that the major pathways induced by these combinations are the inflammatory pathways. We could observe an immune cell death induction demonstrated by an increase of ATP and HMGBA release and calreticuline exposure. We showed cGAS/STING pathway activation as evidenced by an increase of P-IRF3 protein level and interferon stimulated genes (ISGs) expression. We demonstrated an inhibition of the ISGs induction after treatment by our chemotherapies when cells are treated with an ATM inhibitor or in UMUC-14 deleted for STING.

These results indicate that the combination of platinum salts + gemcitabine induces inflammation via a non-canonical STING pathway dependent on ATM activation in UMUC-14.

P302

Proteolytic protein repression mediates tumor T cells infiltration and anti-tumoral immune response: Drug-repurposing approach

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Immune checkpoints, such as programmed death (PD-1), play important roles in regulating T cell responses. They were proven to be effective targets in treating various cancers; however, prolonged stimulation of T cells due to chronic infections or cancer results in gradual suppression of the cell's effector function. The discovery that inhibitory receptors serve as an immune checkpoint, which regulates T cell effector function, was rapidly exploited for the treatment of various solid and hematologic cancer. Although therapies targeting PD-1 were clinically effective in various preclinical models and cancer patients, several patients with solid tumors are refractory to these treatments. Solid tumors evade anti-cancer immune control by establishing immune-privileged niches within the tumor microenvironment that reduce proliferation, viability, and/or activity of cytotoxic T lymphocytes (CTL). Interestingly, a wide range of proteins involved in these pathways require proteolytic cleavage of their protein precursors by furin to be biologically active.

Using computer-aided virtual screening and repurposing approved drugs against furin, we generated a collection of molecules acting in different therapeutic areas that we tested in vitro and in vivo. We found that 14 drugs were able to inhibit the activity of the furin in vitro using enzymatic digestion assay. In cells they were able to repress the cleavage of the known furin substrate PDGF-A. Of these molecules, two namely I0 and I13 induced a potent repression of PD-1 expression in T cells activated by PMA/Io or CD3. Subcutaneous inoculation of mice with syngeneic cancer cells revealed the anti-tumoral efficacy of these two drugs that associated increased intratumoral T cells infiltration in the developed tumors. In addition, the treated mice showed improved overall survival while compared to controls.

These and other findings highlight the potential use of drug repositioning process for the identification of safe furin inhibitor able to repress PD-1 expression in T cells and FDA-approved drugs as a novel immunotherapeutic approach to inhibit tumor progression.

P303

"Epitranscriptomics": a promising source of biomarkers for personalized medicine

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Diffuse gliomas are among the most common tumors of the central nervous system, with a high morbidity and mortality and very limited therapeutic possibilities. They are characterized by a variability in the histological and molecular features, the ability to transform into a higher grade and/or to disseminate, and response to treatment. Most particularly, grade II (low-grade) and III (high grade) gliomas cannot be easily distinguished, as intratumor heterogeneity of the tumor grade is not rare in patients treated with extensive surgical resection.
Identification of accurate biomarkers, through molecular profiling in healthy and cancer patient samples, could improve diagnosis and promote personalized medicine. While genetic and epigenetic alterations of DNA are currently exploited as cancer biomarkers, their robustness is limited by tumor heterogeneity. Further, defining a set of biomarkers instead of one would maximize the prediction performance. Recently, cancer-associated alteration of RNA marks has emerged as a promising source of diagnostic and prognostic biomarkers.
RNA epigenetics (a.k.a "epitranscriptomics") is an emerging field that encompasses more than 150 chemical modifications in all types of RNA. These modifications fine-tunes gene expression and play a role in key cellular processes in both physiological and pathological contexts. Therefore, it comes at no surprise that a growing number of studies have connected variations of specific modified nucleoside levels in solid/liquid biopsies with cancer onset and progression.

Others and we have associated several chemical marks with cancer evolution, adaptation as well as response to conventional therapy. Building on these observations, epitranscriptomics landscape may evolve along with cancer progression and grading. Our goal is to exploit multiplex targeted mass spectrometry in order to establish "epitranscriptomics signatures" that could be used for diagnosis and/or prognosis.

Total RNA was extracted from a cohort including 59 RNA samples from tumor biopsies (glioma / glioblastoma patients at different stages of the disease (grades II, III, IV)) as well as 19 "control samples" using TRIzol reagent (Invitrogen). RNA was digested into nucleotides and dephosphorylated into nucleosides. The nucleosides were quantified by using a LCMS-8060 mass spectrometry in MRM mode.

Among the 35 RNA modifications implemented in our LC-MS/MS method, we successfully detected 25 modifications. We designed an experimental pipeline dedicated to feed a bioinformatics process with both experimental and clinical data. MS data was merged with that containing the grade information. Then we applied statistical analysis methods to (1) assess the variability of any chosen nucleoside quantity with the tumor grade (0 for controls; II, III or IV for glioma patients according to WHO classification) and (2) investigate whether the variation of nucleoside quantities among samples reflect the distinct tumor grades, for instance using Principal Component Analysis (PCA). We could distinguish three categories of chemical marks -decreased, increased and unchanged. Remarkably, three-dimensional PCA was capable of separating all three grades of glioma, most particularly grade II from grade III. Finally, a machine learning based approach (Support Vector Machine) demonstrated that epitranscriptomics-based grading prediction is accurate over 90%.

In the coming years, cancer studies in the blossoming field of epitranscriptomics will definitively translate into opportunities for clinical applications. Easy to perform, fast, cost-effective, sensitive and reproducible methods are needed to evaluate the power of these potential biomarkers. RNA modification profiling by mass spectrometry could become a powerful tool for identifying biomarkers signatures in cancer which, coupled with machine-learning approaches, may help disease diagnosis as well as clinical decision-making.

P304

Colorectal cancer stem cells respond differently to chemotherapies depending on their original location

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Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths worldwide. Treatment options for CRC include surgical resection, chemotherapy, targeted therapy and immunotherapy. Unfortunately, even after well-directed curative treatment, some patients experience treatment failure and relapse due to multi-drug resistance, which can be attributed to cancer stem cells (CSCs). With their self-renewal and multi-lineage differentiation capabilities, CSCs play a key role in tumor initiation, therapeutic resistance and metastasis development. However, CSCs represent less than 5% of the tumor mass, which is a challenge for their isolation. The sedimentation field-flow fractionation (SdFFF) technique allows the sorting of homogeneous populations of poorly differentiated or undifferentiated cells according to biophysical characteristics.

The objectives of this research project are: (1) to isolate CSCs from cell lines and primary cultures, representative of different stages of CRC using SdFFF, (2) to characterize phenotypically and functionally the sorted fractions (CSC-enriched vs differentiated), and (3) to analyze the response to chemotherapy of these fractions in 2D and 3D models.

Our phenotypic and functional characterization results confirm the relevance of SdFFF to isolate CSC-enriched fractions. Furthermore, our preliminary results have demonstrated a difference in chemotherapy sensitivity between CSC-enriched and control fractions, as well as between cell lines derived from primary tumors and those derived from CRC metastases, in 2D. Chemosensitivity tests are underway in 3D models and the regulatory pathways that may be associated with chemotherapy resistance will be analyzed. The ultimate goal of this project is to study the therapeutic response of CSC-enriched fractions from CRC patient samples in order to predict treatment efficacy.

P305

The Endothelin signaling pathway in Diffuse Low-Grade and High-Grade Gliomas

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Introduction: Adult gliomas represent 40% of all brain tumors and are classified in 3 grades of malignancy with glioblastomas, the deadliest. The incidence of malignant gliomas has been multiplied by 4 those last 30 years. Diffuse Low-Grade Gliomas (DLGG) are slowly-growing which often progress into secondary glioblastomas (GBM). Gliomas contain different populations of tumoral cells with different levels of differentiation as well as tumorigenic cancer stem cells. This intratumoral heterogeneity defeats currently used therapies. The molecular mechanisms and pathway underlying the generation of this cellular diversity remain also partially elucidated.

Aims: Endothelins (ET1 and ET3) are cytokines secreted by vessels in the brain and may play a role in the control of proliferation, migration and differentiation of glioma cells. Two receptors for endothelin, EDNRA and EDNRB, are expressed in the brain. We aimed at investigating the expression and role of the endothelin pathway on the proliferation, differentiation and migration of glioma cells.

Methods: Here, we used cultures of low and high-grade gliomas to explore the expression of EDNRA and EDNRB by qPCR, Western blot and immufluorescence thanks to new monoclonal antibodies generated against these receptors. We used immunohistochemistry to study the expression of these receptors on tumor cryosections. We investigated whether the expression of these receptors is regulated during differentiation of glioblastoma cancer stem cells notably by using glioma database mining. Finally, we explored the effect on endothelins on glioma cells properties.

Results: We found that EDNRA and EDNRB receptors are both expressed in glioma cells but at different levels. Stainings performed on live and fixed cells showed that these receptors are distributed between the surface of the cells and the cytoplasm. By treating high- and low grade glioma cells with ET-1 and ET-3, we found that these cytokines affect proliferation and possibly migration and differentiation.

Conclusion: Endothelin receptors are present in low- and high-grade gliomas and regulate properties of glioma cells. Our current work aims at deciphering the downstream signaling cascade and target genes mediating endothelin effects on glioma cells.

P306
Programming lactic acid bacteria for colorectal cancer therapy

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In the recent years, bacteria have been genetically engineered to detect and treat several pathologies in vivo, including infections, metabolic disorders and inflammatory bowel diseases. Recently, numerous studies have been conducted to modify bacteria to treat cancer. The strategy of anti-cancer bacteria consists in genetically modifying bacteria in order to make them recognize, colonize, and proliferate in the tumor microenvironment and finally produce in situ therapeutic molecules in a controlled manner. A potential advantage of using bacteria as cargo is to counter the side effects of chemotherapy and immunotherapy treatments, which are still generally delivered systemically.

Our project is to engineer *Lactobacillus gasseri* as a cargo to treat cold solid tumors using colorectal cancer as model. As precision engineering of LAB is currently limited by the lack of tools enabling reliable control of gene expression, a part of this project aims at building a collection of well-characterized genetic parts to control transcription, translation and secretion levels. In parallel, we are optimizing the production of cytotoxic (*Cytolysin A, Azurin*) and immunomodulatory proteins (*VHH- aPDL1, VHH VEGF*, interleukins,...) in *Lactobacillus gasseri*. Ultimately, bacterial therapeutic activity will be controlled by sensors responding to signals from the tumor microenvironment. In order to test, improve and validate our recombinant strains, we are combining in vitro spheroid-based screening with animal models.

P307

Discovery of Soluble Pancreatic Cancer Biomarkers Using Innovative Clinical Proteomics and Statistical Learning

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Pancreatic ductal adenocarcinoma (PDAC) late diagnosis is primarily attributed to its asymptomatic progression combined with absence of any reliable screening markers. This leads to one of the deadliest cancer with a 5-years survival rate less than 10%[1]. Diagnosis is provided by endoscopy-guided fine-needle biopsy (EGFNB) only, which is invasive, risky and with a poor level of negative predictive value (NPV). Nevertheless, EGFNB remains the gold standard for diagnosing PDAC and enabling the right treatment for the patients.

In this proof-of-concept study we developed a novel proteomic approach which recovers the soluble proteins in the EGFNB that remains a rich source of potential biomarkers[2]. Proteomic analysis of the soluble proteins led to over 2500 identifications, which were subjected to subsequent statistical analysis. To build the subsequent protein signature score (PSS), we used several resampling methods[3-6] at different steps of the analysis and an algorithm derived from microarray analysis techniques[7,8].

We followed 58 patients that underwent pancreatic EGFNB, of which 43 were diagnosed as PDAC while 15 had non-cancerous lesions. The PSS achieved 0.917 and 0.853 of sensitivity and specificity rates respectively. We then linked the PSS with clinical data to provide a decision algorithm achieving 100% of positive predictive value and 92.3% of NPV.

Due to their soluble nature, the newly discovered protein biomarkers bare the potential to be detected in the patient serum. This will enable the development of non-invasive blood-sample based assays to a larger patient cohort, leading to the hope of promoting a population-based screening test, allowing for quicker management at an earlier stage.

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P308

Repression of liver colorectal metastasis by the naturally occurring inhibitor of Furin (ppFurin)

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At present, surgical resection is the only curative option for liver colorectal metastasis, and it produces roughly between 20% 5-year disease-free survival rate. Indeed, although the liver represents a common site of spread from many tumor types isolated hepatic metastases most commonly occur from colorectal tumors. Thereby, a better understanding of the cellular and molecular biology of colon cancer and its hepatic metastases will facilitate the development of new effective prognostic and/or therapeutic strategies that may be used alone or with conventional treatments.

To date, the proteolytic processing and activation of various precursor proteins involved in colon cancer are known to be mediated by the convertase Furin and its targeting by gene therapy are suggested in ongoing clinical trials. In this study, we evaluated the repression of the malignant and metastatic phenotype of cancer cells by the prodomain of Furin (or ppFurin), a natural inhibitor of this convertase. The overexpression of ppFurin in cancer cells considerably reduces the furin enzymatic activity and furin ability to activate substrates involved in cancer such as PDGF-A and IGF-1R. Inhibition of the cleavage of these substrates affects their signaling pathways, as well as invasion of tumor cells while increasing their sensitization to apoptotic agents. In mice, intrasplenic inoculation of colon cancer cells induces the formation of hepatic metastases and this effect is repressed by ppFurin. Similarly, the use of synthetic ppFurin of 83 amino-acid mediated the inhibition of tumor cells proliferation and migration/invasion. In parallel, the analysis of Furin expression in colorectal and/or liver metastasis patients revealed high expression of this convertase in colon tumors, and this expression is further upregulated in their corresponding liver metastasis. Taken together, these findings demonstrate that ppFurin may constitute a potential strategy for the prevention of liver colorectal metastases.

P309

Modelling 3D tumour microenvironment in vivo using live imaging technique : a tool to predict cancer fate

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[2] LPHI, CNRS, INSERM, Univ Montpellier - Multiscale analysis of blood stem cells production in vivo

The tumour microenvironment (TME) characterisation has become over the years a major topic in the understanding of tissue tumorigenesis and novel treatments development. The tumour niche is a very dynamic region where different cell types coexist and interact with cancer cells, conditioning their fate. Among them, vessels play an important role as a pathway for cancer dissemination and oxygen and nutrient supply. Similarly, the recruitment and infiltration of immune cells in the tumour mass, such as macrophages, have been described as having a direct impact on cancer progression. Moreover, the composition of the tumour niche is nowadays used as a mean of diagnosis and indicator of good or bad prognosis. Therefore, the 3D dynamic visualization of the TME appears as a predictive tool of cancer cell behaviour in terms of intravasation, invasiveness and metastasis. However, to study in detail the molecular and cellular mechanisms of cancer progression, innovative methods based on in vivo models are essential. In recent years, zebrafish embryos have emerged as a relevant tool in oncology. Among many advantages, its transparency allows to image and visualize in real time conserved cellular processes involved in tumour dissemination. We report for the first time a cutting-edge methodology to visualize in 3D live imaging the interaction between TME and cancer cells to study cancer fate. Through a xenograft model of human glioblastoma and melanoma cancer cells in zebrafish embryos, we highlight two major actors of TME, macrophages and vessels. The 3D reconstructions of the tumour niche show a massive macrophage recruitment and infiltration in the tumour mass. Simultaneously, they demonstrate the active role of vasculature in tumour progression through neo-angiogenesis and vessel co-option events. This method can be implemented to study different cancer types and TME players. Finally, with the exponential interest in precision medicine, this method will represent a powerful tool to predict tumour fate in patients and to screen new efficient therapies.

P310

New Imidazo[1,2-a]quinoxalines compounds for Pancreatic Ductal Adenocarcinoma treatment : mechanism of action and target identification.

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In 2020, the survival rate for patients with Pancreatic Ductal Adenocarcinoma (PDAC) is dramatically poor with a five-year survival rate less than 10%. The research of new treatments, which could complement the current therapeutic arsenal constitued only by non-specific treatments such as gemcitabine or FOLFIRINOX, associated with innovative diagnostic strategies, is a major challenge.

IBMM F16 team's developed new Imidazo[1,2-a]quinoxaline compounds with potent activities against cancer cells. Among them, EAPB02303 exerts nanomolar activities in pancreatic cancer cells. The objective of the IRCM and IBMM collaboration project is to elucidate the precise mechanism of action of EAPB02303 and to assess its anticancer potential in pancreatic cancer.

We characterized EAPB02303 effect on cell viability on a panel of pancreatic cancer cells including Patient Derived Xenograft (PDX) and Cancer Associated Fibroblasts (CAF). Moreover, we looked for synergy with other pancreatic cancer treatments and found a potent synergy with paclitaxel. Now, these results will be confirmed in pancreatic Patient Derived Xenografts models. A pharmacokinetic study will help us to define the best way of injection of EAPB02303.

Furthermore, we found by flow cytometry and immunofluorescence that EAPB02303 induces mitosis arrest and mitotic catastrophe after 24h treatment. We also showed that cells undergo apoptosis after 48h treatment.

We used chemical proteomic methods for target identification of EAPB02303 based on affinity purification using compounds-immobilized beads. This quantitative proteomic technique helped us to identify potential targets of EAPB02303. Now we are analyzing mRNAseq and Reverse Phase Protein Array data of pancreatic cancer cell lines treated with EAPB02303 6 or 24h. These data will allow us to identify signaling pathways and key proteins implicated in EAPB02303 effect. All these potential proteins and pathways will be confirmed by knockdown models and western blot.

P311

Association of oxaliplatin-based chemotherapy and ATR inhibitor in pancreatic cancer

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Pancreatic Ductal Adenocarcinoma (PDAC) is an extremely aggressive disease with no efficient treatment. Recently, a new polychemotherapy oxaliplatin-based (FOLFIRINOX) has been approved but associated with toxicity and limited efficiency. Most of the drugs induce their toxicity by provoking DNA damages and replication stress leading to the activation of DNA repair pathways. Recently, a PARP inhibitor has been approved by the FDA for patients with BRCA mutated PDAC showing the potential of this type of therapy. Therefore, in this project, we added an ATR inhibitor (ATRi) to FOLFIRINOX to increase responses and analyzed the effect of this combination on the tumor microenvironment.

Viability matrix in 2D & 3D co-culture of tumor cells with primary CAFs were carried out and DNA damage repair pathways, cell death and autophagy were analyzed. In vivo, immunodeficient mice xenografted with ATCC and Patient Derived Xenograft models were treated with FOLFIRINOX and ATRi to evaluate the effect on tumor progression.

A synergistic effect of the combination was demonstrated in pancreatic models in co-culture with CAFs independently of the DDR deficiency. A higher apoptosis and DNA damages were observed in tumor cells treated with the combination associated with a decrease of DNA repair pathways and an inhibition of the autophagy flux. A protective effect of the CAFs on tumor cells was observed and secretome of CAF analysed. In vivo, the combination inhibits significantly the tumor growth compared to each treatment alone.

Now, a validation of this polychemotherapy in vivo using co-culture models in immunodeficient mice is crucial to confirm the therapeutic potential of this new treatment for PDAC.

P312
Characterization of β-catenin translation factories

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The Wnt / β-catenin pathway plays important roles in embryogenesis and in tissue homeostasis in adults. It is also very important in cancer. Indeed, nearly all colorectal tumors have an alteration of this pathway and about a third have a strong β-catenin signature. β-catenin is known for its dual function as a transcription factor in the nucleus and in adherent junctions at the cell membrane. This protein is tightly regulated in terms of its stability. β-catenin located at adherent junctions is stable whereas the one present in the cytoplasm is rapidly degraded by the proteasome. However, this protein is stabilized in the presence of a Wnt signal, and it can then be transported to the nucleus where it activates transcription. The key factors involved in the degradation of β-catenin are APC, Axin and the kinases CK1a and GSK3b. Altogether, these proteins form the "destruction complex". In the absence of Wnt, this complex binds β-catenin and degrade it. In the presence of Wnt, this complex binds to the Wnt receptor at the plasma membrane and no longer interactions with β-catenin, which is thus stabilized.
We have discovered a new regulatory mechanism in which β-catenin is translated in specialized cytoplasmic foci concentrating β-catenin mRNA, which we have called "translation factories". These foci concentrate the destruction complex and allow a co-translational degradation of β-catenin.They thus play an important role in the control of its expression.

The aim of this project is to study in detail these β-catenin translation factories and to address their possible roles in tumorigenesis. For this, we used quantitative proteomic approaches in order to characterize the biochemical composition of these factories as well as the polysomes translating β-catenin. Using APC as a bait in HEK293 cells, we found that the interaction of APC with β-catenin is translation dependent. This result confirms our hypothesis about the co-translational degradation of β-catenin.

Moreover, we found that APC interacts with many members of the CTHL complex. This complex is known for its E3 ubiquitin ligase function. This suggests a possible role of this complex in β-catenin foci formation and maybe in its degradation.

Since Wnt pathway is related to colorectal cancer, we tried to determine if these foci are also present in colonic cells. Using smFISH essays on mouse tissue sections, we found that these foci are present in wild-type colonic tissue. Moreover, these factories seem to have a specific localization pattern among the colonic crypt. Many are found to be formed in the differentiated epithelial cells, whereas stem cell and proliferative cells may lack them.

This completion of this project will provide detailed knowledge of β-catenin translation foci and it will clarify the role of these foci in normal or cancer cells. In particular, our experiments could determine a new function for the tumor suppressor APC, which would be to organize these translation foci and thus to control the synthesis and the fate of the nascent β-catenin protein.

P313

The effect of metformin on the survival of colorectal cancer patients with type 2 diabetes mellitus

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Introduction: Colorectal cancer (CRC) is the second most deadly cancer worldwide and the third most diagnosed globally. Evidence from previous studies suggests a protective effect of metformin in patients with CRC. The aim of this study was to examine the associations between metformin use and overall survival (OS) and disease-free survival (DFS) in CRC patients with type 2 diabetes mellitus.

Method: This was a historical cohort study. It included diabetic patients who underwent surgery for CRC at Limoges' University Hospital between 2005 and 2019. Data on the characteristics of patients, CRC, comorbidities, and drug exposure were collected from the patients' electronic medical records. The exposure was the use of metformin. Patients were followed for two years after surgery. The outcomes were overall survival (OS) and disease-free survival (DFS). All cases of CRC recurrence were confirmed by computed tomography (CT) scan or magnetic resonance imaging (MRI) and verified by biopsy. Multivariate analysis using the Cox model was performed to adjust various confounding factors (age, sex, body mass index (BMI), tumor site, cancer stage, Charlson comorbidity index, diabetes complication, the use of hypolipidemic and antihypertensive drugs) and all statistical analyses were done with IBM SPSS Statistics 22.

Results: Of the 1605 patients operated for CRC, 290 patients were identified with type 2 diabetes mellitus. Half of the diabetic patients were treated with metformin (49.7%)
The 2-year OS rate for metformin users was 86.9±2.9% and 71.0±4.0% for metformin non-users (p=0.001). The Cox regression model showed a 64.0% reduction in all-cause mortality (adjusted hazard ratios (aHR), 0.36; 95% confidence interval 95%CI 0.17-0.73) among metformin users compared with non-users. Furthermore, metformin users had better DFS than non-users (aHR, 0.31; 95%CI 0.19-0.52).

Conclusion: The use of metformin may improve OS and DFS in diabetic patients with CRC. Further prospective studies are also recommended to better explore the effect of this drug.

P314

Uncovering phenotypic heterogeneity of drug-tolerance in EGFR-mutated non-small cell lung cancer in vivo and in patients

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Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKI) are effective therapies for advanced lung cancer patients bearing EGFR-activating mutations but are not curative due to the inevitable apparition of resistance. Recent in vitro studies suggest that resistance to EGFR-TKI may arise from a small population of drug-tolerant cells through non-genetic reprogramming. Here, we show that combination of EGFR-TKI with a farnesyl transferase inhibitor efficiently delays the relapse in EGFR-mutated patient-derived xenograft (PDX) models. We highlight phenotypic heterogeneity and different adaptive resistance mechanisms by single-cell RNA-sequencing from PDX samples after EGFR-TKI or combined treatments. We also propose to deepen our understanding of drug tolerance in patients by monitoring circulating tumour DNA and by performing phenotypic and molecular characterisation of circulating tumour cells in a cohort of EGFR-mutated adenocarcinoma patients treated with EGFR-TKI.

P315

Repurposing the old antihelmintic drug niclosamide to sensitize colon cancer stem cells to chemotherapy

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Cancer chemoresistance and tumor recurrence are often attributed to cancer stem cells. We previously demonstrated that down-regulation of Pregnane X Receptor (PXR, NR1I2) decreases the chemoresistance of cancer stem cells and could prevent colorectal cancer recurrence. However, currently, there are no pharmacological means to down-regulate PXR expression or activity.

Here, we identify key targetable elements upstream of PXR signaling in cancer stem cells. We first confirmed that PXR is downregulated by the microRNA, miR-148a, which when over-expressed decreased PXR expression in cancer stem cells and impaired their relapse after chemotherapy in mouse tumor xenografts. We then developed a fluorescent reporter screen for miR-148a activators among the 1280 known drugs in the Prestwick library. In vitro validation of the ten most promising candidates confirmed the anti-helminthic drug niclosamide as by far the most potent inducer of miR-148a expression. Niclosamide also decreased PXR expression and cancer stem cell numbers in colorectal cancer patient-derived cell lines. Furthermore, xenograft studies demonstrated that niclosamide synergizes with classic colorectal cancer chemotherapeutic agents to downregulate PXR and prevent cancer stem cell chemoresistance and tumor recurrence.

Our study provides the first evidence that small molecule-mediated up-regulation of miRNAs is a viable strategy to down-regulate target proteins with no known pharmacological inhibitor, and illuminates niclosamide as a viable neoadjuvant repurposing strategy to block PXR activity in cancer stem cells and prevent tumor relapse in colon cancer.

P316

Patient Derived Lymphoma Spheroids in personalized medicine: a new model to test immunotherapies

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Follicular lymphoma (FL), the second most common type of non-Hodgkin lymphoma, is composed of malignant cells derived from germinal center B cells, both centrocytes and centroblasts, with a follicular growth pattern. In most cases, FL cells exhibit the t(14;18) translocation leading to the expression of the antiapoptotic Bcl-2 protein. FL usually has an indolent course and excellent overall survival. However, the disease remains incurable with conventional approaches and is characterized by repeated relapses. Thus, FL research focus on the development of new and more efficient molecules. To this end, the development of relevant in vitro models is crucial for testing new drugs in preclinical settings. Here we present a simple worflow to produce Patient Derived Lymphoma Spheroids established from FL biopsies in 96-well plates adapted for medium/high throughput imaging and/or screening. Morphological features and cell growth behavior were evaluated and response to treatment with different drugs (anti- CD20 Obinutuzumab, anti-PD1 Nivolumab) was evaluated to determine the robustness of the model with 2D/3D imaging and flow cytometry. These 3D models exhibit characteristics close to FL biopsies. By 2D and 3D imaging, we were able to analyze their morphology and the 3D distribution of proliferation and cell death in CD3+ and CD19+ cells. Detailed characterization of PDLS composition by multiparametric flow cytometry analyses showed inter-patient variability with potent differences in the B/T ratios.

Moreover, we determined precisely for each PDLS the expression of immune checkpoint on T cells. Finally, we tested drug screening by using anti-CD20 and anti-PD1 mAbs combination and evaluating the effect on PDLS morphology but also on lymphoma cell death. To our knowledge, this is the first 3D FL model exempt of matrix, or other compounds which are classically used to keep aggregated tumor cells, relevant to the pathology with the same pattern of histology and presenting strong evidences for their use as preclinical model in personalized medicine objectives.

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Targeting cancer cells with basal-like, mesenchymal phenotype with oncolytic virus to inhibit the growth of pancreatic cancer.

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Pancreatic cancer (PDAC) is soon to become the second cause of death by cancer in the western world and remains one of the most aggressive of all cancers due to the lack of efficient treatment or diagnostic markers. Recently, molecular investigations revealed two main tumor phenotypes that stratify patients with PDAC. While gene expression classifications proved prognostic value, PDAC molecular subtyping is yet to inform precision medicine strategies. Oncolytic virotherapies are fastly emerging as credible anticancer agents for the treatment of numerous cancer entities. Oncolytic viruses have already shown great safety during clinical trials. Among them, the fibrotropic minute virus of mice prototype (MVMp) shows promise, but its oncolytic potential has not been explored in PDAC models. We report here that MVMp specifically targets and kills primary pancreatic cancer cells with a mesenchymal, basal-like profile, both from mouse or patient origin, in vitro and in vivo, when cells with more classical plhenotype were left unarmed. Molecular investigations indicate that RhoC is critical for MVMp infection of cancer cells. Systemic MVMp injection recruits and synergizes with the immune system to provokes tumor growth inhibition in orthotopic syngeneic models of PDAC with basal-like, mesenchymal phenotype. Agzain, tumor cells with classical phenotype were moderately impacted by oncolytic treatment. Collectively, we demonstrate herein for the first time that MVMp is specific and oncolytic in PDAC tumors with mesenchymal, basal-like profile, paving the way for precision medicine opportunities for the most aggressive form of PDAC tumors.

Posters – Axe 4 “Cancers : enjeux individuels et collectifs”

P401

RHESOU (Registre de l'Hérault spécialisé en Onco-Urologie) 1er registre urologique français : 2 ans d'expérience (2017-2018)

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Introduction

L'Hérault est un département du sud de la France avec plus de 1,1 million d'habitants. Depuis plus de 35 ans, il existe un registre général de cancers dans ce département où sont diagnostiqués plus de 9 000 nouvelles tumeurs chaque année dont 1961 tumeurs urologiques et des organes sexuels masculins (soit 21,4% de l'ensemble des tumeurs de l'Hérault). Le cancer de la prostate représente 26% des cancers masculins. Devant l'importance et la recrudescence des cancers urologiques, nous avons créé un registre spécialisé (RHESOU) en novembre 2018 pour tous les cancers urologiques et génitaux masculins diagnostiqués depuis le 01/01/2017 chez les personnes qui résident dans l'Hérault au diagnostic. Ces informations permettront de fournir des analyses pertinentes et des données exhaustives.

Organisation et méthodologie

Les cas inclus sont tous les cancers incidents de l'appareil urinaire de l'homme et de la femme et des organes génitaux masculins chez les personnes de plus de 18 ans, diagnostiqués après le 01/01/2017. Des fiches de renseignement ont été réalisées et validées pour chaque organe: prostate, rein, vessie, voie excrétrice supérieure, testicule, urètre, pénis. Elles ont été établies selon les recommandations 2018/2020 du CCAFU (Comité de Cancérologie de l'Association Française d'Urologie) et sont adaptables en fonction de l'évolution des recommandations. Une base de données a été créée sur le serveur du registre des tumeurs et les fiches de recueil ont été informatisées. Nous avons pu analyser le pourcentage de données manquantes et la description de chaque variable. L'exhaustivité des données se fait à partir des PMSI de toutes les structures publiques et privées prenant en charge les patients de l'Hérault, affections de longue durée (ALD) transmises par les caisses d'assurance maladie, fichiers des réunions de concertation pluridisciplinaire (RCP), comptes rendus des pathologistes de l'Hérault sur les cas de cancersqu'ils ont analysés. Tous les cas signalés font l'objet d'un retour systématique aux dossiers médicaux afin de valider les informations.

Résultats

En deux ans (2017 et 2018), RHESOU a recueilli plus de 3 900 cas de tumeurs urologiques et génitales masculines dont 2065 cancers de prostate, 1180 tumeurs de vessie (TVNIM et TVIM), 124 tumeurs des voies excrétrices supérieures, 460 cancers du rein, 77 cancers des testicules, 13 cancers du pénis et 2 cancers de l'urètre. Pour chaque localisation, nous pouvons décrire les facteurs de risque, les comorbidités, les spécificités tumorales ainsi que la prise en charge et le parcours de soin de chaque patient. Des requêtes spécifiques ont été réalisées. Tous les résultats sont présentés sous forme de tableaux et de graphiques.

Conclusion

RHESOU respecte tous les critères de qualité épidémiologiques exigibles et permet de répondre par des études adaptées aux questions liées aux facteurs pronostiques, à la clinique, aux traitements et au suivi dans l'optimisation des prises en charge des patients. Des essais cliniques et médicaux économiques pourront aussi se greffer sur cette cohorte.

P402

Réalité virtuelle ou relaxation musicale : quelles stratégies distractives privilégier en chimiothérapie ?

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La réalité virtuelle est aujourd'hui un outil thérapeutique éprouvé pour atténuer les symptômes de l'anxiété liés au cancer, améliorer l'observance au cours des traitements et augmenter la satisfaction des soins en oncologie (Bakelis et al., 2017 ; Ryu et al., 2017 ; Ganry et al., 2018). La plupart des travaux ont mis en évidence les bénéfices de l'immersion virtuelle, permettant grâce à son pouvoir de distraction, de détourner l'attention du contexte médical stressant pour se focaliser sur les stimuli agréables de l'expérience virtuelle. Bien que la réalité virtuelle se révèle efficace auprès des patients malgré des dispositifs très divergents d'une étude à une autre (e.g., casques, environnements, modalités immersives, temps d'immersion), le niveau d'implication des patients dans l'univers virtuel (e.g., actif vs. passif) n'a pas encore été appréhendé durant la chimiothérapie. Les stratégies distractives les plus performantes restent encore à déterminer afin de de proposer des accompagnements thérapeutiques appropriés aux situations de soins en oncologie. Ainsi, il convient de définir les bénéfices des modalités immersives au regard d'une technique distractive plus conventionnelle, la relaxation musicale.

Le pouvoir distractif de la réalité virtuelle tiendrait en partie dans son caractère multimodal et hautement interactif permettant d'engager les individus dans le monde virtuel (Chirico et al., 2019). La possibilité de modifier la forme et d'intervenir sur le contenu de l'environnement virtuel augmenterait la prégnance du sentiment de présence en maintenant l'attention de l'individu vers l'expérience virtuelle (Bouvier, 2009 ; Buche et al., 2021). À ce titre, l'immersion participative impliquant l'utilisation de manettes pour effectuer des actions dans l'environnement virtuel, devrait être plus efficace pour améliorer l'état émotionnel qu'une immersion contemplative. Par ailleurs, la réalité virtuelle devrait être une stratégie distractive plus efficace que la musique tant cette dernière ne nécessite qu'un engagement attentionnel passif de la part des patientes.

Ces hypothèses nous ont conduits à mettre en place un protocole quasi-expérimental dans un service d'oncologie auprès de 120 femmes atteintes d'un cancer du sein. Lors d'une séance de chimiothérapie, nous avons réparti au hasard 4 groupes de 30 patientes en vue de tester différents dispositifs distractifs durant les 10 premières minutes de leur séance de chimiothérapie : un groupe a été placé sous une immersion participative où les patientes avaient la possibilité d'agir dans un environnement naturel relaxant (e.g. contrôler la météo, planter des arbres ou nourrir des animaux) à l'aide d'un casque de réalité virtuelle monté sur la tête et ses deux manettes (Occulus quest 2); un groupe a été placé sous une immersion contemplative de ce même environnement où les patientes avaient pour seule consigne de naviguer en observant la nature ; un groupe écoutait une musique classique via casque audio sans fil (Beats solo pro) ; tandis qu'un groupe ne disposait d'aucun dispositif distractif.

L'impact de l'implication des patientes dans l'univers virtuel et la pertinence des modalités immersives ont été appréhendés à travers l'évaluation du sentiment de présence (ITC-SOPI). Un intérêt particulier a été porté sur l'évaluation du niveau d'anxiété (STAI) et l'induction émotionnelle (SAM).

Les résultats confirment l'efficacité du pouvoir de distraction en induisant un sentiment de présence spatial par intégration multisensorielle qui favorise la diminution du niveau d'anxiété et apaise l'état de tension émotionnel. La réalité virtuelle est une stratégie distractive multimodale qui se révèle plus efficace que la musique pour induire une émotion positive durant la chimiothérapie lorsque l'immersion est participative.

P403

L'apport des tables de mortalité ajustées sur la comorbidité pour corriger la survie nette: l'exemple des patients atteints de cancers ORL

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Dans l'étude de la survie des cancers, disposer de tables de mortalité aussi proches que possible de celles de la population dont sont issus les cas est crucial pour corriger la survie nette. Ceci est particulièrement vrai lorsque le profil des cas de cancer est différent de celui de la population générale, par exemple en raison de la distribution des comorbidités comme dans les cancers ORL.

Nous avons évalué l'impact, sur des données de « vraie vie », d'une correction plus ou moins correcte de la mortalité attendue (observée en population générale) pour l'estimation de la survie nette, fréquemment utilisée par les registres de cancers, en prenant l'ajustement des comorbidités comme exemple.

Les cas incidents de cancers ORL entre 2011 et 2014 (n=633) ont été identifiés à partir d'un échantillon 1/97 de la base de données de l'Assurance maladie française (EGB) sur la base des codes CIM10 (C00-14, C30-C32) pour les diagnostics hospitaliers, la consommation de médicaments et les procédures médicales, avec un suivi maximal de 5 ans. Ces données ont été aussi utilisée pour identifier les comorbidités, selon un algorithme déjà publié pour construire l'indice de comorbidité de Charlson (CCI) à partir de données de consommation de soins. Le score CCI a été ensuite catégorisé : nul (cci=0), modéré (cci=1 ou 2), intermédiaire (CCI = 3 ou 4), et sévère (CCI >= 5). L'incertitude sur la validité des cas identifiés de métastases associées à un autre cancer primitif nous a conduit à les exclure du calcul du score de comorbidité. La survie globale et la survie nette ont été estimées à 1, 3 et 5 ans. Nous avons utilisé les tables de survie par âge, sexe et comorbidité que nous avons construites pour la France métropolitaine dans la population générale de l'EGB pour corriger la survie nette. Tout d'abord, nous avons décrit la survie globale de la population des cancers ORL en utilisant les estimateurs de Kaplan-Meier et de Cox. Ensuite, nous avons utilisé l'approche de Dickman basée sur l'estimateur Ederer II pour corriger la survie nette en utilisant soit les tables de survie classiques, soit les tables de survie par âge, sexe et comorbidité.

Nous retrouvons, dans l'échantillon des cas de cancers ORL dans l'EGB, une fréquence de comorbidité plus élevée que dans l'ensemble de la population EGB. La survie globale [95%CI] à 1, 3 et 5 ans était respectivement de 0,77 [0,72 ; 0,80], 0,54 [0,49 ; 0,58], 0,46 [0,41 ; 0,50] chez les hommes et de 0,78 [0,71 ; 0,84], 0,64 [0,56 ; 0,71], 0,58 [0,50 ; 0,65] chez les femmes. Pour la survie nette, nos estimations corrigées à l'aide des tables de mortalité classiques retrouvaient à 5 ans 50% [45% ; 55%] chez les hommes et 66% [57% ; 74%] chez les femmes. L'utilisation des tables de mortalités ajustées sur la comorbidité pour corriger la survie nette entrainait une augmentation de 4% de la survie estimée chez les hommes, 3% chez les femmes.

Nos résultats soulignent l'importance d'un ajustement des tables de survie aussi proches que possible de la population étudiée, surtout lorsque les caractéristiques de la population cancéreuse diffèrent vraisemblablement de celles de la population générale, comme c'est le cas ici. En excluant les autres cancers métastatiques du calcul du score de comorbidité, nous sous-estimons le poids de la comorbidité dans la mortalité attendue et surestimation la mortalité en excès liée aux cancers ORL. Les valeurs de 4% et 3% que nous trouvons respectivement pour les hommes et les femmes correspondent à une estimation basse de l'impact de la comorbidité sur la mortalité attendue. La prise en compte d'une fenêtre temporelle plus longue pour la définition des cas et des comorbidités, permise par le travail sur les données quasi-exhaustive de l'assurance maladie, permettra de limiter les erreurs de classement et de proposer des estimations plus précises.

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Diagnostic tardif des cancers colorectaux chez les personnes déficientes intellectuelles : Une série héraultaise de 14 patients

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Introduction Les cancers du côlon et rectum sont plus fréquents chez les personnes déficientes intellectuelles (DI) que dans la population générale. La DI est définie comme un retard mental (QI inférieur à 70) associé à des troubles adaptatifs avant l'âge de 18 ans. Les caractéristiques de ces tumeurs au diagnostic ne sont pas connues. Aucune série n'a jusqu'à présent été rapportée dans cette population. L'objectif de cette étude est de déterminer les stades au diagnostic et les modalités de prise en charge des cancers colorectaux des patients DI résidants dans l'Hérault.

Méthode Dans le cadre de l'étude CHAID (Cancer Hérault Adultes Déficience Intellectuelle) une base de données des personnes DI a été croisée avec celle du registre des tumeurs de l'Hérault afin de déterminer les patients DI diagnostiqués avec un cancer du côlon ou du rectum entre le 01/01/2012 et le 30/06/2021. Ces résultats ont été ensuite comparés à la population générale.

Résultats Au total, 14 patients (5 hommes et 9 femmes) DI avec un diagnostic de cancer du côlon (n=10) ou du rectum (n=4) ont été identifiés entre 2012 et 2021. L'âge médian était de 66,5 ans (extrêmes : 32-85 ans).

Pour 13 des 14 patients où l'information était disponible, un cancer a été découvert dans le cadre d'une surveillance pour antécédents familiaux. Douze patients étaient symptomatiques au diagnostic : douleurs et vomissements (n=5), amaigrissement et altération de l'état général (n=3), occlusion intestinale (n=3), rectorragies (n=1). Treize (93%) patients présentaient une atteinte ganglionnaire et 9 patients (64%) des métastases viscérales synchrones. La répartition par stades des patients DI était en faveur d'une augmentation des stades IV au diagnostic par rapport à la population générale (64,3% vs 26,8%) respectivement, celle des stades III était similaire (28,6% vs 21,9%). Seuls 7,14% des patients DI étaient diagnostiqués au stade I/II vs 51,3% pour la population générale (p=0,0009, 95%).

Concernant la prise en charge thérapeutique, 3 des 10 patients porteurs d'un cancer colique ont été traités par chirurgie exclusive et 3/10 ont reçu une chimiothérapie sans chirurgie préalable. Parmi les 4 cancers du rectum 1 patient a été traité par radio-chimiothérapie préopératoire. La moitié des patients (n=4 tumeurs du colon et n=3 tumeurs du rectum) ont été orientés vers une prise en charge palliative, sans chirurgie.

Au 30 septembre 2021, dix patients sont décédés avec un délai moyen entre le diagnostic et le décès de 13,1 mois et une médiane de 2 mois (extrêmes : 1-96 mois).

Discussion Les personnes DI communiquent peu et expriment leurs symptômes de façon différente. Les tumeurs sont souvent révélées par des modifications de comportement (hyperactivité, repliement sur soi). Ces personnes peuvent ne pas s'inquiéter devant des rectorragies. Toutes les tumeurs sauf une ont été diagnostiquées à un stade III ou IV, seulement la moitié des patients ont bénéficié d'un traitement curatif. Ces importants délais diagnostiques, sont responsables d'un mauvais pronostic pour ces personnes DI.

Conclusion Cette première série de cancer du côlon et du rectum chez les personnes déficientes intellectuelles montre des tumeurs découvertes à des stades plus avancés que dans la population générale. Il est important d'inclure les personnes DI dans le dépistage de masse organisé des cancers colorectaux, et d'avoir de façon générale une attention particulière pour les symptômes pouvant révéler une tumeur abdominale.

Etude soutenue par l'institut du cancer (INCa) et l'Association Française pour l'Epargne Retraite (AFER).

P405

Development of Odor Kits dedicated to recovery of olfactory memory in chemobrain cognitive disorder. Case of alcoholic extracts, hydro-alcoholic gels and inhaler sticks

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Temporary loss of smell, called anosmia, is the main neurological symptom and one of the earliest and most commonly reported indicators for chemobrain sensory disorders observed after chemotherapy treatment.
Post-Chemotherapy Cognitive Impairment (PCCI), aka chemofog, affected up to 30% of patients and main effects described were changes in memory, fluency, and other cognitive abilities that impeded their ability to function as they had pre-chemotherapy, especially for odor detection.
In the framework of an in-house generic R&D program on Olfactotherapy, we focused on the recovery of olfactory memory in chemofog sensory disorder by developing dedicated Odor Kits to be used in hospitals (CHEMONOSE Project).

First, we selected 12 naturals biosourced scents (essential oils, floral waters and natural extracts) illustrating both the 5 continents (North/South America, Africa, Asia, Europa and Oceania) and the main odorous notes of the french olfactory referential "The Field of Odors": fruity (banana, orange, citrus), floral (rose, lavender) and spicy (cinnamon, dills, cloves, anise, coriander, coffee).

Secondly, 3 kinds of Odor Kits were developed for evaluation: scents diluted in alcohol to be used with perfume tips (n°1) or in hydro-alcoholic solution pasted on hands (n°2) and drops of pure scents deposit on a cotton wick positioned inside an inhaler stick (n°3).
Experiments were conducted among panel of 72 volunteers of INP Toulouse in order to identify the odor and evaluate its intensity: up to 75% of panelists identify the submitted odor whatever the support and concentration was while 40% of the panel declare equally easy to use inhaler stick and perfume strip while 50% identify alcoholic extract having the highest efficiency for odor recognition.

Finally, tests with Odor Kits n°1 and n°3 will be performed in November at home among a panel of 7 persons presenting chemobrain cognitive disorder, including 1 Researcher-Patient, while further tests among patients in Department of Oncology at Toulouse Universitary Hospital will be set-up beginning of 2022.

P406

Survie après un cancer du sein selon la régularité de participation au dépistage organisé en Gironde

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Introduction: Le dépistage du cancer du sein et ses évolutions reste un sujet discuté. Lors d'une précédente étude, nous avons pu observer que les femmes dont le cancer du sein avait été diagnostiqué dans le cadre du dépistage organisé (DO) avaient des taux de survie nette à 5 ans plus élevés que celles dont le cancer avait été découvert en dehors de ce dépistage. De plus, les taux de survie étaient significativement différents entre les 2 groupes pour les femmes vivant dans les zones les plus défavorisées. Cependant, dans cette première étude, seule la dernière mammographie avait été prise en compte pour déterminer la participation au DO. Il semblait donc intéressant de regarder parmi les femmes dont le cancer avait été découvert dans le cadre du DO si la régularité de participation à ce dernier avait un impact sur la survie après le cancer.

Objectif: Décrire la régularité de participation au DO des femmes diagnostiquées avec un cancer du sein entre 2009 et 2015 en Gironde et estimer la survie nette à 5 ans selon la régularité.

Méthode: Les femmes de 50 à 74 ans diagnostiquées avec un cancer du sein entre 2009 et 2015, résidant en Gironde et dont le cancer avait été découvert dans le cadre du DO ont été inclus. Les données de cancers ont été extraites de la base de données du registre général des cancers de la Gironde. Grâce à un croisement avec les données de la structure de gestion des dépistages du département, nous avons pu obtenir l'historique de participation au DO pour chacune des femmes de notre population. A partir du nombre de mammographies réalisées et le nombre théorique de mammographies que les femmes auraient dû faire depuis leurs 50 ans, nous avons obtenu un indicateur de suivi, identifiant le nombre de mammographies manquantes. Nous avons également étudié les délais entre chaque mammographie et nous avons distingué les femmes pour lesquelles tous les délais étaient inférieurs à 3 ans et celles qui avaient au moins 1 délai supérieur à 3 ans. Ainsi, à partir du croisement de ces 2 indicateurs (de suivi et de délai), nous avons déterminé la régularité de participation des femmes, à savoir: celles qui participaient de manière régulière versus celles qui participaient de manière irrégulière au DO. Nous avons ensuite décrit pour chacun des 2 groupes les variables relatives aux patientes et aux tumeurs et nous avons estimé la survie nette à 5 ans dans chacun des groupes et selon les quintiles de défavorisation, selon la méthode de Pohar-Perme.

Résultats: Parmi les femmes de 50 à 74 ans diagnostiquées avec un cancer du sein entre 2009 et 2015, résidant en Gironde et dont le cancer avait été découvert dans le cadre du DO, 74,2 % d'entre elles avaient participé de manière régulière au DO avant leur diagnostic de cancer et 27,8% avaient participé de manière irrégulière. La proportion de décès à 5 ans était plus importante parmi les femmes irrégulières et celles-ci présentaient des tumeurs de plus mauvais pronostic que celles qui avaient participé régulièrement au DO. Concernant la survie nette à 5 ans, elle était plus élevée pour le groupe des femmes régulières avec un taux de 97,3% versus 94,5% pour le second groupe. Selon les quintiles de défavorisation, seul dans le quintile 5 (le plus défavorisé) une différence de survie s'observait mais de manière non significative.

Conclusion: Cette étude permet de voir que, parmi les femmes dont le cancer est diagnostiqué dans le cadre du DO, celles qui participent de manière régulière à ce programme de dépistage ont une survie plus élevée par rapport aux femmes qui y participent de manière irrégulière. Cette étude permettra des analyses plus fines dans le cadre d'une étude plus large sur la survie après un cancer du sein en fonction des différents dépistages et surveillances.

P407

Étude à partir des registres des cancers du réseau Francim: évolution de la survie des personnes atteintes de cancer en France métropolitaine 1989-2018 (Tumeurs solides)

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Les données de survie suite à un cancer sont essentielles à connaitre par tous les décideurs et les soignants qui prennent en charge les patients atteints de cancer, afin d'optimiser toutes les décisions à prendre en matière de prévention, de dépistage, et de soins. L'objectif de cette étude, réalisée à partir des données du réseau des registres de cancers français (réseau Francim), est d'analyser la survie des personnes ayant eu un cancer diagnostiqué entre 1989 et 2015 en France métropolitaine.

Toutes les personnes, âgées de 15 ans ou plus au moment du diagnostic, résidant dans un des départements métropolitains couverts par un registre de cancer et ayant eu un diagnostic de cancer entre 1989 et 2015 ont été incluses dans l'étude. Leur statut vital a été mis à jour au 30 juin 2018.
Les analyses ont permis d'estimer la survie nette à 1 et 5 ans, des personnes diagnostiquées sur la période récente entre 2010 et 2015, ainsi que la survie nette à long terme (20 ans) des personnes diagnostiquées entre 1989 et 2000. Des analyses ont aussi été réalisées afin de décrire les tendances de la survie nette à 1, 5 et 10 ans selon l'année de diagnostic, des personnes diagnostiquées entre 1990 et 2015 et âgées de moins de 75 ans au diagnostic. Les modèles utilisés pour estimer la survie nette reposent sur une nouvelle approche basée sur des splines multidimensionnelles pénalisées pour modéliser les taux de mortalité en excès. Cette étude a porté sur 50 tumeurs solides (28 localisations principales et 22 sous-localisations anatomiques ou histologiques).

Les résultats montrent une grande disparité de la survie nette entre les différentes localisations. La survie nette standardisée (SNS) à 5 ans varie de 96 % pour les cancers de la thyroïde à 10 % pour les mésothéliomes. Pour la presque totalité des localisations cancéreuses, la SNS à 5 ans est plus élevée chez la femme. Le pronostic est aussi fortement lié à l'âge pour toutes les localisations. Dans la majorité des cas, le risque de décéder est élevé dans l'année après le diagnostic et tend à décroître ensuite au cours du suivi. Le risque de décéder juste après le diagnostic augmente avec l'âge et est maximal chez les personnes âgées pour toutes les localisations. Généralement, plus l'âge au diagnostic est élevé, plus la survie est basse. L'étude des tendances de la survie nette sur l'ensemble de la période d'étude montre une amélioration globalement significative de la survie nette à 5 ans pour une majorité des localisations.

Ces résultats de survie en population générale permettent de visualiser les progrès réalisés ou encore à faire dans le système de soins à la fois dans la détection des cancers mais aussi dans leur prise en charge post diagnostic. Connaitre la survie de chaque cancer et leur évolution dans le temps peut en effet aider les décideurs et les soignants dans leurs prises de décisions durant le parcours de santé des patients.

P408

Evolution de la survie des hémopathies malignes en France de 1989 à 2018 en population générale : Étude à partir des registres des cancers du réseau Francim

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Introduction

Indicateur-clé pour la surveillance épidémiologique, la survie permet d'apprécier l'amélioration globale du pronostic des personnes atteintes d'un cancer, résultant à la fois des progrès thérapeutiques et des améliorations des parcours de soin en cancérologie. Il s'agit de la 4eme étude de survie réalisée à partir des données des registres de cancers métropolitains du réseau FRANCIM (Partenariat service de Biostatistique-Bioinformatique des Hospices civils de Lyon, Santé publiqueFrance et l'Institut national du cancer). Avec 45000 nouveaux cas estimés en 2018 en France, et un taux d'incidence en augmentation pour certains sous-types, l'actualisation des estimations de survie par sous-type d'hémopathie maligne (HM) est essentielle.

Organisation et méthodologie

L'ensemble des HM diagnostiquées de 1989 à 2015 (variable selon leur entrée dans la classification OMS), âgées de 15 ans ou plus au diagnostic et résidant dans une zone couverte par un registre de cancer sont inclus (Date de point au 30 juin 2018). Quatorze sous-types d'hémopathies lymphoïdes et neuf sous-types d'hémopathies myéloïdes ont été définis selon la CIM-O-3. La survie nette (i.e. survie attachée à la mortalité en excès dû à la pathologie), a été estimée jusque 20 ans du diagnostic en utilisant une nouvelle approche de modélisation du taux en excès basée sur l'utilisation de splines multidimensionnelles pénalisées permettant désormais de visualiser les tendances de survie selon l'âge et l'année de diagnostic.

Résultats

Entre 2010 et 2015, 45 % des HM ont un pronostic favorable à 5 ans avec une survie nette standardisée sur l'âge (SNS) supérieure à 65 %. La Leucémie aigüe myéloïde (LAM) reste la seule hémopathie de pronostic défavorable (SNS de 27 %). La survie à 5 ans diminuait avec l'âge pour tous les sous-types avec un écart maximal pour la LAM et la Leucémie lymphoïde chronique.

L'amélioration la plus marquée de la survie de 1990 à 2015 étaient observées pour les Leucémies myéloïdes chroniques (LMC) avec +42% quel que soit l'âge tout comme pour les lymphomes diffus à grandes cellules B (LDGCB) (> 20 %). Alors que pour certains sous-types, le gain de survie n'est observé que pour les sujets jeunes comme dans la LAM ou le myélome multiple, le pronostic des sujets âgés atteint de Lymphome Folliculaire s'est largement amélioré avec +38 % depuis 1990. Cette tendance à l'amélioration de la survie est plus marquée après 2005 chez les sujets âgés pour le lymphome de Hodgkin et le lymphome du Manteau. Depuis 2005, aucune évolution de survie n'est observée pour la Leucémie myélomonocytaire chronique et autres SMM et les syndromes myélodysplasiques, la survie restant peu élevée chez ces patients quel que soit l'âge.

Conclusion

L'amélioration de la survie pour la grande majorité des HM étudiées est une combinaison entre des diagnostics plus précis grâce à des outils plus performants et une amélioration des thérapeutiques (nouveaux traitements, meilleur contrôle de leur toxicité, soin de support). Les résultats positifs observés chez les personnes âgées peut être une conséquence des différents Plans cancer successifs dont l'un des objectifs était d'améliorer la prise en charge de ces patients par des recours plus systématiques à l'évaluation gériatrique. L'actualisation de la survie en population générale permet de mettre en évidence les profils de patients qui nécessitent un besoin urgent d'amélioration en adaptant au mieux leur parcours de prise en charge.

P409

Survie nette des patients avec un diagnostic de sarcome à court et long terme : données du réseau des registres des cancers FRANCIM

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Introduction : Les sarcomes sont un groupe hétérogène de tumeurs malignes rares (> 100 histotypes) pouvant survenir à tout âge et siéger dans la plupart des sites anatomiques. Le diagnostic des sarcomes est aussi très complexe et nécessite très souvent une relecture d'un pathologiste expert pour confirmer le diagnostic. En raison de cette hétérogénéité et des possibles erreurs dans le diagnostic, les données de survie sont rarement décrites avec précision. L'objectif de cette étude était de fournir des données de survie nette (SN) à 1, 5 et 10 ans selon le sous type histologique, le profil génomique et la topographie en s'appuyant sur les données des registres des cancers qui bénéficient généralement de l'accès à cette relecture.

Méthodes : Ont été inclus dans cette étude tous les patients atteints de sarcomes âgés d'au moins 15 ans entre 2000 et 2015 et suivies jusqu'au 30 juin 2018. Les données collectées provenaient des registres du réseau FRANCIM. La SN a été estimée à l'aide des modèles flexibles du taux de mortalité en excès avec des splines pénalisées.

*Résultats :*Durant la période d'étude 12 662 sarcomes ont été analysés. En ce qui concerne la localisation, la SN à 5 ans allait de 41 % (Intervalle de confiance à 95 % - IC95 % [38-44]) pour les organes génitaux de la femme à 92 % (IC95 % [89-94]) pour la peau. Des survies intermédiaires ont été observées pour les tumeurs des tissus mous, des os et gastro-intestinales, avec un NS à 5 ans de 58 % (IC95 % [56-59]), 64 % (IC95 % [61-66]) et 73 % (IC95 % [70-75]). Selon le profil histologique, les mêmes disparités ont été observées avec une SN à 5 ans allant de 100 % (IC95 % [86-100]) pour le dermatofibrosarcome à 25 % (IC95% [21-30]) pour l'angiosarcome. S'agissant du profil génomique, la SN à 5 ans allait de 56 % (IC95 % [54-58]) à 79 % (IC95% [76-81]) respectivement pour les sarcomes non défini/altérations diverses et les sarcomes avec mutations. La variation de SN entre 1 et 10 ans allait de -48 % à -5 %, de -62 % à 0 % et de - 59 % à - 24 % respectivement pour la topographie, le sous type histologique et le profil génomique.

Conclusion : Cette étude fournit les premières données de survie à long terme des patients atteints de sarcome et montre de grandes disparités de survie selon leur localisation et leur sous type histologique.

Posters – Axis 5 “Health Technologies”

P501

Microfluidic Lab-On-Chip for UHF-Dielectrophoresis Discrimination of Glioblastoma Undifferentiated cells

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Glioblastoma (GBM) is one of the most aggressive solid tumors, particularly due to the presence of cancer stem cells (CSCs). Today the characterization of this type of cells with an efficient, fast and low-cost method remains an issue. Hence, we have developed a microfluidic lab-on-a-chip based on dielectrophoresis (DEP) single cell electro-manipulation to measure the two crossover frequencies: fx01 in low frequency range (below 500 kHz) and fx02 in Ultra High Frequency (UHF) range (above 50 MHz). Experiments were performed on ex vivo GBM cells from patients' primary cell culture in order to reflect clinical conditions. We demonstrate that the usual exploitation of low frequency range DEP does not allow the discrimination of the undifferentiated from the differentiated phenotypes of GBM cells. However, the presented study highlights the use of UHF-DEP as a very promising tool with the great potential to discriminate cell according to their internal biological properties. Our microfluidic system allows the identification and the discrimination of aggressive and resistant cells from a tumor. In the future, the early detection of CSC subpopulation in glioma tumor with UHF-DEP approach could have a prognosis value on therapeutic response and might allow to adapt therapeutic strategy following diagnosis.

P502

Bioinformatical analysis of tumor cell with stroma crosstalk that impact aggressiveness of pancreatic ductal adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive cancers due mostly to its high metastatic and chemoresistance features. Hence, 5-year life expectancy is below 9% with a 6-month mean survival after diagnosis. PDAC is characterized by highly invasive pancreatic cancer cells, immerged in an exuberant stroma, which represents up to 80% of the tumor volume. Both tumor and stromal cells have to interact in order to survive in this harsh micro-environment.
Two major molecular (transcriptomic) subtypes of PDAC were clearly described through bio-informatical sequencing analysis of RNA extracted from patient tumor regions enriched with cancer epithelial cells: a dedifferentiated and more aggressive (associated with poor outcome) "basal" subtype, and a "classical" subtype presenting more general differentiation features and a better outcome. Molecular heterogeneity of the PDAC stroma was also studied through bio-informatical deconvolution analyses of bulk RNA sequences (extracted from tumor samples composed of both cancer epithelial and stromal cells); these analyses also stratified PDAC within two prognostic stromal subgroups, which however don't mirror the two identified epithelial (basal / classical) subgroups.

By performing bioinformatical analysis on transcriptomic data (RNAseq) from PDX (Patient Derived Xenograft), a hybrid tumor model from which human-derived tumor cell sequences are distinguishable from murine-derived stromal cell sequences, we searched for different stromal behaviours linked to the aggressiveness of the tumor, to be then validated in published PDAC patient databases.

According to NMF (Non-negative matrix factorization) and GSEA (Gene Set Enrichment) analyses, we identified two components allowing us to classify the samples according to a stromal gradient. Interestingly, these components are characterised by distinct functional signatures of aggressiveness and they are highly prognostic (correlated with survival). In addition, these components stratify patients independently on the described molecular tumor (basal / classical) classification. The results obtained (functional signatures as well as the impact on survival) were then validated on other PDAC published databases. Moreover, ligand-receptor bioinformatics analyses identified novel players of the TGFB superfamily in the most aggressive component.

Further analyses (bioinformatical and experimental) will now enable us to identify specific stromal therapeutic targets to be functionally tested using already available patient-derived cell models, including patient-derived tumor cell organoids and cancer-associated fibroblasts. Therapeutic targeting of the stroma must take into account its functional heterogeneity, and the functional validation of the newly identified players should define a promising axis for targeting the most aggressive PDAC.

P503

Implantable NMR microcoil coupled to microdialysis : application to tumor diagnosis and therapy

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Introduction: To be able to study locally and in real time the effect of compounds administered in a tissue or more particularly in a tumor remains a major challenge in oncology.
A device, coupling an implantable NMR microcoil and a microdialysis membrane, would allow on one hand to improve the low sensitivity of nuclear magnetic resonance (NMR), by miniaturization and implantation of the probe[1], and on the other hand to administer a compound of interest in a specific location. The device presented in this study is precisely designed to explore, by magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS), regions of interest of very small volume inferior to the microliter and the effect of compounds administered by the microdialysis membrane directly at the implantation site.

Materials and methods: The device consists of a microcoil made with copper wires of 150 µm diameter forming an elliptical loop with outer dimensions of 3 mm in length, 700 µm in width, and 150 µm in thickness and a microdialysis membrane of 2 mm in length and 240 µm in diameter, placed in the center of the microcoil. The tuning and matching circuit of the microcoil is exported outside the organ.
In a first step, the device was used to obtain spectra on healthy rats and glioma-bearing rats and thus evaluate the ability of the device to distinguish tumor tissue from healthy tissue based on the metabolite concentration obtained from the NMR spectra[2].

Then, in vitro and in vivo experiments were performed to evaluate the correct functioning of the two joint entities: optimal NMR detection of the microcoil and optimal diffusion of the compound through the microdialysis membrane. Temporal follow-ups of the diffusion of a compound were obtained by the implantable microcoil in MRI (contrast agent) and MRS (DMSO) in in vivo and in vitro conditions.
For the in vivo experiments, prior to the insertion of the device into the cortex of the Wistar rats, cannulas were positioned by stereotactic surgery the day before the NMR acquisitions.

Results/Discussion: A succession of in vivo spectra acquired with the implantable microcoil during a DMSO perfusion (30 mM) with a flow rate of 1 µL/min through the microdialysis membrane could be observed. The appearance of the DMSO resonance peak at 2.70 ppm is visible during the acquisitions. These spectra were acquired using the PRESS localized spectroscopy sequence (256 averages - acquisition time: 8 min) at 7T. The main resonances of the brain metabolites (NAA, Cr, Tau, Glu, Gln) and the DMSO resonance are easily identified.

Conclusion: The preliminary results presented here demonstrate the potential of this device, comprising an implantable NMR microcoil (for in vivo NMR data acquisition on sub-microliter volumes such in tumor for instance) and a microdialysis membrane (for the injection of a chemical compound such as an anti-tumor drug), in the real time evaluation of the effect of the compound of interest.
The first application and validation envisioned for this device is the early evaluation of the response and impact on metabolism of an anti-tumor molecule on gliomas in small animals. It could also be used in different neurodegenerative diseases or in healthy animals in neurosciences with the quantification of intra and extra cellular cerebral chemical molecules like neurotransmitters for instance.

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P504

Towards a novel framework for large scale RNAseq data analysis in human health

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With its ability to reveal both altered gene expression levels and the production of aberrant transcripts, RNAseq is popular in the field of precision medicine. An increasing number of clinical trials uses this technology in order to discover functionally relevant alterations. Driven by myriads of projects, public RNAseq databases are exploding, to date, there is over 164,000 RNA-seq on SRA for human. This huge body of publicly available RNAseq libraries is a precious resource to identify specific transcriptional events. However, the challenges lie in the complexity of RNA biological content and the exponential increase in data volume. We want to make RNAseq data easily accessible, providing a capture of the whole transcriptome complexity, in the context of human health applications. Therefore, we developed a new framework based on a k-mer approach, constructed with several modules: 1/ a new RNAseq indexing structure that will serve as an efficient platform to request any transcribed information, 2/ a complete module to generate unique k-mers as signature of transcripts, 3/ a supporting web site to facilitate the queries for the biologists.

The indexing step uses Reindeer, a new k-mer based indexation structure. To our knowledge, it's the first method capable of performing fast mapping-free quantification of variant transcripts in thousands of RNAseq libraries [1]. The methodology is already efficiently implemented for several biological applications based on public datasets (from ten to thousand of RNAseq corresponding to 100Go to 10To of data). The k-mer designing module uses Kmerator, a tool developed to extract specific k-mers (https://github.com/Transipedia/kmerator) [2]. Finally, the web application is already available to facilitate large RNAseq datasets queries by the biologists with their sequences of interest as input (fasta format).

Concerning medical applications, we already requested and identified in selected public datasets, genes co-expressions, tissue specific biomarkers, as well as tumor specific signatures comparing normal and tumoral samples. As an example, we recovered known translocations and mutations in RNAseq Acute Myeloid Leukemia (AML) samples and identified new specific biomarkers (long non-coding RNAs…).

With the addition of advanced Machine Learning approaches, our framework could be used to select the best signatures and to improve diagnosis and prognosis models in cancers.

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P505

Aptamers as diagnostic tools for the detection of Human Epididymis protein 4 (HE4), clinical biomarker of ovarian cancer

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Ovarian cancer is the most deadly gynecological cancer and eight leading cause of cancer-related deaths among women globally. When it is diagnosed in early stages, 5-year survival is approximately 90 %. However, due to the silent nature of the disease and lack of sensitive diagnostic methods, the majority of the patients are diagnosed in advances stages, with a 5-year survival of only 15 - 40 %. Unfortunately, there are no early-stage or screening methods available, so late diagnosis remains the main factor contributing to the high mortality. Recently, aptamers with affinity to molecular tumor biomarkers have emerged as useful diagnostic tools. Aptamers are non-coding, synthetic single-stranded DNA or RNA oligonucleotides that bind with high-affinity to the wide range of ligands, including cancer proteins. Similar as antibodies, they form unique secondary and tertiary structures that enable them recognition of the target. Due to their characteristics of high specificity, low toxicity, complete development *in vitro* and easy and reproducible synthesis, they could offer advantages over antibodies.

Herein, we present a discovery method for high-affinity DNA aptamer sequences targeting Human Epididymis protein 4 (HE4). Human Epididymis protein 4 (HE4) is a protein overexpressed in ovarian cancer, but not in benign gynecological conditions or healthy individuals. Elevated HE4 levels are found in the blood and urine of ovarian cancer patients. Therefore, HE4 has an important role as clinical biomarker in the management of ovarian cancer. HE4 is currently used in the differential diagnosis of women with pelvic masses, prognosis and follow-up of ovarian cancer patients. The high-affinity single-stranded anti-HE4 DNA aptamers are selected from random, highly diverse DNA library using *High-Fidelity Systematic Evolution of Ligands by EXponential enrichment* (HI-FI SELEX) method. This diagnostic aptamer discovery method utilize sequence amplification by sensitive digital droplet Polymerase Chain Reaction (ddPCR). The selected anti-HE4 sequences and structures are identified using bioinformatics analysis. Described DNA aptamers will be further characterized, in order to be potentially used as molecular probes in the development of aptamer-based diagnostic methods, biosensors or Point-of-care testing (POCT) devices for ovarian cancer.

P506

Nanotherapy of pancreatic adenocarcinoma by targeted magnetic hyperthermia: efficacy and mechanisms

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Magnetic nanoparticles (MNPs) are already widely used in nanomedicine, notably as MRI contrast agents or in magnetic hyperthermia therapy. A clinical trial was conducted to treat high-grade brain tumors in 2011, and a clinical trial is currently being conducted in the United States on prostate cancer, in combination with radiotherapy. However, the benefit on life expectancy remains minimal and neither radiotherapy nor magnetic hyperthermia can distinguish between normal and cancerous tissues, leading to adverse effects.

Our strategy is based on the design of iron oxide MNPs capable of specifically recognizing target cells and therefore specifically treating cancerous tissue with targeted magnetic hyperthermia via the application of an external high frequency alternating magnetic field (AMF), while minimizing damage to healthy tissue. This targeted magnetic hyperthermia consists of specifically delivering MNPs into the lysosomes of target cells. The AMF exposure will then specifically eradicate these cells, without macroscopic temperature elevation. We have chosen as a model pancreatic adenocarcinoma, particularly resistant to conventional therapies and characterized by the presence of a large and dense microenvironment with a key role of CAFs (Cancer-Associated Fibroblast) cells secreting extracellular matrix proteins (collagen) that limits the penetration and efficacy of treatments (chemotherapy and radiotherapy). Cancer cells as well as pancreatic CAFs can overexpress the CCK2 receptor (membrane receptor that internalizes after binding of its specific agonist: gastrin).

We have developed MNPs with high thermal power, termed "NanoFlowers" (NF), decorated with fluorophore and gastrin molecules allowing respectively their detection and targeting. These MNPs (NF@Gastrin) specifically target cancer cells (MiaPaca2-CCK2) and CAFs (CAF-CCK2) expressing the CCK2 receptor, internalize and accumulate in their lysosomes. The AMF application (275 kHz, 30 mT) kills up to 45% of cancer cells and CAFs that have internalized NF@Gastrin, slows down their proliferation, and sensitizing them to Gemcitabine (a chemotherapy used for the treatment of pancreatic adenocarcinoma), without affecting cells lacking the nanoparticles. In parallel, we are studying the mechanisms that cause cell death. The hypothesis is that the MNPs temperature rise induced by AMF may trigger the release of ferric ions (Fe3+) from the MNPs, which catalyze the production of ROS (reactive oxygen species) in the lysosomes; then, ROS peroxide the proteins and lipids of the lysosomal membrane, induce the lysosome permeabilization leading to lysosomal cell death.

Our results confirm this hypothesis since NF@Gastrin generate ROS, induce the lysosome permeabilization and cell death after AMF exposure, while NF-SiO2@Gastrin covered with a silica shell preventing Fe3+ ion release do not induce cell death. We are now studying the impact of the targeted magnetic hyperthermia on cell migration, as well as on the expression of Damage-Associated Molecular Pattern (DAMP) proteins able to induce an anti-tumor immune response. We will then validate the efficacy of this new therapeutic strategy in a pre-clinical study on mouse models of pancreatic adenocarcinoma.

P507

On the dynamics of TAM formation in Chronic Lymphocytic Leukaemia: a multi-scale approach

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Mathematical and network modelling of individual cells and cell populations offer a powerful approach to analyse complex biological systems at a multi-scale level, integrating theoretical and experimental knowledge into a single representation. In tumours, identifying the mechanisms that establish the immune cell - cancer cell interactions is crucial in understanding the system behaviour and dynamics. In this work, we focus on the ecology of the tumour microenvironment, and particularly on the differentiation of monocytes into tumour-associated macrophages (TAMs) in Chronic Lymphocytic Leukaemia (CLL). TAMs are known to play a critical role in the survival of cancer cells. In CLL, they protect the leukemic B cells from spontaneous apoptosis and contribute to their chemo-resistance. Here, we propose an integrated modelling approach of monocyte differentiation into TAMs in in-vitro monocyte-CLL co-culture, including molecular and inter-cellular interactions. Firstly, we apply a Boolean model on a macrophage gene regulatory network to identify the molecular pathways that lead to TAM formation in the presence of tumoural signals. Secondly, we build an agent-based model to explore the cell population spatio-temporal dynamics and identify the key processes controlling the stability of this multi-cellular system, in which cell behaviour is determined both by cell interactions and their internal molecular regulation. With this coupled approach we recapitulate a broad spectrum of macrophages, ranging from pro- to anti-tumoural phenotypes, while highlighting the effect of TAMs protecting role in CLL cell population dynamics.

P508

Remote magneto-mechanical destruction of pancreatic cancer cells using targeted ultra-small superparamagnetic iron oxide nanoparticles and low frequency magnetic fields

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Pancreatic ductal adenocarcinoma (PDAC) represents more than 80% of pancreatic cancer malignancies and is considered one of the most devastating difficult-to-treat cancers. Although surgery, radiation or chemotherapy are available treatment options, PDAC has a dismal prognosis with a median survival of 6 to 12 months. Hence, there is an imperative need to develop an efficient and safe therapeutic platform that could improve pancreatic cancer prognosis and enhance its survival rates. Cancer cells destruction using mechanical activation of magnetic nanoparticles by low-frequency magnetic fields (LFMF) constitutes a new approach that is technically easier and with potentially fewer undesirable effects than magnetic hyperthermia. We developed ultra-small superparamagnetic iron oxide nanoparticles (6-nm USPION) decorated with gastrin peptide (USPION@gastrin), which successfully targeted MiaPaCa-CCK2 pancreatic cancer cells, chosen as a model, and accumulated within their lysosomes. LFMF exposure to MiaPaCa2 CCK2 cells have uptaken the USPION@gastrin decreased their cellular proliferation, and caused more cell death compared to treatment with USPION@gastrin or exposure to LFMF only. Further investigations are currently being conducted to investigate the mechanical destruction effect on cancer cells migration, clonogenicity and stimulation of expression of damage-associated molecular patterns (DAMPs) such as heat shock proteins (HSPs) and calreticulin R. This study could establish proof-of-concept that targeted USPIONs can disrupt tumor cells through mechanical forces generated by LFMF, opening new opportunities for PDAC therapy.

P509

A pipeline based on k-mers to select specific biomarker candidates in a tumor microenvironnement context

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Publicly available human RNA-sequencing (RNAseq) datasets are precious resources for biomedical research. Indeed, RNA-seq is widely used to identify actively transcribed genes or any transcribed alterations, and quantify gene or transcript expression. RNA-seq analysis also substantially contributes to our understanding of the processes involved in human disease. Then, the need for tools enabling fast and specific quantification of candidate sequences in large RNA-seq datasets is more and more required. Lately, approaches relying on k-mers from raw sequencing files have emerged and are used for the query of transcriptomic data. We therefore developed tools based on a k-mer approach (Riquier et al, 2021; https://github.com/Transipedia/kmerator) that propose a new way to explore RNAseq data and can be used for fast and in-depth exploration of transcriptomes.
In this context, we focus on tumor microenvironment measures in cancer patient cohorts. Indeed, many studies characterizing the tumor microenvironment have been published as well as prognosis associations of tumor immune and stromal response measures. However, the proposed gene signatures are generally very large and shared by different cancer types. Collection of signatures adapted to a specific tumor are often lacking, particularly in hematopoietic malignancies. Moreover, the quantification of relevant microenvironnement markers can give a score of the tumor contamination that is a crucial parameter for further analysis on primary cancer biopsies.

We propose a pipeline to select relevant candidate signatures specific for one cancer type. In a first step, the tumor microenvironment known genes/transcripts were submitted to Kmerator tool [1] to design their specific k-mers, and the RNA-seq cancer patient cohorts are indexed with the Reindeer software [2] which enables an ultra fast k-mer counting. Then, we developed a script that identifies biomarker candidates in the datasets, provided from the previous step, using machine learning methods to test the biomarker candidates for specific cancer predictions. We are working to improve the script with a feature selection step that will allow several possible selection criteria, including the use of appropriate cancerous cell lines. According to some preliminary results, our pipeline seems to be a flexible method capable of extending to different contexts and helping to compare several published microenvironment gene signatures.

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P510

Using nanoparticle tracking analysis (NTA) for appreciation of up and down extracellular vesicle secretion

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Extracellular vesicles consist in heterogenous small vesicles, bounded by portions of plasma membrane, and secreted by most eukaryotic cells, being healthy or pathological. They are classically divided in microvesicles, exosomes and apoptotic bodies; the classification being mainly based on biogenesis, size and composition of the vesicle. Due to the variety of origin, the size range is comprised between 30 and 1000 nm, exosomes being known to be the smallest (30-150 nm) (Kurian TK, 2021). The content of these vesicles is both the reflect of the donor cell's unsorted composition and a result of selection, in addition to non-specific exosomal constitutive proteins. So, they have functions in cell signaling, modifying their surrounded cells' behavior. This capacity is particularly important in tumor development (Mashouri BMC, 2019). The RAB family, especially RAB27A and RAB27B, has been described to be crucial for exosome secretion (Van Niel et al 2018 ; Auger et al ; Brunel et al, 2021). Both isoforms are responsible for the docking and fusion of MVBs on plasma membrane, the last step before exosome release. When working with such EVs, or more strictly with exosomes, one of the difficulties relies on the reproducibility and standardization of techniques used for their characterization (editorial, JEV 2020).

In the present study, our initial aim was to evaluate effects of exosome secretion inhibition in two tumoral models, glioblastoma and colorectal cancer. To reach this goal, we performed transfection of two representative cell lines, respectively U87-MG and HCT116, with 3 different shRNA targeting RAB27a. EVs were isolated following an adaptation of Théry et al.'s protocol (Théry et al., 2006) using differential ultracentrifugation's. The final pelleted and washed EVs are then resuspended either in PBS for Nanotracking Analysis (NTA), or in cell lysis buffer for Western Blot analysis. As an alternative, the cell culture supernatants were also analyzed after only the 300 x g and 2 000 x g centrifugations (named crude supernatant). EVs quantification was evaluated using NanoSight NS300® (Malvern Panalytical Ltd, UK) with specific parameters according to the manufacturer's user manual (NanoSight NS300 User Manual, MAN0541-01-EN-00, 2017).

Whereas tanscriptomic and proteic analysis confirmed RAB27a down-regulation, we did not detect any EVs quantitative variation with NTA analysis, neither after EVs purification nor in crude supernatant. In a second step, we aimed to reduce EVs secretion either by transient transfection with siRNA RAB27A or pharmacological inhibitors (nexinhib20 or indomethacin). Same lack of quantitative variation was observed with the 3 different approaches.

In light of these data, we wondered if NTA allows monitoring of EVs secretion's enhancement. To this purpose, we either treated both cell lines with 0.5 µM rotenone (Wu et al., 2015, Neuroscience) or we cultured them in hypoxia (1% O2) for 48h vs normoxia (20% O2). The EVs secretion in crude supernatants was significantly enhanced in rotenone-treated pLKO (control: scramble shRNA) cells for both HCT116 and U87-MG, without modification of the mean size. Concerning hypoxic conditions, the NTA analysis showed that both cell lines produced significantly more EVs after 48h of 1% O2 culture. Such an enhancement of EVs secretion started to appear straight after 24h of hypoxia even if it reached significant values only for U87.

Our work emphasized the weakness of using only one characterization method to assess EVs secretion. Western blotting is ongoing in order to evaluate the EVs content, which could explain a variation in the nature of EVs rather than in the quantity when inhibiting the exosome secretion.

Posters – “Technological facilities & Industrial partnerships”

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PhenOtypic scrEening TechnICal (POETIC) platform

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The ARTiSt group has set up a PhenOtypic scrEening TechnICal (POETIC) platform which allows us the automatization of miniaturized phenotypic screening assays, to understand the molecular mechanisms that regulate cell transformation and tumour growth. The POETIC platform all the equipment, software and support needed to facilitate a broad spectrum of screening projects. Whether you would like to screen the whole genome or simply automate image acquisition, we have fully automated systems available.

POETIC is arranged into three main sections :
1. The JANUS automated liquid handling workstation (PerkinElmer) capable of si/sh/CRISPR or compound addition as well as automated cell seeding, medium changes, cell fixation and staining in 96 or 384 well formats.
2. The EnVision Plate Reader (PerkinElmer) for ELISA, Luminescence, Fluorescence Assays.
3. Automated high throughput image acquisition using Cytation™3 imaging system (BioTek) which is a cell imaging multi-mode microplate reader that combines automated digital microscopy and conventional microplate detection.

To date, POETIC has successfully completed a number of phenotypic screening assays [1-6] (high-content miRNA, genome-wide siRNA, compound screens, ...).

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P602
cfDNA size profiling directly from plasma, without DNA extraction

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We have introduced recently the BIABooster, a new system designed to concentrate DNA molecules in a capillary device, and to separate them according to size, like with capillary gel electrophoresis [1]. It is based on µLAS technology, which relies on a pressure driven flow of a viscoelastic fluid in a capillary, in which DNA is carried away and subjected to counter-electrophoresis. The combined action of the laminar, viscoelastic flow and the counter-electrophoresis produces a lift force which drives DNA molecules towards channel walls [2; 3]. The force intensity depends on shear stress, electric field, and on the size of the DNA. When the lift force is high enough, DNA molecules are so close to the walls, where the flow velocity is low, that they crawl backward by electrophoresis. This phenomenon allows to concentrate DNA above a given size at the junction between two capillaries of sufficiently different diameters.

The on-line concentration function confers an unrivalled sensitivity to the technology; indeed as low as 10 pg/ml per fragment can be readily analyzed, 100-1000 fold moresensitive than state-of-the-art capillary electrophoresis. The BIABooster system has shown promising results for the characterization of purified cell free circulating DNA (cfDNA) samples and the use of cfDNA as a cancer biomarker [1].

The on-line DNA concentration can also be used to purify DNA from biological samples. DNA is a large and highly negatively charged molecule. All the other components of biological samples are much smaller, or neutral, or positively charged, or less negatively charged. They are therefore washed away during the concentration step.

We make use of this feature for analyzing unpurified cfDNA; starting from only 100 µL of plasma, and after digestion by proteinase K, the quantified cfDNA size profile could be assessed directly within 70 minutes, without needing to extract and purify DNA.

The method has been evaluated on plasma samples from a cohort of 20 healthy subjects and 20 cancer patients. We have compared the cfDNA size profiles obtained with this new method and with a method including DNA extraction. There is a good correlation between the two methods for the size profile; correlation
coefficients are respectively 0.75 and 0.98 for the size of the main, mononucleosomal peak, and for the size of the second, dinucleosomal peak. The correlation is also good for DNA concentration, with a correlation coefficient of 0.96.

The method presents an excellent repeatability and reproducibility, both for cfDNA concentration and for cfDNA size distribution. In repeatability, the standard deviation of the mononucleosomal peak size is 1 bp, and that of the dinucleosomal peak is 1.1 bp. The coefficient of variation of cfDNA concentration is 9%.The differences in terms of size profile and concentration between patients and healthy subjects are also similar between the two methods.

We expect this method will greatly facilitate studies aiming at exploring the potential of cfDNA size profiling for cancer monitoring.

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FONDATION ARC POUR LA RECHERCHE SUR LE CANCER

Reconnue d'utilité publique

BIEN INFORMÉ, ON EST MIEUX ARMÉ CONTRE LE CANCER

3 COLLECTIONS

COMPRENDRE & AGIR

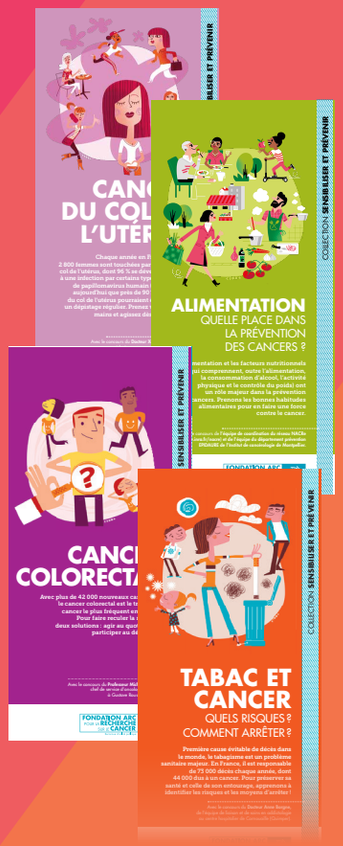
Pour informer le patient et son entourage pendant la prise en charge.

MIEUX VIVRE

Pour améliorer la qualité de vie du patient et de ses proches pendant et après les traitements.

SENSIBILISER & PREVENIR

Pour sensibiliser le grand public aux risques et à la prévention des cancers.



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LA FONDATION ARC
POUR LA RECHERCHE SUR LE CANCER

CHIFFRES CLÉS 2020

74,1 %
des dépenses (26,73 millions d'euros) consacrés au soutien à la recherche et à l'information du public

315
projets de recherche soutenus pour un montant de 23,3 millions d'euros

164 600
donateurs

35,1 M €
collectés auprès du public dont près de la moitié issue des libéralités (legs et assurances-vie)

Reconnue d'utilité publique, la Fondation ARC est 100 % dédiée à la recherche sur le cancer. Grâce à la générosité de ses donateurs et testateurs, elle alloue chaque année de l'ordre de 25 millions d'euros à des projets de recherche porteurs d'espoir pour les malades. Son objectif : contribuer à guérir 2 cancers sur 3 en 2025.

La Fondation ARC a pour mission de **lutter contre le cancer par la recherche.** Forte d'une expertise nationale et internationale, elle met en œuvre une politique scientifique visant à **accroitre les connaissances sur tous les cancers**, à **favoriser l'innovation thérapeutique** et à **créer les conditions d'une recherche d'excellence.**

Aujourd'hui, elle entend notamment accélérer en priorité le développement de la **médecine de précision** (thérapies ciblées, immunothérapies, chirurgie mini-invasive…) et l'amélioration de la prise en charge des **enfants et adolescents atteints de cancer.**

Menée en toute indépendance et sur l'ensemble du territoire, son action est guidée par l'intérêt général et l'excellence scientifique : elle identifie, sélectionne, finance et accompagne des programmes de recherche prometteurs.

La Fondation ARC œuvre également à informer le public sur les avancées de la recherche afin d'apporter à chacun les moyens de **mieux prévenir, mieux prendre en charge et mieux comprendre la maladie.**

La Fondation ARC est financée par la générosité du public ; c'est le soutien de ses donateurs et testateurs qui lui permet de mener son action en faveur de la recherche. Elle est agréée par l'organisme de contrôle le « Don en confiance » depuis 1999.

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La **Fondation d'Entreprise SILAB - Jean PAUFIQUE** a pour vocation, depuis 2007, d'apporter un soutien actif et constant à la recherche fondamentale et appliquée dans les pathologies cutanées, et tout particulièrement dans le domaine du cancer de la peau.

Elle alloue à de jeunes chercheurs, doctorants ou post doctorants, une somme annuelle de 20 000 euros sur une période maximale de 3 ans.

FONDATION d'entreprise Silab Jean PAUFIQUE

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FRANCE

CIBLER LE CANCER TRANSFORMER LES THÉRAPIES

UNE ATTENTION CONSTANTE À L'ONCOLOGIE DEPUIS PLUS DE 23 ANS

Seagen a été fondée en 1998 à Seattle par le scientifique, Dr Clay Siegall. Seagen a pour mission de découvrir, développer et commercialiser des thérapies ciblées innovantes à fort potentiel de changer le pronostic et la prise en charge des patients atteints de cancer.

Aujourd'hui, Seagen est présente dans plus de 10 pays et compte plus de 2 000 employés, dont la majorité sont des chercheurs et des scientifiques.

Seagen a été élue Biotech de l'Année par BioPharma Dive en 2020.

DÉDIÉE À L'AMÉLIORATION DE LA VIE DES PERSONNES ATTEINTES D'UN CANCER

Trois produits issus de la R&D de Seagen sont déjà commercialisés au niveau international et l'entreprise poursuit ses efforts pour répondre aux besoins non-satisfaits de patients atteints de cancers à un stade avancé. Seagen a conclu des partenariats avec des sociétés biotechnologiques et pharmaceutiques de premier plan, pour accélérer la mise à disposition de ces innovations aux patients

Le lancement de son premier médicament en nom propre en Europe démontre l'engagement continu de Seagen envers les professionnels de la santé et les patients.

LEADER INDUSTRIEL DE TECHNOLOGIES DE POINTE

La croissance de Seagen est basée sur son agilité et sa science de pointe. Seagen est propriétaire et leader industriel de la technologie ADC (anticorps conjugués), qui a permis le développement de plusieurs anticancéreux innovants ciblés.

Seagen est aussi propriétaire de la technologie des anticorps non fucosylés (SEA) qui améliorent la réponse immunitaire contre le cancer.

UNE ENTREPRISE AXÉE SUR LA RECHERCHE & LE DÉVELOPPEMENT

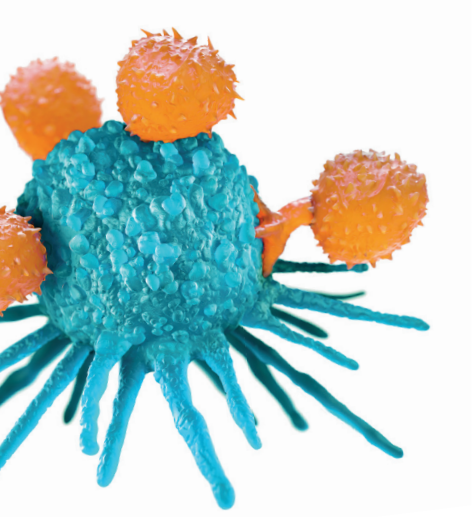
Seagen est une entreprise axée sur la recherche, qui investit lourdement en R&D.

En 2020, nous avons réinvesti plus de 80 % des revenus issus de ses produits dans la R&D.

La recherche clinique en cours comprend 18 programmes, avec près de 40 essais cliniques allant de la phase 1 à la phase 3 à travers plus de 18 pays.

EUM/COR/2021/0023

GSK, un acteur de santé guidé par la science



En oncologie, notre mission est axée sur l'amélioration de la survie et de la qualité de vie pour que chaque être humain soit plus actif, se sente mieux et vive plus longtemps.

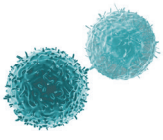
LE PATIENT AU CŒUR DE NOS PRIORITÉS

Nous nous donnons pour objectif d'accompagner les patients tout au long de leurs parcours de vie : de l'annonce du diagnostic, en passant par la prise en charge thérapeutique, le bon usage et la préservation de la qualité de vie.

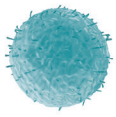
LA R&D AU SERVICE DES PATIENTS

Notre objectif est d'assurer une mise à disposition continue de nouvelles thérapies à partir d'un portefeuille diversifié de molécules en développement.

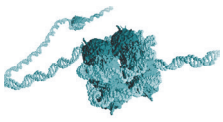
Dans la lutte contre le cancer, nous concentrons nos efforts de R&D sur 4 axes de recherche innovants :



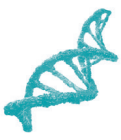
l'immuno-oncologie



thérapies ciblant les cellules tumorales



la thérapie cellulaire



la létalité synthétique

Au travers de thérapies mieux ciblées et personnalisées, nous nous engageons à aider les patients à faire plus, à se sentir mieux et à vivre plus longtemps.

gsk.fr

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