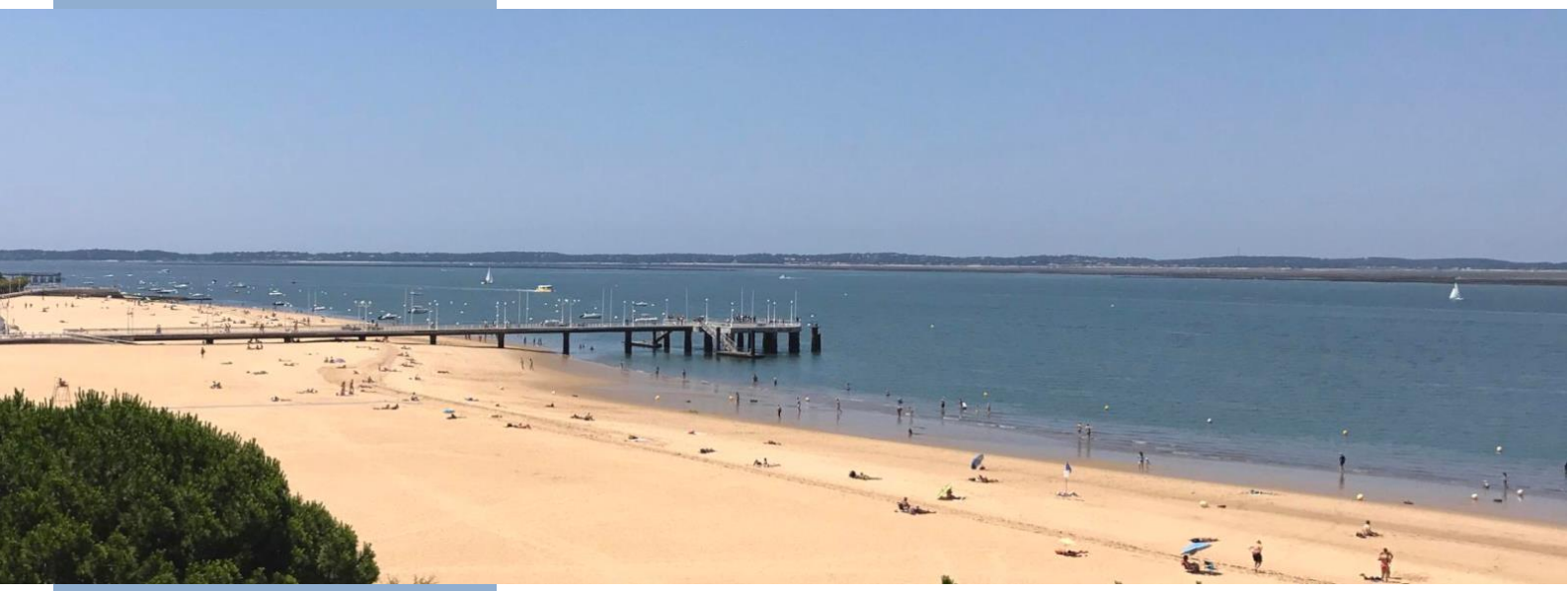
19th

Annual Meeting

Cancéropôle Grand Sud-Ouest

November 22-24, 2023

Congress Center / Arcachon



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 coordonnateurs et les membres des Comités de Pilotage des Axes,**
- **les copilotes et les membres des Bureaux des Groupes de Travail,**
- **les membres du Comité de Pilotage Scientifique,**

pour leur participation et leur implication dans l'élaboration du programme de ces 19èmes Journées et la qualité scientifique des présentations.

Comité de Pilotage Scientifique

E. Assénat, N. Bonnefoy, O. Calvayrac, N. Christou, S. Croce, P. Cordelier, M. Del Rio, C. Delpierre, C. Franchet, V. Gigoux, A.M Gué, E. Julien, B. Liagre, V. Moreau, N. Moya, J. Pannequin, V. Randrian, F. Saltel, P. Soubeyran

Comités de pilotage des Axes

Axe 1 – Signalisation, microenvironnement et ciblage
B. Bessette, **G. Bossis**, D. Gomez, N. Larmonier, A. Maraver, **V. Moreau**, A. Penna, M. Poupot, C. Sirac, F. Vergez

Axe 2 - Dynamique et expression du génome
JC. Andrau, O. Gadal, **E. Julien**, G. Legube, L. Linarès, D. McCusker, V. Pancaldi, S. Péron, H. Seitz, PYJ. Wu

Axe 3 – Innovation thérapeutique et biomarqueurs
N. Bakalara, A. Bobrie, T. Chardès, E. Chatelut, S. Dabernat, E. Deluche, M. Dufresne, **V. Gigoux**, W. Jacot, AM. Khatib, F. Lalloué, L. MBacthi, MA. Poul, B. Segui, I. Soubeyran, **D. Tougeron**

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

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

C'est avec grand plaisir que nous vous accueillons dans cette agréable ville balnéaire d'Arcachon pour cette 19ème édition des Journées Annuelles du Cancéropôle Grand Sud-Ouest.

Après une année de maturations des groupes thématiques, les coordonnateurs de ces groupes se sont associés aux pilotes des axes scientifiques du Cancéropôle Grand Sud-Ouest pour vous proposer un programme d'une qualité exceptionnelle. Je tiens à exprimer mes sincères remerciements à tous ceux qui ont contribué à la conception de ce programme, que ce soit au niveau des sessions des axes ou des présentations en plénière. Cette année également une ville est mise à l'honneur...alors n'hésitez pas à faire acte de candidature pour l'année prochaine.

Ce programme vous offrira l'opportunité d'assister à de nombreuses interventions de la part de chercheurs et cliniciens de notre interrégion, ainsi que de prestigieux conférenciers invités venus d'horizons variés. Parmi nos collègues frontaliers, nous aurons le plaisir d'accueillir des experts renommés tels que Francis Barr d'Oxford, Marc Chadeau-Hyam de Londres, Roger Gomis de Barcelone, Irmela Jeremias de Munich, Dario Neri de Zurich, sans oublier Joan Seoane de Barcelone. Parmi nos collègues d'autres Cancéropôles, nous aurons l'honneur de recevoir Charles-Antoine Dutertre de l'Institut Gustave Roussy, Agathe Figarol de Besançon, ainsi que Guy Launois de Caen, dont les présentations promettent d'être passionnantes. Nous irons encore plus loin afin de comprendre la physiopathologie du cancer, dans son environnement et ses interactions avec l'hôte, vers de nouvelles pistes thérapeutiques.

Nous aurons le privilège d'accueillir en conférence de Prestige, Jacques Arnould, conseiller Ethique au CNES, dont la conférence « le patient est-il un extraterrestre ? » ne manquera pas de vous interpeller.

L'année 2023 marque également les 20 ans de la création du Cancéropôle Grand Sud-Ouest. Tous les acteurs de la recherche du Grand Sud-Ouest ont relevé ce défi avec enthousiasme et ont contribué au succès remarquable du Cancéropôle Grand Sud-Ouest, qui est avant tout votre succès. Vingt années de rencontres, de discussions et de partages ont permis de construire année après année un plan d'actions qui répond à vos attentes. Je tiens à exprimer ma profonde gratitude à tous ceux qui ont contribué à cette réussite tout au long de ces années. Nous aurons l'occasion de le célébrer à la soirée de gala, où des surprises vous attendent.

Je vous remercie sincèrement d'être présents et réunis pour ces Journées, devenues incontournables dans la vie de notre Cancéropôle. Je souhaite que ces journées soient riches en informations et en discussions. Je suis convaincue qu'elles seront également l'occasion de rencontres informelles et de moments de convivialité, afin de perpétuer la dynamique qui nous anime depuis de nombreuses années.

Je souhaite à chacun d'entre vous de passer d'excellentes 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 2024 - 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	25 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	25 k€ maximum par projet

EMERGENCE DE CONSORTIUM THEMATISE

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	Inscription dans une dynamique de mutualisation des expertises (trans- ou inter-axes) en lien avec les groupes de travail 2023-2027.
FINANCEMENT	30 k€ maximum par projet

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

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.

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 2024 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
- **Cancer du pancréas** en 2022

PROCHAINE EDITION EN 2024

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.

*PLUS D'INFOS 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 EN 2024

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**
- **2022 : Génome dynamics and Cancer**
- **2023 : Signaling, Microenvironment and Targeting**

Prochaine édition : Nanomedicine in Cancer, du 7 au 8 décembre 2023 à Carcassonne

LES GROUPES DE TRAVAIL

ACTUALITES ET ACTIONS REALISEES

Contexte et Objectifs

Dans le cadre de la stratégie scientifique pour les années 2023-2027 décidée par son Comité de Pilotage Scientifique, le Cancéropôle GSO a mis en place **5 groupes de travail sur des thématiques transversales**, en pleine évolution, et incontournables dans un futur proche.

Les objectifs de ces groupes de travail sont de **favoriser les approches pluridisciplinaires et les projets collaboratifs**, **améliorer le partage des expertises présentes et les renforcer, faciliter l'émergence de nouveaux talents et de nouvelles technologies, et ainsi renforcer la structuration inter-régionale**.

Biologie spatiale

Un workshop a été organisé le 7 septembre afin de rassembler les utilisateurs (actuels et futurs) du Grand Sud-Ouest ayant besoin d'informations sur les approches de biologie spatiale basées sur la transcriptomique et l'imagerie. Les présentations avaient pour objectif de discuter la faisabilité des technologies déjà implémentées dans le GSO ou bientôt disponibles. Une cartographie des expertises disponibles est en cours de préparation et des webinaires sont à l'étude pour 2024.

Chimie et Cancer

Un workshop a été organisé les 19 et 20 juin afin de rassembler les communautés autour de courtes présentations scientifiques et de posters ayant pour objectif de présenter des offres de services ou de collaborations portées par des plateformes ou des équipes de recherche, mais aussi de permettre à des chimistes de proposer de nouveaux outils pour répondre à des questions biologiques ou, pour les biologistes, d'exprimer des besoins liés à des problématiques qu'ils n'arrivent pas à résoudre. Une liste de diffusion est en cours de création afin de favoriser les recherches de collaboration. Un nouveau workshop est prévu à Toulouse au printemps 2024 et à Bordeaux à l'automne.

Stress environnemental

Le bureau du groupe de travail s'est réuni à plusieurs reprises afin d'échanger sur la notion de « stress » qui peut être abordée sous différents angles. La conférence présentée par Marc Chadeau-Hyam lors de la session plénière des Journées Annuelles permettra d'aborder les travaux sur l'exposome. D'autres animations se dérouleront courant 2024.

Modèles alternatifs à l'expérimentation animale

Un workshop a été organisé le 31 mai afin de favoriser la transdisciplinarité et faire émerger des projets collaboratifs entre équipes biologiques/cliniques et porteurs de technologies. Cette journée a permis de présenter des infrastructures nationales visant à structurer la thématique des organoïdes et des nouveaux modèles alternatifs. Des offres de services ou de collaborations portées par des plateformes ou des équipes de recherche du Grand Sud-Ouest ont été aussi mises en avant.

Méthodes, Management et Analyses de données

Le bureau du groupe de travail prépare des interactions avec les autres groupes de travail en fonction des besoins qu'ils peuvent exprimer, notamment dans l'analyse des données. Un workshop thématisé sera organisé en 2024. A noter que le club SMAC (Statistiques et Mathématiques Appliquées à la Cancérologie) organise ses 13èmes journées à Montpellier les 14 et 15 mars 2024.

Si vous souhaitez participer aux actions des groupes de travail, n'hésitez pas à contacter l'équipe de coordination.

Program

Wednesday 22nd November

12h30 – 13h45 Welcome lunch

13h45 – 14h00 Opening ceremony

Nadine HOUEDE, Scientific director of Cancéropôle Grand Sud-Ouest

14h00 – 15h45

Chairs: Julien FAGET & Frédéric LOPEZ

Lecture: **Charles-Antoine DUTERTRE**, *Gustave Roussy Institute, Villejuif* - **Heterogeneity and spatial organisation of Tumour-Associated Mononuclear Phagocytes**

- **Hamid-Reza REZVANI**, *Bordeaux Institute of Oncology (BRIC), Bordeaux* - Single-cell analysis of skin pre-cancerous and cancerous lesions reveals differences in their metabolic profile and immune cell types
- **Abdelmounim ESSABAR**, *Toulouse Cancer Research Center (CRCT), Toulouse* - Tools for analysing spatial data in the context of immuno-oncology

15h45 – 16h05

16h05 – 17h15 Poster session & Coffee break

17h15 – 18h45

Chair: Guillaume BOSSIS & Eric JULIEN

Lecture: **Irmela JEREMIAS**, *Helmholtz Zentrum München, Munich (Germany)* - **Characterizing drug targets using CRISPR/Cas9 screens in PDX leukemia models *in vivo***

- **Aurélie BEDEL**, *BoRdeaux Institute of Oncology (BRIC), Bordeaux* - CRISPR-Cas9 side effects: risk of genomic instability
- **Dominique HELMLINGER**, *Centre for Biochemical and Macromolecular Research (CRBM), Montpellier* - New insights into transcription co-activator functions from conditional perturbations
- **Samuel AMINTAS**, *BoRdeaux Institute of Oncology (BRIC), Bordeaux* - The CRISPR/CAS Technology for the detection of rare KRAS mutant alleles

Chairs: Fabrice LALLOUE & Véronique GIGOUX

- **Diego BARBA,** *Montpellier Cancer Research Institute (IRCM), Montpellier* - Development of pH-modulated antibodies, that recognize immune system checkpoints.
- **Marie BOUTAUD**, *Control of cell Activation in Tumor Progression and Therapeutic Resistance, (CAPTuR) Limoges* - Metformin Treatment Reduces CRC Aggressiveness in a Glucose-Independent Manner: An In Vitro and Ex Vivo Study
- **David BRACQUEMOND**, *Montpellier Cancer Research Institute (IRCM), Montpellier* - Overcoming osimertinib resistance in EGFR-driven-MET amplified lung cancer
- **Lydia DIF**, *Bordeaux Institute of Oncology (BRIC), Bordeaux* - Fascin-1 inhibitors decrease hepatoblastoma cells tumorigenicity via YAP 1
- **Isabel GALEANO-OTERO**, *Bordeaux Institute of Oncology (BRIC), Bordeaux* - Evaluation of tumor heterogeneity in colorectal cancer and its implication in metastatic process
- **Gaëlle GUILLON**, *Montpellier Cancer Research Institute (IRCM), Montpellier* - Targeting the TNBC tumour microenvironment with Exatecan-conjugated anti-Cathepsin-D ADC
- **Leila KHAJAVI,** *Cancer Research Center of Toulouse (CRCT), Toulouse* - Transcriptomic Profiling of the Non-Small Cell Lung Cancer (NSCLC) Microenvironment Identifies a Duality in Natural Killer Cell Behavior
- **Fang LIU**, *Montpellier Cancer Research Institute (IRCM), Montpellier* - Improving the TA99 immunomodulatory effect with combination therapies using a new controlled delivery technology in oncology

- **Nicolas MATTEI**, *Pharmacology and Structural Biology Institute (IPBS), Toulouse* - Study of irreversible electroporation-induced cell death in spheroids derived from a murine hepatoma cell line
- **Alexia MIRANDOLA**, *Montpellier Cancer Research Institute (IRCM), Montpellier* - Persistence of netosis in resected colon cancer patient and impact on circulating DNA applications and post-surgery colon cancer management care
- **Léa RIMAILHO**, *Cancer Research Center of Toulouse (CRCT), Toulouse* - Role of CD39 on γδT cells as a positive marker of anti-CD20-induced ADCC of non-Hodgkins lymphoma cells
- **Mathis TRIQUARD**, *PROgression et DIssémination CErébrales des cellules Tumorales (PRODICET) Poitiers University, Poitiers* - Role of Exosomes in the Brain Dissemination of Tumor Cells derived from Breast Cancer (Locoregional and Distant)

Session 3C – Health Technologies: Flash Posters 24

Chairs: Audrey FERRAND & Bertrand LIAGRE

- **Israa AL JAMAL**, *Institut de Chimie des Milieux et Matériaux de Poitiers (IC2MP), Poitiers* - Study of cellular communication processes induced by artificial cell-cell interactions for the development of novel cell-based therapy
- **Chloé BAZILE**, *Cancer Research Center of Toulouse (CRCT), Toulouse* - Therapeutic targeting of pro-tumoral Tumor-Associated Macrophage by vectorized anti-folate receptor beta magnetic nanoparticles
- **Manon PORTA**, *LIttoral, ENvironment and Societies (LIENSs), La Rochelle* - Bioactive λ-Carrageenan oligosaccharides coated ferrite nanoparticles as potential anticancer nanodrugs
- **Marcelo HURTADO**, *Cancer Research Center of Toulouse (CRCT), Toulouse* - Transcriptional regulatory networks unravel cell states from immune cell type deconvolution and uncovers cell niches predictive of cancer progression
- **Aurélien MAZET**, *Laboratory for the Bioengineering of Tissues (BioTis), Bordeaux* - Bioprinting early-stage pancreatic cancer models: a new tool to decipher tumor initiation mechanisms
- **Camille DOUILLET**, *Photonics, Numerical and Nanosciences Laboratory (LP2N), Bordeaux* - Gut-in-caps: exploring the biophysical cues that drive self-organization of Caco2 cells into patho-physiological models of the intestinal epithelium
- **Malvina MARKU**, *Cancer Research Center of Toulouse (CRCT), Toulouse* - From gene regulatory network inference to dynamical modelling: revealing cell behaviour through network modelling in Chronic Lymphocytic

18h45 – 20h00 Icebreaker & Poster session

Thursday 23rd November

08h30 – 09h00 Welcome coffee

09h00 – 11h00

Session 4 – Chemistry & Cancer 32

Chairs: Bertrand LIAGRE & Philippe POURQUIER

Lecture: **Dario NERI**, *ETH, Zurich (Switzerland)* - **From encoded combinatorial libraries to clinical-stage targeted therapeutics**

- **Marie LOPEZ**, *ETaC, Toulouse and Institut des Biomolécules Max Mousseron (IBMM), Montpellier* - GRP94 chaperone identified as a new target of adenosine-cytosine bisubstrate analogues in cancer cells using affinity based chemical probes
- **Justine JOURNAUX**, *Toulouse Cancer Research Center (CRCT), Toulouse* - Thermal and mechanical stresses generated by magnetic nanoparticles upon magnetic field exposure induce immunogenic cell death in pancreatic adenocarcinoma
- **David LEGER**, *Laboratoire des Agroressources, Biomolécules et Chimie pour l'Innovation en Santé (LABCiS), Limoges* - Effects of metalation on the PDT activity of arene ruthenium porphyrin-based photosensitizers on prostate cancer
- **Sébastien BRITTON**, *Pharmacology and Structural Biology Institute (IPBS), Toulouse* - Deciphering and exploiting the mechanism of action of a large family of natural and bioinspired cytotoxic

11h00 – 12h00 Poster session & Coffee break

12h15 – 14h00 Lunch break

14h00 – 16h00

Session 5A – Cell Signaling, Microenvironment and Targeting 38

Chairs: Violaine MOREAU & Antonio MARAVER

Lecture: **Roger GOMIS**, *Institute for Research in Biomedicine (Barcelona, Spain)* - **ER+ breast cancer metastasis cell fate mapping**

- **Benoît THIBAULT**, *Toulouse Cancer Research Center (CRCT), Toulouse* - Inflammation-induced epithelial plasticity can be by-passed through Vps34 inactivation to limit pancreatic cancer initiation
- **Jennifer FALCONI**, *Institut de Recherche en Cancérologie de Montpellier (IRCM), Montpellier* - Tumor-derived cytokine, Upd3, promotes gut atrophy in a Drosophila larvae model of cachexia
- **Thomas BOYER**, *Immunoconcept, Bordeaux* - Immunosuppressive Myeloid Cells foster Cancer Stem Cell (CSC) emergence through TGF-β
- **Lucile ROUYER**, *BoRdeaux Institute of Oncology (BRIC), Bordeaux* - Cancer cells transfer invasive properties through tracks
- **Gilles FAVRE**, *Toulouse Cancer Research Center (CRCT), Toulouse* - In silico-based strategy for the discovery of direct small molecule inhibitors of mutant RAS

Session 5B – Genome Dynamics & Expression 45

Chairs: Jean-Christophe ANDRAU & Derek McCUSKER

Lecture: **Francis BARR**, *Trinity College & University of Oxford (Oxford, United Kingdom)* - **Mitotic mechanisms generating and sensing aneuploidy**

- **Frédéric BECKOUET**, *Center for Integrative Biology (CBI), Toulouse* - Mechanisms regulating cohesin dependent loops
- **Nezih KARASU**, *Institute of Molecular Genetics of Montpellier (IGMM), Montpellier* - G-quadruplexes involvement in mammalian genome organization and stability
- **Sophie PERON**, *Control of the Immune B Response and Lymphoproliferations (CRIBL), Limoges* - IgH 3'RR recombination uncovers a non-germinal center imprint and c-MYC-dependent IgH rearrangement in unmutated chronic lymphocytic leukemia
- **Domitille CHALOPIN-FILLOT**, *Institute of Cellular Biochemistry and Genetics (IBGC), Bordeaux* - Exploiting the dark matter of single-cell transcriptomes to encompass suppressive myeloid cell differentiation in hepatocellular carcinoma

16h00 – 17h00 Coffee break

17h00 – 18h30

Chair: Cyrille DELPIERRE

Lecture: **Marc CHADEAU-HYAM**, *Imperial College (London, United Kingdom)* - **Exposome Analytics: Definitions, Challenges and Applications**

18h30 – 19h15

Chairs: Eric JULIEN & Marc PIRCHER

Lecture: **Jacques ARNOULD**, *French Spatial Agency (Paris)* – **Is the patient an extraterrestrial ?**

20h00 Gala dinner (under registration)

Friday 24th November

08h00 – 08h30 Welcome coffee

08h30 – 10h00

Session 8A – Immunotherapy 55

Chairs: Bruno SEGUI & Abdel-Majid KHATIB

Lecture: **Joan SEOANE**, *Vall d'Hebron Institute of Oncology (VHIO) & Universitat Autonoma de Barcelona (UAB), Barcelona, Spain* - **Understanding the impact of the tumor microenvironment on immunotherapies**

- **Nicolas LARMONIER**, *Immunoconcept, Bordeaux* - The multifaceted tumor-promoting functions of myeloid cells
- **Ludovic MARTINET**, *Toulouse Cancer Research Center (CRCT), Toulouse* - T cell Immunotherapy: thinking beyond inhibitory receptors
- **Camille DANTZER,** *BoRdeaux Institute of Oncology (BRIC), Bordeaux* - Repression of exosome secretion by mutated ß-catenin contributes to immune escape in hepatocellular carcinoma

Session 8B – Patients Fragiles ou Patients Vulnérables (1) 60

Modération : Brigitte TRETARRE et Cyrille DELPIERRE

Conférence : **Guy LAUNOY**, *Université de Caen Normandie* – **Inégalités sociales en cancérologie. Approches quantitatives pour éclairer la politique publique**

- **Cyrille DELPIERRE**, *Centre d'Epidémiologie et de Recherche en Santé des Populations, Toulouse* - Accès à l'oncofertilité des femmes atteintes de cancer du sein : de l'observation à l'intervention
- **Isabelle INGRAND**, *CHU, Poitiers*

10h00 – 10h45 Coffee break

10h45 – 12h30

Session 9A – Alternative Models to Animal Testing 64

Chairs: Audrey FERRAND & Gaëlle RECHER

Lecture: **Agathe FIGAROL**, *FEMTO-ST (Besançon)* - **Development of a physiological microsystem, from a blood-brain barrier-on-chip to a vascularized glioblastoma-on-chip**

- **Léa MAGNE**, *Institut de Recherche en Santé Digestive (IRSD), Toulouse* - Mechanical interplays between mitotic spindle orientation of colonic epithelial cells and extracellular matrix deformations
- **Thomas BESSEDE**, *Institut de Recherche en Cancérologie de Montpellier (IRCM), Montpellier* - Sensitizing the PDAC tumor microenvironment to immune checkpoint therapies: characterization of a PDAC 3D model to decipher immune infiltration
- **Margot MACHU**, *Institut de Recherche en Cancérologie de Montpellier (IRCM), Montpellier* - Combination of immune checkpoint inhibitors with chemotherapies in new biological models of upper tract urothelial carcinomas
- **Alexia DE CARO**, *Institute of Pharmacology and Structural Biology (IPBS), Toulouse* - Anti-tumor efficacy of new high-frequency electrical protocols in a 3D in vitro colorectal cancer model
- **Joudi EL MIR**, *BoRdeaux Institute of Oncology (BRIC), Bordeaux* - Xenopus laevis is an attractive model for studying the pigmentary abnormalities in xeroderma pigmentosum type C

Session 9B – Patients Fragiles ou Patients Vulnérables (2) 71

Modération : Brigitte TRETARRE et Cyrille DELPIERRE

Conférence : **Daniel SATGE**, *Institut Debrest d'Epidémiologie et de Santé Publique & Oncodefi (Montpellier)* – **Cancers des personnes avec déficience intellectuelle**

- **Brigitte TRETARRE**, *Registre des Tumeurs de l'Hérault, Montpellier* - Etude CENTRUM: Impact de la centralisation de la prise en charge des cancers du rectum

12h30 – 13h00 Poster awards session - Closing ceremony

13h00 – 14h00 Lunch

Session 1 – Spatial Biology

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Heterogeneity and spatial organisation of Tumour-Associated Mononuclear Phagocytes

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The composition and spatial organisation of the tumour microenvironment is now gaining attention, especially immune cells, with the emergence of immunotherapies. Mononuclear phagocytes (MNP), the major immune cells populating tumours, comprise heterogenous subsets of monocytes (Mo), macrophages (Mac) and dendritic cells (DC). They are key regulators of the anti-tumour immunity. Mo and Mac (MoMac) most often promote tumorigenesis and metastasis. DC, which have the unique functional capacity to prime naïve T cells, can control the pool of tumour infiltrating T cells and, thus, regulate the T cell anti-tumour cytotoxicity.

In a recent study (Mulder, Immunity 2021), MoMac from 41 single cell RNA sequencing (scRNAseq data), and in-lab generated data were integrated to generate the universal "map" of human MoMac in heathy and diseased tissues revealing 17 populations of MoMac including three Mac subsets that accumulated in all solid tumours (tumour-associated macrophages, TAM) studied: (i) TREM2 Mac, strongly biased towards a pro-tumour lipid metabolism; (ii) proliferating Mac; (iii) previously undescribed IL4I1 Mac that express interferon stimulated genes, molecules involved in T cells regulation (PD-L1, PD-L2, MHC-II, CD40 and CD86), and that activate the aryl hydrocarbon receptor (AHR) through IL4I1 and IDO1 expression. Our analyses predicted that IL4I1 Mac arise from monocytes through engagement of CD40L expressed by CD4+ T cells and their stimulation by IFNg expressed by CD8+ T cells. IL4I1 Mac could attract regulatory T cells (Treg) by secreting CXCL9/10/11, suppress anti-tumour T cell functionality via PD-L1/L2 and via the IDO1/IL4I1-mediated AHR pathway activation.

We also integrated 38,293 DC from 13 tissues to generate a DC single-cell RNA compendium (DC-VERSE) (Mulder, in revision). We found that all neoplastic tissues studied contained CD207+ DC2 where their expansion: (i) inversely correlated with CD8+ T cells and T cell clonality; (ii) inversely correlated with tumour CD8+ resident memory T cells (TRM); (iii) positively correlated with terminally differentiated effector memory "exhausted" CD8+ T cells (TEMRA); (iv) was associated with lower overall survival in ICB-treated patients.

Following these scRNAseq-based studies, we now aim at evaluating the localisation and potential interactions of MNP populations, defined in our recent studies, within the different niches of carcinomas, focusing on breast and lung tumours where both IL4I1 Mac and CD207+ DC2 were detected. Indeed, the spatial distribution of immune cell populations within the different tumour niches is a major factor impacting the quality of the anti-tumour immune response, especially of cytotoxic effector CD8+ T cells, whose presence within tumours is associated with a good prognosis. To address this, we employed immunofluorescence histology and MERFISH, a single cell spatial transcriptomic technology. While most DC populations accumulated within the tumour stroma in contact with lymphocytes, CD207+ DC2 were mostly embedded within carcinomatous tumour nests. Interestingly, we confirmed that TREM2 Mac and FOLR2 Mac were detected within very distinct niches, the former detected within immune poor regions and FOLR2 Mac within immune riche stromal regions. IL4I1 Mac were detected both in the tumour stroma and tumour nests and were also forming some grape-like structures where they were in contact of T cells, especially Treg.

While scRNAseq allows to grasp the deep transcriptomic programmes of all cells within the tumour microenvironment, data obtained using single cell spatial transcriptomic allow to evaluate their localisation and the cells they potentially interact with. Such information could lead to the discovery of prognostic markers and the development of therapeutic approaches to (i) deplete or inhibit the differentiation of pathogenic myeloid cell populations; (ii) disrupt their pathogenic interactions with stromal and immune cell populations.

1 / 2

Single-cell analysis of skin pre-cancerous and cancerous lesions reveals differences in their metabolic profile and immune cell types

Lea DOUSSET[1], Domitille CHALOPIN[2,3,4], Vitalina BASHYNSKA[2,3], Jean-Philippe GUEGAN[5], Julie GIRAUD[2,3], Fanny BELTZUNG[1,6], Maya SALEH[4], Marie BEYLOT-BARRY[1,7], Macha NIKOLSKI[2,3], **Hamid-Reza REZVANI[1]**

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Cutaneous squamous cell carcinoma (cSCC) can manifest as a spectrum of progressively advanced malignancies, ranging from precursor actinic keratosis (AK) to in situ, invasive, and finally metastatic cSCC. Despite a very good prognosis for the majority of the patients, around 1.5 to 5.2% of cSCC recurred in a very aggressive manner (called "advanced cSCC", acSCC) with local and perineural invasion, metastatic spread and high mortality rate. However, little is known about the difference between cellular compositions and spatial architecture of precancerous lesions and acSCC. To this end, we combined single-cell RNA sequencing with spatial transcriptomics (ST) on a series of primary human locally acSCCs and their matched precancerous surrounded lesions. To decipher the tissue segmentation, we performed immunostaining using pancytokeratin (PanCK) for epithelial cells, vimentin for cell in mesenchymal transition on the invasion front of the tumor, and CD68 staining for macrophages localized in healthy, pretumoral, in situ or tumoral areas. Differentially expressed genes (DEG) in different clusters of cells were then screened for gene ontology (GO) analysis. Results showed that when epithelial cells started their tumor transformation, they stopped their keratinocyte and epidermal cell differentiation. At the same time, the glycolysis was upregulated and lipid biosynthesis was downregulated. Comparing metabolic features of PanCK+ cells between tumor and invasion front indicated that cells at mesenchymal transition state (PanCK+, vimentin+ cells) rewired their metabolic pathways favoring the oxidative phosphorylation (OXPHOS). We next explored the metabolic heterogeneity among immune cells. Both glycolysis and OXPHOS were upregulated in macrophages located in the invasion front compared to macrophages in healthy tissue, pointing out that both energetic pathways are activated in tumor-associated macrophages (TAMs).

Since it has been shown that energy metabolism has a direct effect on the immune cell functions, we then compared immune cell types between tumors and their paired precancerous skin lesions using single-cell RNA sequencing (scRNA-seq) integrated with digital spatial profiling (DSP). To this end, we used sequential fluorescence in situ hybridization thanks to Nanostring technologies to directly visualize RNA molecules in their native environment. 32526 single-cell transcriptomes of CD45+ immune cells were characterized from 2 tumors and their paired precancerous skin lesions. Results showed that some clusters of macrophages and Treg lymphocytes were enriched in the tumor, while, at the other hand, B cells and plasmablasts depleted. Tumor and precancerous lesion exhibited differences in subset of immune cells in G2M phase, pointing out a rapidly growing behavior for some subsets of cells or a senescent state for others.

Altogether, our data indicate that 1) metabolic and immune features of acSCC and precancerous lesions are different and 2) metabolism rewiring plays a key role in cSCC progression. Indeed, both tumor transformation of precancerous lesions and epithelial-mesenchymal transition process require specific metabolic demands, which will be achieved through metabolic rewiring mechanisms. Given the key contribution of metabolic reprogramming in carcinogenesis, targeting tumor bioenergetics vulnerabilities may be promising targets as an innovative therapeutic strategy in cSCC.

1 / 3
Tools for analysing spatial data in the context of immuno-oncology

Abdelmounim ESSABBAR[1], Alexis COULLOMB[1], Marcelo HURTADO[1], Leila KHAJAVI[1], Véra PANCALDI[1,2]

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Immunotherapies are imposing themselves as a revolution in cancer treatment, giving hope to many previously incurable patients. They aim to restore the natural defences mounted by the body against cancerous cells, such as lymphocytes, which are often disarmed by the tumour. Despite the promising potential, around 30% of patients do not benefit at all from immunotherapy. Better characterising the inter-cellular interactions in the tumour microenvironment (TME) will be key to propose new kinds of immunotherapy.

Given the recent discoveries about the importance of specific interactions between immune and cancer cells, understanding the spatial properties of tumors at single-cell resolution becomes crucial. We have recently developed computational tools to describe spatial patterns and clustering of specific cell types in tissues using network theory. These approaches allow us to extract statistical properties from imaging or spatial-omics datasets that can be used as biomarkers.

In particular, we have developed tools to extract information from biological images in the form of cellular networks, in which cells are nodes and edges are present if the cells are close to each other in the tissue. Our tysserand python package (tysserand, Coullomb and Pancaldi 2021, https://github.com/VeraPancaldiLab/tysserand) is better and faster at reconstructing these cellular networks than previous approaches allows us to perform a quantitative analysis of the topology of these networks.

Briefly, the nodes of this network represent single cells and can be associated to a cellular phenotype, which can be either a cell type, as can be easily achieved using single markers for specific cell types in the image) or a cell state, which can be defined based on multiple markers. So far we have developed methods to quantify the spatial localization of different immune and cancer cells within the tumour microenvironment as identified, for example, by multiplex Immuno-fluorescence experiments (a few markers per panel).

Making these networks can be useful to compare samples, extract biomarkers, and also make simulations of the tissues with realistic statistical distributions.
We also developed mosna (https://doi.org/10.1101/2023.03.16.532947), a Python package that exploits these networks to analyze spatially resolved experiments and discover patterns of cellular organization.

We apply concepts from network theory (assortativity) to measure the extent to which cells of a particular type/state cluster with each other and with cells of different cell types/states (mixing patterns). We are thus able to spot whether the presence of T-cells in a tumour involves close contacts between them and the cancer cells or if other cells (for example macrophages or myeloid suppressive cells) are preventing this direct contact. Published approaches mostly rely on distance between cell types as measure on the images to predict survival or response to therapies, or sometimes ratios of distances. We have show on public data that the cell type assortativities can be more predictive.
The detection of preferential interactions between specific cell types can also be used to populate tissue simulations for in-silico cancer models.
We have tested the method on different types of spatial proteomics and transcriptomics and have used public data from cancer patient samples annotated with clinical response to immunotherapy. *mosna* can identify a number of features describing cellular composition and spatial distribution that can provide biological hypotheses regarding factors that affect response to therapies.
Finally, *mosna* uses the Neighborhood Aggregation Statistics method to assign a feature vector to each cell, describing the composition of its neighborhood with different statistics. Applying clustering of the cells based on these neighborhood features, we can identify niches, specific subsets of interacting cells, that can also be used to predict response to immunotherapy.

Session 2 - Cancer research in Poitiers at a glance

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Poitiers is a city of more than 90,000 inhabitants located halfway between Paris and Bordeaux. It is the capital of the Vienne department and the former capital of the Poitou-Charentes region. In 2016, Poitiers has joined the Nouvelle-Aquitaine region and is now one the 4 city-university dynamic hubs of the Oncosphere Regional Research Network of Nouvelle Aquitaine and one of the 6 city-university hubs of the Great South West Cancéropôle network.

We will present the research units and hospital departments constituting the Poitiers interdisciplinary ecosystem of cancer research.

Session 3A – Genome Editing & CRISP'R Screen

3A / 1

Characterizing drug targets using CRISPR/Cas9 screens in PDX leukemia models in vivo

Irmela JEREMIAS

Helmholtz Center Munich, German Research Center for Environmental Health Head Research Unit Apoptosis in Hematopoietic Stem Cells Munich, Germany

Background:
Targeted therapies address specific proteins in a cell which harbor an essential function for that tumor cell so that the tumor shrinks if the target is inhibited. As underlying concept, a gene with a proven essential function might predict activity of an inhibitor targeting the respecting protein. Here we aimed to identify additional genes with essential function in patient leukemias which might serve as future therapeutic targets for novel targeted therapies.

Methods:
We developed a CRISPR-Cas9 screening approach for functional analysis of molecules. For a highly patient-related approach, we used patient-derived xenograft (PDX) AL models in vivo. Size of CRISPR library was determined by genetic barcoding. Stable expression of fluorescently labelled Cas9 and sgRNA constructs in two PDX samples. Enrichment of double positive cells by MACS and injection into NSG mice. Gene depletion analysis using MAGeCK algorithm to screen and functional competitive in vivo assays to validate the candidates. Characterization of the KO or drug inhibitor treated cells for engraftment capacity by homing assay, frequency of leukemic stem cells by competitive limiting dilution transplantation assay (LDTA), sensitivity towards routine chemotherapy by in vivo competitive chemotherapy trials in both lineages. Rescue assay by reconstitution of protein variants in functional competitive in vivo assays.

Results:
When performed several customized CRISPR-Cas9 in vivo screens targeting about 100 candidate genes per screen, in PDX models of both acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). The screens allowed identifying novel therapeutic targets, such as the surface molecules ADAM10 (Bahrami et al., Molecular Cancer 2023), WT1 and DNMT3A (Ghalandary et al., Blood 2023) and BCL2 overcoming drug resistance (Wirth et al., Leukemia 2022). In vivo screens are specifically suitable to study molecules mediating the interaction between tumor cells and the normal environment.

Summary/Conclusion:
In summary, we established CRISPR-Cas9 drop-out screens in PDX models in vivo as technology to explore patient-specific tumor dependencies. Our data revealed a yet unknown function of several proteins to maintain leukemia cells alive. The respective gene products might represent targets for inhibitory drugs for putative treatment of leukemia patients in the future.

3A / 2

CRISPR-Cas9 side effects: risk of genomic instability

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The CRISPR-Cas9 system has revolutionized our ability to precisely modify the genome. Gene editing is classically used to invalidate genes, to model or correct mutations. Clinical trials using this technology are already on going to treat monogenic diseases and cancers. However, this powerful tool can induce adverse genomic events. The off-target genotoxicity is well described, predictable, detectable, and can be resolved by the use of new generations of Cas9 nucleases with high fidelity. In contrast, the ON-target genotoxicity due to a DNA double-strand break at the targeted locus is still underestimated1. Comprehensive analysis of gene editing products at the targeted cut-site has revealed a complex spectrum of outcomes by incorrect or ineffective DNA repair and DNA damage response. ON-target genotoxicity is underestimated with standard PCR-based methods and necessitates appropriate and more sensitive detection methods.

Using cytogenetic approach after cell cloning (FISH, CGH-array, SNP-aray), we discovered the appearance of megabase-scale rearrangements in cell lines2 and in primary cells3. We next developed two Fluorescence-Assisted Megabase-scale Rearrangements Detection (FAMReD) systems that enable the detection, quantification, and cell sorting of edited cells with megabase-scale loss of heterozygosity (LOH). These tools offer highly sensitive readouts to decipher the short-term (murine FAMReD) and long-term (human FAMReD) risk and to find solutions to limit it.

We showed that LOH frequency depends on p53 status and cell division rate during editing. Importantly, G1-cell cycle arrest (by palbociclib) during editing suppresses the occurrence of LOH without compromising editing 4. These data were confirmed in human stem/progenitor cells, suggesting that clinical trials should consider p53 status and cell proliferation rate during editing to limit this risk by designing safer protocols. In particular, cell cycle blockade by CDK4/6 inihibitors could offer opportunities to make nuclease-based gene therapy protocols safer.

1 Boutin J et al. CRISPR J 2022
2 Cullot G et al. Nature com 2019
3 Boutin J et al. Nature com 2021
4 Cullot et al. Nature com 2023

3A / 3

New insights into transcription co-activator functions from conditional perturbations

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Gene regulation is crucial to generate phenotypic diversity between cells of identical genotypes, for example during cell fate decisions or in response to environmental changes. A critical step in gene expression is transcription, which is controlled by many distinct epigenetic regulators, such as chromatin-modifying and -remodeling complexes. These complexes integrate regulatory information from promoter elements, transcription factors, and chromatin marks. Despite the advent of functional genomics, understanding how these factors establish specific gene expression programs remains challenging, likely because our view of their function is static. Using a combination of CRISPR-Cas9 editing and functional genomics approaches in cancer cell lines, we initiated a project aimed at identifying the principles governing the gene-specific effects of chromatin-regulatory factors, focusing on the multifunctional SAGA and TIP60 co-activator complexes as paradigms. We will present our efforts to establish innovative tools to measure the effects of co-activators on the temporal dynamics of transcription, nucleosome modifications, and general transcription factor assembly, allowing the ordering of mechanistic events. Overall, we will discuss how these data contribute to better understand the roles of SAGA and TIP60 in the transcription addiction of cancer cells to oncogenic transcription factors such as MYC.

3A / 4

The CRISPR/CAS Technology for the detection of rare KRAS mutant alleles

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Introduction: The diagnosis of pancreatic ductal adenocarcinoma necessitates tumor biopsy by endoscopic ultrasound fine needle aspiration (EUS-FNA), which shows insufficient negative predictive value. As mutations in the KRAS oncogene are very common in PDAC, their detection from circulating tumor material may provide a powerful diagnostic tool for PDAC formal diagnosis but this requires a level of sensitivity challenging the available molecular tools. We assessed the emerging CRISPR/Cas13a SHERLOCK technology promising the high discrimination of genetic alterations, from large intragenic rearrangement to single nucleotide polymorphism.

Methods: Guides hybridizing various positions on the KRAS target were designed to detect G12V, G12C and G12C mutant alleles. Mismatches around the single mutation of interest, as well as hairpin spacer sequences were tested to prevent hybridizing the wild-type allele. Mutant allele detection was tested on matrix containing known concentrations and compared to Q-PCR, allele-specific PCR and ddPCR. The sensitivity of a large intragenic rearrangement and a 15nt deletion of the EGFR were compared to that of single KRAS mutation. The combination of an allele specific PCR and a CRISPR/Cas detection (AS-SHERLOCK) of the related products was finally set-up and tested with pancreatic cancer and non-small cell lung cancer liquid biopsy patients' samples.

Results: The position of RNA guide affected the ability of Cas13a to detect KRAS alleles and the possibility to discriminate between different alleles. We observed efficiency variations between mutations, possibly related secondary structures of the matrices and the nature of the mismatches between the guide and the matrix. Hairpin spacer strategy only slightly improved specificity for KRASG12D mutant detection. The detection of a large intragenic rearrangement and a 15 nt deletion mutation in the EGFR gene reached a total specificity and a sensitivity similar to the one of ddPCR. Moreover, AS-SHERLOCK reached performance similar to ddPCR for KRASG12D mutant detection. Finally, AS-SHERLOCK technology allowed the efficient detection of KRAS and EGFR mutant in patients' samples.

Conclusion: As other sensitive tools, CRISPR/Cas13a technology is challenged to detect mutant variants outnumbered by WT alleles. However, the use of highly discriminant guides reached or outperformed the gold standard ddPCR for the detection of rare alleles. It is implemented with a simple workflow, without expensive equipment, and applicable to patients' samples analysis. Efforts are still needed to increase the specific detection of single mutations to avoid initial allele-specific PCR.

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Session 3B – Therapeutic Innovation and Biomarkers: flash posters

3B / 1

Development of pH-modulated antibodies, that recognize immune system checkpoints

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[7] MabDesing
[8] Mabqi

Targeting and blocking the immune system checkpoints using antibodies is revolutionizing cancer therapy, especially in those that generate many mutations such as melanoma. Since these checkpoint targets are not tumor specific, it is common to generate toxicities like autoimmune syndromes. Interestingly, some approved anti cytotoxic T-lymphocyte associated protein 4 (CTLA4) antibodies showed increased life expectancy of patients with melanoma. Nonetheless, the systemic autoimmune-related adverse effects due to T-Regulator lymphocyte depletion outside the tumor do not allow most patients to continue their treatment. Hence, it would be desirable to promote the activation of T-Effector and eliminate the T-Regulators only in the tumor site to reverse cancer progression. Thus, we can rely on the fact that tumor tissues have a more acidic pH than healthy ones due to the metabolic switch stated by the Warburg effect.

Therefore, we plan to generate pH-dependent antibodies able to bind and inhibit the CTLA4 checkpoint only at the tumor acidic microenvironment to promote the activation of the immune system, but not at physiological pH in normal tissue. We will obtain anti-CTLA4 antibodies with reduce systemic toxicities by a phage display selection with two different types of synthetic scFvs libraries, one enriched in histidine residues and one without enrichment, to acquire antibodies against different epitopes of CTLA4. Subsequently, we will reformat and produce the selected pH-sensitive scFvs fragments into full IgG formats to characterize them. After having found the pH-dependent anti-CTLA4 scFv antibodies, we will change their format to IgG and perform further tests. Initially analyzing their capacity to fix on human T-lymphocytes positive for CTLA4 with acidic pH conditions. So that afterwards, the high-profile selected antibodies can be tested in "Knock-in" mice for human CTLA4 to see if they are able to deplete the T-Regulatory cells at the tumor site and not in healthy tissues (e.g., Spleen). Finally, we will study their overall tumor distribution and ability to reduce the tumor mass compared to other published and FDA approved controls.

3B / 2

Metformin Treatment Reduces CRC Aggressiveness in a Glucose-Independent Manner: An In Vitro and Ex Vivo Study

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As per the World Health Organization (Globoccan, 2020) report, colorectal cancer (CRC) ranks as the third most commonly diagnosed cancer in males, following lung and prostate cancer, and as the second most prevalent in females, trailing breast cancer. Despite advancements in diagnostic and screening technologies, CRC remains a formidable adversary, with recent upticks in occurrence among individuals under 50 in high-income nations. Furthermore, CRC frequently reoccurs even when detected at its initial stages. The TNM (Tumor Node Metastasis) classification categorizes CRC into five stages, ranging from 0 to IV. In stage 1, the mucosa is the sole area affected, progressing to stage 2 with involvement of the muscularis, stage 3 with lymph node invasion, and stage 4 with the presence of metastases.

Epithelial-mesenchymal transition (EMT) is chiefly characterized by the loss of epithelial markers like E-cadherin and the activation of cell movement, partly attributed to extracellular matrix modification by metalloproteinases such as MMP2 and MMP9 (1). EMT is a pivotal process promoting the dissemination of cancerous cells, particularly in epithelial cancers like CRC. The reduction of epithelial markers, including E-cadherin, facilitates EMT and is linked to the aggressiveness of CRC (2). **Metformin**, primarily employed in the management of type 2 diabetes due to its actions on mitochondrial metabolism and AMPK, has been investigated for its inhibitory effects on EMT in various cancer types, although its impact on colorectal cancer remains unexplored (3).

This research aims to investigate the influence of metformin on the suppression of EMT-related genes, migration, and invasion in colorectal cancer cell lines (HCT-116 and SW-620), along with a cohort of 23 patients. Special attention is given to its effect on E-cadherin and MMPs. In addition, it was investigated whether this was due to the action of metformin on AMPK. To assess the effect of glucose on metformin-induced EMT inhibition ***in vitro***, all experiments were conducted under **two glucose conditions**, mirroring fasting blood glucose (7.8 mM) and hyperglycemic conditions (17.5 mM). The ***ex vivo*** experiments involve **patients in stage 1, 2, and 3 of CRC**, either with or without diabetes-metformin treatment.

The results indicate that metformin gives favourable results and can prevent the early stages of colorectal cancer. Metformin appears to influence E-cadherin cleavage during EMT, notably by acting on MMP2 and MMP9 through activation of AMPK. Under *ex vivo* conditions, metformin shows promise in the early stages of colorectal cancer. However, further experiments are needed to validate these results.

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3B / 3

Overcoming osimertinib resistance in EGFR-driven-MET amplified lung cancer

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Patients diagnosed with EGFR- mutated lung adenocarcinoma are treated with specific EGFR-inhibitors such as osimertinib. However, inevitably, most patients relapse. A subpopulation of cells called Drug Tolerant Persisters (DTP) seems to be responsible for the tumour recurrence. About 20% of relapse is due to the amplification of the oncogene MET with currently no therapeutic alternative than conventional chemotherapy. Our aim is to establish whether DTPs are associated with the NOTCH pathway, a crucial pathway in lung adenocarcinoma and/or MET.

EGFR-driven cells with or without MET amplification were treated with a combination of EGFR, NOTCH or MET inhibitors. We infected these cells with the FUCCI system to monitor HES1 expression (read-out of Notch pathway activity) during the cell cycle by cytometry upon different drug treatment. Tet-on-EGFRT790M/L858R transgenic mice have been treated with the combination of EGFRi and NOTCHi by oral gavage 5 days per week.

Upon EGFRi treatment, we observe a fluctuation of the NOTCH pathway activity over time. Initially, HES1 drops drastically in DTPs while Notch1 intracellular domain increases significantly. Then, once DTPs are expending, the trend is reversed. In vitro, EGFRi/NOTCHi combination delays the expansion of DTPs compared to EGFRi alone and additionally, Met-amplified osimertinib-resistant cells demonstrate strong sensitivity to EGFRi/METi and EGFRi/NOTCHi combinations.

EGFRi treatment affects the NOTCH pathway activity and targeting NOTCH in combination with EGFR brings therapeutic benefit as it delays the relapse in vitro in EGFR-mutated cell lines. A preclinical in vivo evaluation is currently on going. In the presence of MET amplification, EGFRi/METi and EGFRi/NOTCHi treatments showed moderate therapeutic benefits, what about tritherapies with a NOTCH pathway inhibitor?

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Fascin-1 inhibitors decrease hepatoblastoma cells tumorigenicity via YAP 1

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Hepatoblastoma (HB) is a liver tumor that arises in children. It's a sporadic malignancy that is often very aggressive. The current treatment consists of chemotherapy. However, chemotherapy in young patients has disastrous and long-term side effects such as ototoxicity, cardiomyopathy and infertility. Thus, alternative strategies are needed. One hint is to target the most common mutations in HB. It has been demonstrated that 90% of HB tumors are mutated for the Wnt pathway effector ß-catenin. This mutation leads to an aberrant constitutive activation of Wnt/ß-catenin signaling. Here, we investigate one of ß-catenin transcriptional targets, Fascin-1 that is found up-regulated in many tumors. Fascin1 affects actin organization into bundles and this leads to cell migration and invasion. Whereas Fascin-1 is absent from normal hepatocytes, we found its expression associated to the poor prognosis C2 subtype of HB. In both human and murine HB samples, Fascin-1 is associated to undifferentiated tumor cells. We further demonstrated that Fascin-1 expression modulates tumor hepatocyte differentiation status through gene expression. In this study, we investigate how Fascin-1 is able to regulate tumor cell plasticity and whether Fascin-1 is a druggable target in HB tumors.

Method: We use two classical HB model cells Huh6 and HepG2 and 3 Patient-Derived-Xenograft cell lines. We explore the effect of Fascin-1 actin-bundling activity impairment by using inhibitors NPG2044 and BDP13176, on invasion and migration using Trans-well and wound-healing assays. We follow proliferation and cell death by Flow cytometry and investigate gene expression by PCR and reporter assay. We investigate Fascin modulation of the kinome with PamGene technology.

Results: We show that the inhibition of Fascin actin-binding activity decreases cell invasion and migration as well as proliferation. We show an increase of cell death in Huh6/HepG2 cells but not in the PDX models. Differentiation genes are overexpressed and EMT genes are repressed. Yap expression, is downregulated; Yap promoter activity is downregulated and Yap is found translocated into the cytoplasm upon Fascin-1 inhibition. These data suggest that Fascin inhibition effects on cells are mediated via the Hippo pathway.

Conclusion: Fascin-1 is an interesting target in hepatoblastoma, commercialized phase-2 drugs are available and this study will confirm the potential use of those drugs in HB treatment and elucidate by which mechanism Fascin-1 inhibition impacts tumors.

3B / 5

Evaluation of tumor heterogeneity in colorectal cancer and its implication in metastatic process

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Colorectal cancer (CRC) remains the 3rd most diagnosed cancer in Europe. Liver represents the most frequent metastatic site of colorectal cancers and about 50% of patients with colorectal cancer develop this kind of metastases, despite therapeutic advances and new medico-surgical strategies. Consequently, better understanding of the cellular and molecular biology of colon cancer and liver colorectal metastasis are urgently needed. This study was designed to determine the key process and proteins associated to metastatic dissemination in CRC, in order to identify potential biomarkers for accurate prognosis and establishment of personalized therapies. Thereby, transcriptomic (RNAseq) analysis were first performed on 60 pairs of primary colorectal tumors and liver metastases from the same patients and the more significant markers were validated using RT-qPCR and immunostaining. Some of the identified genes were previously reported linked to cancers but never linked to colon cancer. Others genes were specifically dysregulated in metastatic tissues are with unknown function, never reported in cancer. The functional validation of the identified genes in the metastatic process will help not only for patient stratification and prediction of CRC patients that will develop colorectal liver metastasis but also in the potential development of pertinent therapeutic options for each patient.

3B / 6

Targeting the TNBC tumour microenvironment with Exatecan-conjugated anti-Cathepsin-D ADC

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Triple Negative Breast Cancers (TNBC), which account for 15% of Breast Cancer (BC) patients (Estrogen Receptor-negative, Progesterone Receptor-negative, HER2 non-amplified), are mainly treated with chemotherapy and urgently require new targeted therapies. The aspartic protease Cathepsin D (Cath-D), a poor prognosis marker in BC including TNBC, is overexpressed by BC cells and hypersecreted in the tumour microenvironment. In BC extracellular Cath-D displays protumour activities by proteolysis at acidic pH and also acting as a ligand on stromal fibroblasts. Cath-D is an eligible target for ADC (Antibody Drug Conjugate) therapy in TNBC because it is associated with the tumor cell membrane in 85.7% of 147 TNBC samples analyzed (Ashraf*, Mansouri* et al, JITC, 2019). In addition, a previous study showed that extracellular Cath-D bound to anti-Cath-D monoclonal antibodys (mAbs) is endocytosed together with the mAb by TNBC and also by stromal fibroblasts (Laurent-Matha et al, J Cell Sci, 1998). Therefore, anti-Cath-D ADCs should allow the intracellular release of the payload in all cell components of the tumor (e.g., TNBC and stromal cells). The anti-Cath-D F1M1-187 was conjugated to Exatecan (topoisomerase I inhibitor).

It binds specifically to secreted Cath-D (EC50 = 2.4nM), internalizes within 3h and induces a 2D cytotoxic effect in vitro on cell lines recapitulating different subtypes of TNBC. ADC F1M1-187 could be a new therapeutic option for the treatment of TNBC patients.

3B / 7

Transcriptomic Profiling of the Non-Small Cell Lung Cancer (NSCLC) Microenvironment Identifies a Duality in Natural Killer Cell Behavior

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Cancer is the second leading cause of death globally. Lung cancer is the leading cause of cancer death worldwide, with a survival rate of 7 (small cell lung cancer) to 28% (non-small cell lung cancer) after 5 years. Our current understanding of the complex processes defining cancer is insufficient to treat a majority of patients effectively. In a clinical setting, bulk transcriptomics is often the assay of choice to screen and characterize groups of patients quickly and effectively in a cost-effective fashion. Here, we applied a computational immunology approach involving differential expression and pathway analyses, immune cell proportion quantification by deconvolution, transcription factor activity inference and immune score estimation in order to better characterize bulk RNAseq dataset of lung adenocarcinoma (LUAD) samples.

This analysis allowed us to identify biomarkers of disease progression and potential immune infiltration patterns across disease stages. Through our methodology and novel feature integration pipeline, we identified a duality in the behavior of natural killer (NK) cells in the tumor microenvironment (TME), suggesting a potential association with immune response or dysfunctional states. We validate these findings in an independent LUAD cohort with bulk and scRNAseq samples, allowing us to further characterize the NK cell subsets into dysfunctional (reduced cytotoxic potential), peripheral and tissue-resident NK populations.

3B / 8

Improving the TA99 immunomodulatory effect with combination therapies using a new controlled delivery technology in oncology

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Melanoma is the most aggressive form of skin cancer at metastatic stages, the overall survival rate is 32% (American Cancer Society). The first line treatments for the unresectable and metastatic melanoma are anti-PD1 immunotherapies or anti-BRAF and anti-MEK targeted therapies for BRAF mutated patients. While the patient response rate to immunotherapies is often low (around 40%) and unpredictable, the resistance to targeted therapies is prevalent at almost 100% occurrence post-treatment. Combined therapeutic approaches are more effective, however the associated increased drug toxicity and the severe side effects often cause the suspension of the treatment. Our main objective is to overcome these treatment burdens by exploring new combined therapeutic approaches using a biodegradable delivery system, which is able to locally deliver therapeutic molecules. In addition to the improved efficiency, drugs will be released in a controlled manner thereby aiming to avoid systemic drug toxicity.

We previously demonstrated in the B16F10 subcutaneous melanoma that intraperitoneal administration of TA99, a monoclonal antibody (mAb) targeting melanoma cell specific surface antigen TYRP1, was able to induce partial tumor protection trough immunomodulatory mechanisms (They et al, Oncoimmunology, 2017). In this new study, we used the B16F10 wild type melanoma syngeneic mouse model to study how an immunomodulator currently used in clinic, may synergize with TA99, to generate a sustained and protective antitumoral immune response. Combined therapy was compared to monotherapies by evaluation of safety, tumor growth and survival rate. Tumor challenge consisting of B16F10 cells graft in the opposite flank of the survival mice was performed to evaluate anti-tumoral immune response.

Next steps will consist of understanding the anti-tumor mechanisms and how anti-tumoral immune response is impacted following this therapeutic protocol.

3B / 9

Study of irreversible electroporation-induced cell death in spheroids derived from a murine hepatoma cell line

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Liver cancer, and predominantly hepatocellular carcinoma, is the third leading cause of cancer death. The most common ablation method, namely radiofrequency ablation, is a thermal method. When the tumor is located close to a vital structure, like the portal vein, the risk of damage is a major drawback thus more selective methods should be used. In this context, Irreversible Electroporation (IRE) has emerged as a novel non-thermal ablation method. Electroporation is the phenomenon of cell membrane permeabilization, caused by the application of short and intense electric fields, resulting in cell death in the case of IRE. IRE has proven its effectiveness and safety by eliminating tumors while preserving surrounding tissues, yet cases of relapse among certain patients are still an issue. One potential reason for that is the heterogeneity of the electric field effectively covering the tumor. Additionally, on its own, IRE may not elicit an immune response substantial enough to achieve complete disease remission. Combining IRE with a chemotherapeutic or immunotherapeutic agent could kill remaining cancerous cells. In addition, finding the optimal time frame at which damage associated molecules or proteins are released, could improve the efficacy of immunotherapy. In this regard, we explored IRE-induced cell death, its amplification by chemotherapy (bleomycin) and its immunogenicity. Unlike classical experiments, which rely on 2D in vitro cellular models, our experiments were made on 3D multicellular spheroids, which are morphologically closer to small avascular tumors.

In this work, we have applied pulses of electric fields to spheroids composed of murine hepatoma-derived cells (Hepa 1-6), stably expressing the green fluorescent protein (GFP). Through fluorescent imaging, we have analyzed the growth of spheroids over a period of 4 days after the treatment (80 monopolar pulses of 100 µs duration, applied at a pulse repetition rate of 1000 Hz with various electric field intensities). Immediately after the treatment, the spheroids undergo a quick and transient swelling. We observe a complete permanent loss of GFP fluorescence at 2000 V/cm and above. Remarkably, at 1500 V/cm, the spheroids transiently lose almost all fluorescence, but a subset of cells survives and is capable of proliferating rapidly. In this condition, the addition of bleomycin, an anticancer drug, completely inhibits cellular growth.

To understand the immunogenic potential of IRE-induced cell death, we studied the release of major known Damage Associated Molecular Patterns: ATP and HMGB1. A strong ATP release was observed right after the IRE treatment, while HMGB1 was detectable in the medium after 3 to 6 hours, depending on the level of electric field applied. This disparity of events in time was also observed when we looked at the activation of the caspases 3/7, a hallmark of apoptosis. Indeed, in our experiments, we observed a peak of caspase activation 3 and 6 hours after treatment, at 2500 and 1500 V/cm respectively.

IRE is a complex treatment that requires the careful insertion of multiple needle-shaped electrodes around the tumor. In this context, it can be difficult to reach a sufficient intensity of electric field covering the entire tumor. As our findings revealed, a sub-therapeutic electric field leads to a strong relapse. In our experiments, the addition of bleomycin was able to compensate for the inefficacy of the electric field, killing residual cancer cells. Furthermore, we have observed the release of ATP and HMGB1, two DAMPs capable of stimulating an immune response. These results should be considered in the future for the optimization of the timing of combined therapy involving IRE and immunotherapeutic agents. Further in vivo studies performed by our group will validate these results in more complex models (hetero- and orthotopic tumors).

3B / 10

Persistence of netosis in resected colon cancer patient and impact on circulating DNA applications and post-surgery colon cancer management care

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Role of CD39 on γδT cells as a positive marker of anti-CD20-induced ADCC of non-Hodgkins lymphoma cells

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T lymphocytes play a critical role in anti-tumor immunity. γδ T cells have emerged as a key immune cell type in cancer biology, representing very attractive and promising candidates for cancer immunotherapy[1,2]. They recognize tumor cells independently of the major histocompatibility complex and have the ability to eradicate them. γδ T cells can express the Fc receptor CD16 that enable antibody-dependent cell cytotoxicity (ADCC) and through their particular properties be used in allogeneic condition. This represents a major interest especially in non-Hodgkin's lymphomas (NHL) in which γδ T cells and especially vγ9vδ2 T lymphocytes participate in the anti-cancer response[3]. γδ T cells expressed CD39[4,5], an ectonucleotidase playing an important role in the adenosine pathway[6], that is involved in immuno-escape[7] and antibody-dependent cell phagocytosis mechanisms in NHL[8]. However, the role of CD39 in ADCC induced by an anti-CD20 therapeutic antibody has never been assessed. Here, we endeavored to fully characterize vγ9 T cells during their culture and evaluate the role of CD39 in their anti-tumoral activity in presence of anti-CD20 antibody.

We examined 12 blood samples from healthy donors during the culture after activation by BrHPP in presence of IL2. We determined vγ9 T cell phenotypes and evaluated the expression of CD16 and different immune checkpoints whose CD39. In parallel, we measured their ability to promote ADCC in NHL co-cultures in the presence of an anti-CD20 antibody. All these experiments were carried out with fresh and thawed PBMC, in order to compare their properties for future *in vitro* use.

PBMC were followed and characterized from day 0 to day 30 of culture. We observed that:
-vγ9 T cell lines can be established if their basal percentage in the PMBC was superior to 0.7%
-Highest percentage of vγ9 T lymphocytes was reached after 13 days of culture
-CD16 expression varies between donors (from 0 to 55%)
-TIM-3, BTLA and LAG-3 were highly expressed compared to PD-1, CD39 and TIGIT

Functional experiments allow to demonstrate that ADCC (B cell depletion) of NHL cells co-cultured with vγ9 T cells is enhanced by anti-CD20, and is positively correlated with CD39 expression and LAG-3.

We showed that activation of either thawed or fresh PBMC with BrHPP/Il2 enables the establishment of functional vγ9 T cell lines. Moreover, with the full characterization of ICP expression, it appears clearer how vγ9 T cell lines can be used for *in vitro* preclinical studies dedicated to immunotherapy screening. Finally, we identified CD39 as a positive marker in ADCC of NHL cell lines. Obviously, its role needs to be clarified by using specific inhibitory strategies to open new therapeutic perspectives.

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3B / 12

Role of Exosomes in the Brain Dissemination of Tumor Cells derived from Breast Cancer (Locoregional and Distant)

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Breast cancer is the most common cancer in women, with approximately 2.3 million new cases worldwide each year. Currently, due to extended disease control through therapeutic advancements, an increase in the incidence of brain metastases is observed (5 to 30% of patients depending on the country). The development of brain metastasis is a poor prognostic factor, and at present, the mechanisms explaining the cerebral tropism of breast cancer remain poorly understood.

It is now known that exosomes, small extracellular vesicles secreted in large quantities by cells, including cancer cells, are involved in the establishment and maintenance of the tumor niche. In this context, tumor-derived exosomes (TDEs) are particularly interesting because their content (mRNA, microRNA (miRNA), proteins, and lipids) is specific to the secreting cell. Recently, it has been demonstrated that cancer stem cells (CSCs) are capable of secreting exosomes. These exosomes enter the bloodstream and, due to their content, contribute to the formation of pre-metastatic niches in secondary organs. Migrating cancer stem cells can thus establish themselves in these secondary targets and form metastases. To date, few studies have examined the role of breast CSC-derived exosomes in tumor progression and the formation of brain metastases.

To carry out this project, we will first study the stem cell characteristics of cells obtained from brain metastasis samples of breast cancer patients. These samples are collected at the University Hospital of Poitiers, in the neurosurgery department. Cell lines are then established, and their in vitro characterization is performed. In parallel, the concentration of exosomes in the samples will be determined using the ZetaView by Particle Metrix (Nanoparticle Tracking Analysis), as well as the presence of exosomal and/or stem cell markers using a double fluorescence colocalization module (488/660 nm).

In the second phase, the exosomal content will be described for proteins, RNAs, and lipids. We hope to obtain an exosomal signature (biomarker) in patients that can subsequently be searched for directly in exosomes isolated from blood samples of patients with different types of breast cancer (hormone receptor status, HER2 status).

This potential new biomarker may enable long-term monitoring of patients during chemotherapy treatments and in the remission phase of the disease to address possible recurrence or therapeutic resistance. Finally, understanding the mechanisms involved in the brain dissemination of cancer stem cells from breast cancer will be necessary for the development of effective targeted therapies for patients.

Session 3C – Health Technologies: Flash Posters

3C / 1

Study of cellular communication processes induced by artificial cell-cell interactions for the development of novel cell-based therapy

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Cancer represents a leading cause of death worldwide.Many novel strategies are explored constantly in order to fight this disease. Therefore, scientists are currently investigating more selective therapeutic approaches with the aim to limit side effects caused by existing treatments such as chemotherapy.

Within this framework, the design of innovative cell membranes engineering strategies is of prime interest to develop novel cell-based therapies in the field of cancer. over the past decade, genetic engineering was the most utilized approach for the reshuffle of cell membranes, leading recently to the approval of chimeric antigen receptor (CAR) T cells for the therapy of B cell malignancies. Based on the incorporation of an artificial receptor at the surface of T cells, thereby allowing them to recognize and kill cancer cells, this approach sheds the light on the potential brought by the control of intercellular interactions for the development of new cancer treatments. However, genetic engineering techniques are time consuming and produce variable results often with unpredictable efficiency.

Recently, we explored a different approach based on the use of fully artificial cell surface markers that can be introduced by bioorthogonal chemistry on the membrane of cells, previously functionalized by metabolic glycan labelling. In this case, cell-cell interactions are driven by a pair of complementary molecular recognition partners allowing the selective adhesion of different type of cells through non-covalent click chemistry As proof of principle, we coated tumor cells (A549) and T lymphocytes (Jurkat) with complementary surface markers and we demonstrated that their forced interaction activated the natural killer (NK) cells to kill tumor cells.

In the present study, we aim to visualize the appearance of a possible intercellular exchange resulting from this artificial association of Jurkat T and A549 tumor cells. Thus, we studied the impact of this non-natural binding on the proliferative, invasive and migratory properties of tumor cells.

The results of our investigations in this field will be presented in this communication.

3C / 2

Therapeutic targeting of pro-tumoral Tumor-Associated Macrophage by vectorized anti-folate receptor beta magnetic nanoparticles

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Tumor-associated macrophages (TAM) are well known as protecting tumor cells against apoptosis, immune attacks and therapies. Elimination of these pro-tumoral TAM remains a challenge in cancer therapies. Several ways of TAM targeting exist such as the receptor of CSF-1 or the use of bisphosphonates. However these strategies are not specific to pro-tumoral TAM, potentially leading to serious adverse effects.

We produced and patented a monoclonal antibody called 6-25 capable to specifically recognize the nurse-like cells (NLC), TAM of the chronic lymphocytic leukemia (CLL), and TAM from different solid cancers. We showed that the 6-25 antibody recognizes the folate receptor beta (FRβ) at the surface of these cells and is internalize in these cells without inducing any toxicity. The FRβ is also expressed by the M2 monocytes-derived macrophages (M2M) but not by the M1 monocytes-derived macrophages (M1M) or other myeloid cells.

The goal of the project is to produce a tool that specifically targets and kills pro-tumoral TAM in the tumor in order to sensitize cancer cells to chemo- or immunotherapies.

In cancer treatment, magnetic hyperthermia represents an emerging approach with promising therapeutic potential. The magnetic hyperthermia induces an increase of the temperature of the tumor containing magnetic nanoparticles (MNPs), leading to cell death, in response to a high frequency alternating magnetic field (AMF) application. Iron oxide MNPs are highly biocompatible and non-toxic (rapid degradation and iron cations recycling), which allows their combination with conventional therapies.

Thus, we develop a magnetic nanoparticle based on a PEGylated iron oxide MNP functionalized with the 6-25 mAb (MNP-6-25) as a specific tool to target pro-tumoral TAM expressing the FRβ or IgG control as a negative control thanks to a Michael reaction, and a fluorophore, the Cyanine 5, allowing its detection.

For this study, two cellular models were used: M2M as expressing FRβ at their surface, and M1M as negative control without FRβ at their surface. M2M and M1M were obtained by the culture of monocytes from healthy donors in the presence of appropriate cytokines cocktails.

First, we showed that MNP-6-25 were not toxic toward M1M and M2M up to 64 µg Fe_2O_3/mL after 72h incubation. Then MNP-6-25 binds specifically M2M but not M1M, with a maximum of binding at 48h of incubation at 8 µg/mL Fe_2O_3/mL. Finally, confocal microscopy imaging showed that MNP-6-25 accumulated in the lysosomes of M2M.

Secondly, we performed an alternative model to study the penetration and the specificity of MNP-6-25 in a 3D model. We realized 3D co-cultures with M2M and A549 (lung cancer cell line) using the technic of ultra-low-attachment plate for the formation of spheroids. We showed that co-culture with M2M favored proliferation of the cancer cells.

In the perspective, we plan to evaluate the efficacy and the specificity of MNP-6-25 to target and kill M2M in this 3D model upon application of magnetic field and then the in vivo targeting of macrophages in a murine model of non-small cell lung cancer with MNP-6-25.

3C / 3

Bioactive λ-Carrageenan oligosaccharides coated ferrite nanoparticles as potential anticancer nanodrugs

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Polysaccharides (PS) are widely recognized as valuable coatings or scaffolds for the design of multifunctional nanoparticles (NPs) intended for advanced biomedical applications.[1] In addition to providing high colloidal stability to the NPs and facilitate further functionalisation, they can confer additional advantageous features such as targeting abilities or bioactivities of interest.[2] However, these applications typically focus on specific PS varieties, neglecting numerous other families with untapped promises such as λ-carrageenan (λ-CAR). λ-CAR is a galactan-based PS that displays promising antitumoral effects, but its use in vivo as a bioactive coating for anticancer NPs is strongly restrained due to its high viscosity and adverse proinflammatory and anticoagulant properties.[3] The depolymerization of λ-CAR into oligosaccharides (OS) can overcome these issues, providing new candidates with improved innocuity and biological specificity.[4] Surprisingly, such OS have not yet been included as coatings for any NPs. In this study, we initially prepared a λ-CAR OS candidate through a radical-based depolymerisation method and showed the removal of the adverse properties from its native λ-CAR parent was eliminated while retaining a specific anticancer activity-namely the inhibition of heparanase (HPSE), a key enzyme involved in tumour progression. Subsequently, an innovative microwave-assisted synthesis was optimized for the preparation of Mn-doped iron oxide NPs functionalized with this λ-CAR OS coating. A comprehensive physicochemical description was conducted, including size, electronic microscopy (TEM, SEM), advanced spectroscopy techniques (EDS, XPS, Raman, FTIR), magnetic properties and colloidal stability analysis. Next, we confirmed that the favourable balance of biological properties of the λ-CAR OS is effectively transferred when integrated as an NP coating. Finally, we assessed the antitumor performance of the NPs in cell models and, using a mouse model, the in vivo pharmacokinetic (PK) properties via MRI. Results revealed the coveted combination of a prolonged vascular lifetime and relatively fast hepatobiliary clearance. These findings will be discussed in the context of the current challenges for improving clinical translation of nanotherapeutics.

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3C / 4

Transcriptional regulatory networks unravel cell states from immune cell type deconvolution and uncovers cell niches predictive of cancer progression

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The tumor microenvironment (TME) can influence and modulate the physiopathological process of cancer development. Despite great progress to describe this complex system, we do not know yet why some patients react well to cancer therapies while others do not and undergo recurrence. Current technologies such as single-cell approaches have allowed us to estimate cell type proportions inside the TME. However, these methods have a high cost and are complex to perform. Computational algorithms to perform cell type deconvolution from bulk RNAseq have been developed, but the quantity of features and high variability between them hinders their potential use in patient stratification.

Based on the fact that gene regulation is heavily dependent on the action of transcription factors (TFs) and that transition and maintenance of biological states is usually controlled by these regulators, this project aims to propose a novel framework to characterize immune patient profiles by the construction of transcriptional regulatory networks (TRN) based on inferred protein activity and cell type immune deconvolution using bulk RNAseq data to capture multiple possible phenotypes/states of immune cells in patient samples. We performed this analysis on a NSCLC cohort of 76 Early stage samples from Vanderbilt university, another from IUCT-Oncopole with 80 NSCLC patients at varying stages and three public melanoma datasets totaling 180 patients with immunotherapy response. We applied algorithms to estimate TF activity from gene expression data and construct different subnetworks of highly correlated TFs along with the results of different published deconvolution algorithms and signatures that estimated immune cell proportions based on bulk and single cell data.

Data integration captures molecularly distinct cellular subpopulations sharing similar TF activity across patients. We identified specific TF modules which separate certain cell types. Patients were split using the high and low TF activity identified in these modules. Differential expression and gene set enrichment analysis reveal sets of patients with high infiltration of NK and myeloid cells associated with an immune-active behavior and another group of NK cells, cancer and CAF cells associated with an immune-suppressive behavior in NSLSC samples. Single-cell RNAseq data from the Vanderbilt cohort were used to characterize the two NK cell populations. In parallel, two publicly available melanoma datasets (with clinical response) were used to determine whether TF activity can split responders from nonresponders. Results in these datasets show two sets of patients where responders present a high infiltration in groups of CD4, CD8 and B cells highly associated with JAK.STAT pathway ($r = 0.82$, $pval = 1e\text{-}15$) and TFs enriched in interferon and interleukin signaling, while non responders have a high infiltration in NKT, NK resting, cancer and neutrophils cells highly associated with TGFb ($r = 0.43$, $pval = 6e\text{-}4$). These immune cell groups along with their TFs in responders are being used to train machine learning models and potentially predict immunotherapy response in other melanoma datasets.

3C / 5

Bioprinting early-stage pancreatic cancer models: a new tool to decipher tumor initiation mechanisms

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Pancreatic cancer remains one of the most aggressive malignancies with late diagnosis, limited therapeutic options and low survival rates [1], emphasizing the critical need for early detection and improved understanding of its initiation mechanisms. In this regard, the application of 3D bioprinting technology presents a compelling approach for developing physiologically relevant cancer models. This innovative technique allows for the replication of various microenvironments and niches during the early stages of pancreatic cancer development, offering valuable insights into its progression.

In this study, we present the development and characterization of a novel bioprinting methodology capable of replicating distinct matrix stiffness gradients that correspond to various stages of pancreatic cancer [2]. Our approach combines inkjet bioprinting, an extracellular matrix-derived bioink, and primary pancreatic cells extracted from wild-type and genetically modified mice to create highly realistic 3D bioprinted pancreatic models.

Rheological assessment showed our ability to finely modulate the properties of the bioinks, enabling us to accurately replicate the matrix stiffness observed in vivo. Image analysis showcase the successful replication of the bioprinted model while maintaining cell viability. Additionally, we show that the model facilitates large-scale image analysis, highlighting its utility in capturing phenotypic changes with high statistical power. Moving forward, our research aims to delve deeper into the dynamic crosstalk between cancer cells and their microenvironment within the 3D model, utilizing advanced techniques such as secretome analysis and multi-omics approaches.

By closely mimicking the in vivo tumor microenvironment, this model offers a valuable platform for investigating the underlying mechanisms involved in cancer initiation and therefore, pancreatic cell tumor phenotype acquisition.

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Gut-in-caps: exploring the biophysical cues that drive self-organization of Caco2 cells into patho-physiological models of the intestinal epithelium

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Over the last decade, the development of *in vitro*intestinal models has open new possibilities for preclinical drug testing and disease modeling. Using Caco2 colorectal cancer cells, microphysiological systems evolved from flat epithelial barriers to complex gut-on-chips[1]. These biomimetic models replicate the invaginated crypt-villi organization of intestinal epithelium, which maintain stem cells in niches and differentiated cells close to the lumen. Advances in 3D culture of patient-derived cells also led to self-organization of primary intestinal organoids. The latter has enabled to uncover mechanisms of crypt-villus morphogenesis[2] and paved the way for drug screening and personalized medicine. But when it comes to modeling tumors, self-organization of mutated intestinal cells often results in disorganized 3D tumoroids with less known morphogenetic processes[3,4]. Yet, Caco2 colorectal cancer cells have been extensively used to design gut-on-chips reliably mimicking the intestinal crypt-villi architecture. Using a new method to culture Caco2 cells in 3D, we propose a new organoid-like model to investigate the (patho)physiological self-organization of intestinal carcinoma cells *in vitro*.

Using the Cellular Capsule Technology (CCT)[5], we harness microfluidic-based biofabrication to culture Caco2 cells in a 3D alginate shell. Briefly, a microfluidic chip is used to coextrude an alginate solution around cells suspended in a Basement Membrane Extract (Cultrex®). The external solution of alginate gels in a calcium bath, forming an elastic nutrient-permeable shell around the cellular environment. These capsules can then be handled for imaging or sorting for further characterization of the cells they contain. Over a week of culture inside capsules, Caco2 self-organize into either hollow enteroid-like cysts or crowded tumor-like spheroids. We explore the parameters that can trigger one organization or the other, such as the initial number of cells, the matrix, or the mechanical confinement. In parallel, we aim to characterize the cellular phenotypes associated with these 3D organizations, using markers of cancer stemness or intestinal differentiation in RTqPCR and immunofluorescence imaging. Finally, we monitor the aggregate growth rates with medium throughput live-imaging[6], and we investigate their respective mechanisms of growth.

By correlating the 3D cellular self-organizations with the cell phenotypes, we aim to decipher some key-features of *in vitro* intestinal morphogenesis in healthy or tumoral states. In the future, such *in vitro* models could help better understand the biophysical cues that favor intestinal epithelium homeostasis or pathological developments such as in colorectal cancers.

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From gene regulatory network inference to dynamical modelling: revealing cell behaviour through network modelling in Chronic Lymphocytic Leukaemia

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Gene regulatory networks (GRNs) and mathematical modeling are critical for understanding the complex regulatory mechanisms that underlie cancer development and progression, providing insights into the molecular mechanisms that drive cancer, including the identification of key driver genes/pathways, and novel therapeutic targets. In the context of the tumor microenvironment (TME), the complex interactions between immune and cancer cells give rise to a cascade of regulatory processes in different levels, defining the cellular behavior and response to external stimuli. It is shown that, in the presence of cancer cells, several immune cells including macrophages, neutrophils or T cells undergo cell-state transitions toward pro-tumoral phenotypes or exhaustion. While the cell reprogramming and state transition of immune cells is well studied, the detailed molecular description of cancer cell behavior and transition of cell states in response to the interactions with immune cells remains incomplete.

In this project, we aim to investigate how regulatory interactions between genes characterize cancer cell behavior. We use an in vitro culture of Peripheral Blood Mononuclear Cells (PBMC) of Chronic Lymphocytic Leukaemia (CLL, a blood disease characterized by progressive proliferation and accumulation of malignant B lymphocytes) patients as a simplified model of TME to study the functional processes determining the CLL cell interactions and behavior in the presence of macrophages and other immune cells. To do so, we perform in vitro experiments in three conditions: (1) monoculture of CLL cells, (2) co-culture of monocyte-CLL cells, and (3) culture of Peripheral Blood Mononuclear Cell (PBMC) of CLL patients, and obtained bulk RNAseq time courses of CLL cells over 14 days.

We then perform gene regulatory network (GRN) inference for each experimental condition, following a data-driven approach, and highlighting important structural and functional differences between the GRNs in the three conditions. To build the gene regulatory networks of CLL cells, we use dynGENIE3 inference method, which employs non-linear modeling and random forest algorithm to infer putative gene regulations. We concentrate on the interaction network of transcription factors (TFs) to reduce the complexity of the predicted network due to the large number of genes. Next, a general GRN of CLL cells is generated, merging the GRNs from each condition, and validating the inferred network using the CollecTRI database for TF-target gene interactions.

The inferred GRN is then used as an input for Boolean model inference using the CaSQ library, which builds on the network structure to infer the functional relationships between each pair of interacting genes, in the form of discrete logical functions. The output consists of an executable dynamical model, which is further used to perform numerical simulations to study the system's asymptotical behavior in various conditions. Simulating the CLL cell Boolean model, we focus on detecting specific CLL cell states (apoptotic, proliferating, or metabolic shifts), and highlight how the presence of immune cells (particularly, tumor associated macrophages) in the TME affects the likelihood for CLL cells to be in one of these states. Furthermore, performing in silico simulations of KO/OE of intracellular molecules, we identify putative TFs to target, in order to disrupt the re-education of immune cells (particularly macrophages) carried out by CLL cells, and shift their states toward apoptotic ones.

With this data-driven approach, we identify novel transcription factors involved in establishing cellular crosstalk between CLL and immune cells, thus better understanding their behavior and response to drugs.

Session 4 – Chemistry & Cancer

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From encoded combinatorial libraries to clinical-stage targeted therapeutics

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More than 100 years ago, Paul Ehrlich envisaged the possibility of achieving a selective therapeutic intervention by means of "magic bullets" (Zauberkugeln): molecules capable of selective localization at the site of disease, helping spare normal tissues from undesired toxicity.

The dream of Paul Ehrlich has become closer to reality thanks to the conjugation of bioactive payloads (drugs, radionuclides, cytokines) to suitable protein ligands (antibody fragments or small organic molecules), capable of high-affinity binding to accessible protein targets, selectively expressed at the site of disease. The discovery of human antibodies and of small organic ligands has been greatly facilitated by advances in encoded combinatorial library technology [1].

In this lecture, I will show how encoded combinatorial libraries can be used to isolate high-quality antibodies and small organic ligands for in vivo tumor targeting applications [2]. I will also show how these building blocks can be used for the creation of novel biopharmaceuticals, which are exhibiting promising results in advanced clinical trials in patients with cancer.

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GRP94 chaperone identified as a new target of adenosine-cytosine bisubstrate analogues in cancer cells using affinity based chemical probes

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In the context of enzyme inhibition several approaches consist in the **development of bisubstrate analogues** combining within the same molecule both substrates or substrate and co-factor of the targeted enzyme. In our quest to identify DNA methyltransferase (DNMT) inhibitors a series of adenosine-cytosine-based bisubstrate-type derivatives was synthesised and tested against DNMTs.[1] Unfortunately, these compounds did not show any activity against DNA and RNA methyltransferase put some potency against arginine methyltransferases.

In order to **understand the mode of action of these adenosine-cytosine-based bisubstrate-type derivatives**, we decided to applied chemical biology experiments using well-designed chemical tools. We first synthetised **affinity-based chemical probes of cytosine-adenosine analogues**. These probes consist in: 1) a bisubstrate analogue to recognise the target protein, 2) a photo-crosslinking moiety to trap the protein of interest, through covalent binding and 3) an alkyne to enable functionalisation by a biotin-fluorophore-azide trifunctional agent to visualise and purified the target protein. We then use the affinity-based protein profiling (ABPP) strategy in different cancer cell lines and proteomic analysis enable us to identify several proteins of interest. Interestingly only one protein, **the chaperone glucose-regulated protein 94 (GRP94), was significantly over-represented** with our probe *vs* control in all cell lines. The validation of the proteomic analysis showed our chemical probe as a selective inhibitor of GRP94, a target of interest for several types of cancers [2]. Moreover, the identified new scaffold behaves as a GRP94-selective inhibitor over other HSP90 proteins.

This project is a **multi-disciplinary project** involving chemical probe design and synthesis, in cell fishing, proteomic analysis and enzyme inhibition assay for validation experiments. Therefore, as a result of the use of adenosine-substituted cytosine analogues to synthetise affinity probes and their application in a chemical-biology methodology, this work opens the way to the development of **a new family of GRP94 inhibitors that could be of therapeutic interest** [3].

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Thermal and mechanical stresses generated by magnetic nanoparticles upon magnetic field exposure induce immunogenic cell death in pancreatic adenocarcinoma

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Pancreatic adenocarcinoma (PDAC) is a cancer with a very poor prognosis since the 5-year survival rate is less than 10%, with more than 220,000 deaths each year worldwide; it is predicted to be the second leading cause of cancer death within a few years. PDAC is 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). CAFs promote proliferation and progression of the tumor, secrete extracellular matrix proteins forming a physical barrier that limits not only the penetration and the diffusion of therapeutic drug but also the infiltration of immune cells and so an efficient anti-tumoral immune response. Consequently, immunotherapy turned out to be disappointing against PDAC and PDAC was classified as a "cold" tumor or a "immune desert" cancer.

Thanks to their physico-chemical properties, iron oxide magnetic nanoparticles (IONPs) are innovative tool notably as theranostic agent for cancer diagnosis and therapy. IONPs exposed to high frequency magnetic field (AMF) can heat and induce thermal related damages, leading to cell death and tumor regression. A clinical trial was conducted in 2011 to treat high-grade brain tumors and one is currently realized on prostate cancer, combining magnetic hyperthermia with radiotherapy. On the other hand, IONPs exposed to low frequency rotating magnetic field (RMF) can generate mechanical forces that induce mechanical related damages, leading to cell death.

The aim of the study was to investigate whether local thermal (magnetic hyperthermia) or mechanical forces released by IONPs upon AMF or RMF application, respectively, can stimulate immunogenic cell death and improve anti-tumoral response, in the PDAC model. We developed IONPs that specifically target pancreatic cancer cells and CAF expressing the CCK2 (MiaPaca2-CCK2 and CAF-CCK2) receptor to optimize their accumulation in the lysosomes of these cells. We showed that these IONPs vectorized with gastrin, called NF@Gastrin, bind, internalize and accumulate in the lysosomes of MiaPaca2-CCK2 and CAF-CCK2 cells. We demonstrated that AMF or RMF application kills specifically cancer cells and CAFs having internalized NF@Gastrin and slows down their proliferation without affecting cells lacking the nanoparticles, on 2D culture and 3D MiaPaca2-CCK2/CAF-CCK2 spheroid models. Then, we demonstrated that magnetic hyperthermia as well as mechanical forces generated by NF@Gastrin upon AMF or RMF exposure increase the expression of calreticulin and HSP70 proteins, known as Damage-Associated Molecular Pattern (DAMP), at the surface of MiaPaca2 cancer cells and CAF. This effect is associated with a raise of phagocytosis of these cells by human THP1 macrophages. Altogether, these results strongly suggest that magnetic hyperthermia or mechanical forces generated by IONPs upon AMF or RMF application are two potential new strategies capable of inducing immunogenic cell death and restoring anti-tumor response within the PDAC.

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Effects of metalation on the PDT activity of arene ruthenium porphyrin-based photosensitizers on prostate cancer

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In 2020, prostate cancer (PCa) was the second most common cancer in men worldwide with more than 1.4 million cases (Source Globocan 2020). Despite existing treatments as chemotherapy, radiotherapy or hormonotherapy, cancer relapse and many side effects are observed in patients (G. Fallara et al., Int. J. Impot. Res. 2021). Improving the patient's quality of life while preserving surrounding healthy tissues remains a priority. For these reasons, photodynamic therapy (PDT) could play an important role, thanks to its minimally invasive nature and high treatment precision, a reduction in side effects has been observed during clinical trials (A.R. Azzouzi et al., Lancet Oncol. 2017). PDT relies on the use of chemical compounds called photosensitizers (PS) which, only in the presence of light and molecular oxygen, result in the production of reactive oxygen species (ROS) that are toxic to cancer cells.

Many 2nd and 3rd generation of PS used in PDT exhibit low solubility in biological media and, therefore, functionalization or vectorization is necessary to ensure internalization. On the other hand, metal-based anticancer drugs have been used to treat various cancers. So, we decided in collaboration with the University of Neuchâtel to develop 3rd generation of PS vectorized by arene-ruthenium complexes (M. Gallardo-Villagrán et al., Dalton Trans. 2022).

The designed metalla-assemblies can host PSs inside their cavity or be constructed with PS building blocks. First, we wanted to demonstrate the efficiency of our arene-ruthenium compounds and their effects on PCa cells. Then, we wanted to determine if the addition of metals (Mg, Co, Zn) in the center of these PSs plays a role.

For all the in vitro experiments, human PCa cells (PC-3 and DU145) were treated with PS (IC50) and irradiated or not with a 630-660 nm CURElight lamp at 75 J/cm². Phototoxicity was determined using MTT assay 24h after irradiation. ROS production was monitored by flow cytometry right after the irradiation with the cell permeant reagent 2',7'-dichlorofluorescein diacetate (DCFDA). To determine compounds localizations, confocal microscopy using LysoTracker and Mitotracker was performed. For apoptosis detection, Western blotting using apoptosis-related proteins and DNA fragmentation evaluation were performed 24h post-PDT.

Among all the compounds synthesized, we have selected a series of ten new complexes. By all of them, a set of three arene-ruthenium complexes coordinated to tetrapyridylporphin (tpp-2H), Zn-tetrapyridylporphin (tpp-Zn) and Co-tetrapyridylporphin (tpp-Co) were selected to highlight the influence of metalation on the activity of the PS. PDT experiences allowed us to validate the efficacy (IC50 in the nanomolar range) and safety (no effect in the dark) of these complexes. Indeed, it is very important to note that the addition of the metal reduces the effect (Zn) or even annihilates it (Co). Flow cytometry analyses indicated that exposure of cells to our three compounds enhanced intracellular ROS levels only after photoactivation. Confocal microscopy showed a good internalization of these PS inside cells, enabling their intracellular localization into the cytoplasm. Concerning apoptosis signaling pathways, we showed an activation of the caspase-3 correlated with a cleavage of the poly-ADP ribose polymerase (PARP) and a strong induction of DNA fragmentation following irradiation, thus inducting the apoptotic process.

Our results confirm that arene-ruthenium tetrapyridylporphin complexes are excellent candidates for PDT application to vectorized our PS with cytotoxicity to light and safety to obscurity (no effect in the dark). It seems that compounds accumulated into the cytoplasm induced apoptotic process following irradiation. It is also important to note that the addition of the metal shows a decrease (tpp-Zn) or even annihilates PDT effects (tpp-Co).

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Deciphering and exploiting the mechanism of action of a large family of natural and bioinspired cytotoxic lipids

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As a large consortium between biologists from IPBS and chemists form the LCC and the SPCMIB, we combined cutting edge technologies to decipher the mechanism of action of a large family of natural and bioinspired cytotoxic lipids. Using a genetic screen in haploid human cells, we established that these lipids behave as prodrugs selectively bioactivated by HDS17B11, an enzyme of the Short-chain Dehydrogenase/Reductase (SDR) superfamily, into highly-protein reactive electrophiles. Once generated in cells, these electrophiles covalently modify several proteins involved in protein-quality control mechanisms, resulting in their lipoxidation on cysteines and lysines.

For some proteins, we established that this triggers their association to cellular membranes and results in an acute endoplasmic reticulum stress, unfolded protein response activation, ubiquitin-proteasome system inhibition and cell death by apoptosis. Importantly, there are 71 SDRs in human, including some that are overexpressed in cancer and/or responsible for cancer relapse. Therefore, as a proof-of-concept, we designed from this mechanism novel compounds that are specifically bioactivated by three other human SDRs, including one that promotes prostate cancer resistance to androgen deprivation therapy, in order to selectively kill cancer cells that express these enzymes. We propose that SDR-bioactivated prodrugs constitute a valuable reservoir of promising anticancer agents that could be used as targeted therapies.

Session 5A – Cell Signaling, Microenvironment and Targeting

5A / 1
ER+ Breast Cancer Metastasis Cell Fate

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Breast cancers (BCas) are deadly due to their capacity to adapt and thus relapse or resist therapy. BCa metastasize primarily to bone (clinically manageable), but patients ultimately succumb to multi-organ metastases. Recent genomic analyses suggest that most deadly metastases are seeded from secondary bone lesions (metastasis-to-metastasis) rather than from primary tumors. Dynamic adaptation to changing environments during the metastatic process requires more rapid change than supported by purely genetic mechanism. We hypothesize that BCa cell plasticity, imparted by transcriptional and epigenetic adaptive mechanisms, facilitates primary bone metastasis, secondary metastasis to metastasis, and drug resistance, thus resulting in poor patient survival.

Bone metastasis is driven by the interaction between cancer and stroma cells, mainly osteoblasts and osteoclasts, which collectively generate what is defined as an osteolytic vicious cycle. As a consequence of bone resorption, bone matrix growth factors become available and further stimulate metastasis growth. This led to the development of bone modifying agents to manage skeletal related events (i.e. bisphosphonates that inhibit osteoclast activity). MAF gene amplification (16q23), occurring in 20% of BCa, has been clinically validated in two phase III trials to be associated with higher likelihood to develop metastasis. Further, MAF amplification as a biomarker explained for the first time the association between using bone-modifying agents in the adjuvant setting and improved patient outcome, but also the striking harmful effects of bisphosphonates in MAF-amplified particularly in young patients with high systemic E2. These observations suggest that ER signaling may also co-opt metastatic responses to drive dissemination. Given that MAF-amplification is associated with relapse and poor overall survival we hypothesize that uncharacterized epigenetic mechanisms underlie the interplay between ER predisposing signaling and metastasis pathogenesis. We show that metastatic drivers' modification of signal-dependent transcription factors (TFs) may provide cancer cell-type-specific enhancer landscapes, with important implications for understanding the pathology as well as treatment outcome.

5A / 2

Inflammation-induced epithelial plasticity can be by-passed through Vps34 inactivation to limit pancreatic cancer initiation

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Introduction: Vps34 is the sole class III PI3K and is the most conserved PI3K in eukaryotes. This enzyme allows the phosphorylation of phosphatidylinositol (PI) into phosphatidylinositol-3-monophosphate (PI-3-P) and plays a crucial role in autophagy and vesicular trafficking. The basal autophagy is necessary to maintain acinar pancreatic cells homeostasis. Indeed, defects of autophagy are frequent in inflammatory diseases such as chronic pancreatitis which constitute a risk factor for pancreatic adenocarcinoma (PDAC).

Methods: We generated a murine model of inducible Vps34 inactivation specifically in pancreatic acinar cells (ElasCreER/Vps34flx/flx). Phenotypic, histopathologic and single cell RNA sequencing analysis have been realized at different times after Vps34 inactivation. A murine model with a Kras mutation combined with a Vps34 inactivation in all pancreatic cells (KC/Vps34flx/flx) have been also generated to determine the role of this enzyme in the development of PDAC.

Results: Vps34 inactivation (ElasCreER/Vps34flx/flx) induces a heterogeneous increase of lipids and markers of autophagy in the pancreas compared to control mice. The single cell RNA sequencing highlighted that Vps34 inhibition triggers the loss of an acinar subpopulation with a high mitochondrial activity for the benefit of 3 subpopulations expressing Reg proteins, linked with pancreas regeneration. These cells have an altered expression of genes related to canonical autophagy and express embryonic markers. Vps34 inactivation is also responsible for an extended endoplasmic reticulum and a modification of mitochondrial dynamics in favor of fission. The total inactivation of Vps34 in the pancreas protects from the initiation of pancreatic precancerous lesions in an inflammatory context (KC/Vps34flx/flx).

Conclusion: Vps34 is a key protein for pancreas homeostasis which allow to maintain physiological acinar populations. Its inactivation creates a model of steatopancreatitis which is closed to human pancreatic diseases that constitute a risk factor for PDAC. The Vps34 inactivation protects from PDAC, despite the presence of Kras mutation.

5A / 3

Tumor-derived cytokine, Upd3, promotes gut atrophy in a Drosophila larvae model of cachexia

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Cachexia, is an acute involuntary weight loss (>5%), associated with different chronic illnesses, and several cancers (pancreas, colon…). It is a global metabolic syndrome triggering adipose tissue and skeletal muscles atrophy, accounting for 30% of cancer patients' deaths. We have developed Drosophila larvae models of cancer-associated cachexia based on localised Notch-driven wing disc overgrowth. In these models, Notch activation leads to hyperplastic overgrowth without muscle or adipose tissue wasting, while combining Notch activation with epithelial polarity impairment results in neoplastic growth and peripheral tissue wasting. Since these two tumour types are similar in size, these results suggest that neoplastic tumours produce specific pro-cachectic factors. Beside the adipose tissue and muscles, we observed that cachectic tumours also promote the atrophy of the larval gut. This is reflected in smaller width of the gut, and a change in its cellular composition. In particular atrophied guts have fewer stem cells, the adult midgut precursors or AMPs, which are normally put aside for metamorphosis, suggesting an untimely usage and depletion. Screening for the factors secreted by the cachectic tumours identified a role for the Jak/Stat pathway ligand Upd3 in mediating the atrophy of the gut and gut stem cell depletion. Preliminary results in mouse models suggest that gut atrophy is also observed in mammals during cachexia and that gut dysfunction might represent an important feature of cachexia.

5A / 4

Immunosuppressive Myeloid Cells foster Cancer Stem Cell (CSC) emergence through TGF-β

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Cells of myeloid origin represent major components of the complex immunosuppressive tumor microenvironment. These myeloid cells such as tumor-associated macrophages (TAM), neutrophils and so-called "myeloid-derived suppressor cells" (**MDSC**) among others have been widely described for their immunosuppressive properties and their ability to inhibit anti-tumor immune responses. They thus represent major obstacles for efficient immunotherapeutic approaches. However, beyond this cardinal immunosuppressive function, MDSC are also endowed with a broad array of "non-immunological" tumor-promoting functions1. Indeed, accumulating evidences has been provided that these cells can directly promote primary tumor cell survival and proliferation and promote local tissue invasion among others. Importantly, MDSC play a key role in the preparation of the pre-metastatic niches before the arrival of cancer cells, thus contributing to the preparation of the "soil" for seeding by metastatic tumor cells. Evidence has also emerged that tumor-induced immunosuppressive myeloid cells may impact cancer stem cells (**CSC**), a subpopulation of cancer cells within the tumor, defined by self-renewal, asymmetrical division and differentiation properties, giving rise to more or less differentiated cells composing the tumor mass.

Using 3-D tumorsphere formation assays we demonstrate that human monocyte-derived suppressor cells (HuMoSC, a surrogate in vitro model for MDSC) are endowed with the capability to promote stemness features in **breast** and **NSCLC** cancer cells in a cell-to-cell contact dependent manner. Moreover, our data also demonstrate that different subpopulation of MDSC isolated from breast cancer bearing mice, as well as immunosuppressive myeloid cells isolated for breast cancer patients promote acquisition of stemness properties by cancer cells.

To understand the mechanisms by which these HuMoSC promote stemness of cancer cells, we combined the use of transcriptomic (single-cell RNA sequencing) and proteomic (surface proteomic analysis (surfaceome)) technologies to explore potential candidates involved in the physical interactions between the two cell types. We found that the induction of the stemness phenotype was partly mediated through **TGF-β**.

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Cancer cells transfer invasive properties through tracks

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Invasion and migration through the extracellular matrix are prerequisites for metastasis, the leading cause of cancer-related deaths. During tumor development, the extracellular matrix is remodeled, including by overexpressing type I collagen that facilitate cancer dissemination. Most studies have focused on events at the leading edge as cells invade. We describe a new event at the trailing edge, as cells detach from the matrix.

We show that small vesicles containing the collagen receptor DDR1 are left behind on collagen fibrils in the migration path. We named these structures attached to the collagen fibers "collagen-tracks". The vesicles are similar in size to exosomes but lack the exosome markers, and they also are different to migrasomes. We show that collagen-track formation is stimulated by DDR1 and by factors that promote adhesion, including collagen cross-linking. We report the protein, mRNA and miRNA content of collagen-tracks. They contain adhesion proteins, suggesting that they form when membrane fragments containing adhesions are torn from the cell as it migrates along collagen fibrils. We show that collagen-tracks are deposited by breast cancer cells in 3D matrices in vitro and in vivo. Collagen-tracks are stable and can be taken up by surrounding cells, promoting epithelial to mesenchymal transition, matrix degradation and invasion, finally leading to increased lung metastasis of breast cancer cells.

In summary, we have identified and characterized a new vesicle entity directly attached to collagen fibrils that plays a role in cell-cell communication and can transfer invasive properties to surrounding cells. We conclude that cancer-related collagen-tracks are a new player acting locally to drive tumor invasion and metastasis.

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In silico-based strategy for the discovery of direct small molecule inhibitors of mutant RAS

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Session 5B – Genome Dynamics & Expression

5B / 1

A p53-dependent mechanism for the detection of mitotic genome instability

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Chromosome instability, aneuploidy, the removal of centrosomes, or anti-mitotic drugs targeting the microtubule cytoskeleton delay progression through mitosis, triggering a p53 and p21 dependent cell cycle arrest in G1. This response is lost in cancer cells in which p53 has become inactivated by mutation or other mechanisms, including expression of viral oncoproteins. Crucially, G1 cell cycle arrest following prolonged mitosis occurs even in the absence of detectable DNA damage suggesting it has a different cause. In this seminar I will describe how p53 is used to detect stochastic variation in the length of mitosis and triggers cell cycle arrest in G1 under conditions where mitosis is prolonged due to errors in chromosome alignment.

5B / 2
Mechanisms controlling maintenance of cohesin dependent loops

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Cohesin is a ring-shaped complex known to organize chromatin into loops. These cohesin-dependent loops are important for regulating biological processes such as transcription, DNA repair and VDJ recombination. The mechanisms regulating the establishment of cohesin-dependent loops are under active investigation. Cohesin is thought to be established via a process known as "loop extrusion" (LE). During this process, cohesin captures a small loop of chromatin which is then expanded by translocation of cohesin along the chromatin. Although the mechanisms of loop establishment are not fully understood, there is evidence that Wapl inhibits loop enlargement. It is possible that Wapl causes the ring to open, causing the extruded loop cohesin to fall off. If this is the case, artificial closure of the interface opened by Wapl should counteract Wapl's inhibitory effect on loop enlargement. Therefore, we aim to identify which cohesin interface(s) is opened by Wapl during loop expansion. To this end, we intend to artificially close each of the cohesin interfaces using a thiol-specific chemical crosslinking and, using this strategy, identify the interface(s) opened by Wapl during loop expansion.

5B / 3

G-quadruplexes involvement in mammalian genome organization and stability

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G-quadruplexes (G4s) are single-stranded DNA structures which are mainly formed through Hoogsteen base paring of guanine stretches. The basic shape looks like a stem-loop that would fold against itself but different anti-parallel or mixed strand organizations also exists. Although well characterized in vitro, their in vivo detection has been challenging for a long time. More recently, various methods have been developed in order to meet this need and among these stands the in-house developed G4access that relies on fine titration of the Micrococcal nuclease When combined with sub-nucleosomal fraction isolation and bioinformatic filtering, it allows high-resolution mapping of G4s in an unbiased manner, as opposed to peptide-based orthogonal approaches (Esnault et al., 2023). As a long list of epigenetic datasets exists in mouse ES cells, we therefore performed G4access in order to correlate these structures with their surrounding chromatin landscape. Seminal analysis showed a significant co-occurrence between G4s, CTCF and different cohesin sub-units. Even more interestingly, some G4 sites showed high cohesin sub-units accumulation in the absence of CTCF, pointing out to a potentially new insulation or cohesin-loading activity, very similar to the loop extrusion model where the looped out chromatin loop by the cohesin is blocked or stalled by convergently oriented CTCF sites. These cohesin accumulated sites were later found out to be a subset of enhancers containing G4s and having higher intrinsic transcriptional activity. Enhancers are essential for gene expression regulation and are known to be involved in long range contacts with their target promoter(s). Strikingly and more recently some studies have pointed out to the role of G4s in chromatin loops formation (Hou et al. 2019, Yuan et al, 2023). Another parallel observation is that G4s are also known to be hotspots for DNA breaks and mutations (Bossaert et al., 2021), as like CTCF-blocked chromatin loop anchors.

In our work, we aim to decipher this link between G4s, chromatin shaping factors (including candidate G4 binders), DNA break-points and enhancers using datasets from human and murine cell lines. On top of these preliminary bioinformatic analyses, the results of G4access experiments in the CTCF-degron cell line (degradation and restoring of CTCF) aiming to shed light on the interplay between CTCF and G4s will also be presented. As perspectives, we will also detail upcoming strategies for functional in vivo testing, involving knock-in of insulation candidates, enhancers or sub-part of enhancers (like CpG islands) with varying G4 content.

5B / 4

IgH 3'RR recombination uncovers a non-germinal center imprint and c-MYC-dependent IgH rearrangement in unmutated chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL) is an incurable indolent non-Hodgkin lymphoma characterized by tumor B-cells that weakly express a B-cell receptor (BCR). The mutational status of the variable region (IGHV) within the immunoglobulin heavy-chain (IGH) locus is an important prognosis indicator and raises the question of the CLL cell of origin (COO). Mutated IGHV gene CLLs (mCLLs) are genetically imprinted by activation induced-cytidine deaminase (AID). AID is also required for IGH rearrangements: class switch recombination (CSR) and recombination between switch Mu (Sμ) and the 3' regulatory region (3'RR) (Sμ-3'RRrec). The great majority of CLL B-cells being unswitched led us to examine IGH rearrangement blockade in CLL. Our results separated CLLs into two groups on the basis of Sμ-3'RRrec counts per sample: Sμ-3'RRrecHigh cases (mostly umCLLs) and Sμ-3'RRrecLow cases (mostly mCLLs), but not based on CSR junction counts. Sμ-3'RRrec appeared to be ongoing in Sμ-3'RRrecHigh CLL cells and comparison of Sμ-3'RRrec junction structural features pointed to different B-cell origins for both groups. In accordance with IGHV mutational status and PIM1 mutation rate, Sμ-3'RRrecHigh CLLs harbor a non-GC experienced B-cell imprint while Sμ-3'RRrecLow CLLs are from AID-experienced B-cells from a secondary lymphoid organ. In addition to the proposals already made concerning the CLL cell of origin, our study highlights that analysis of IGH recombinatory activity can identify CLL cases from different origins. Finally, on-going Sμ-3'RRrec in Sμ-3'RRrecHigh cells appeared to presumably be the consequence of high c-MYC expression, as c-MYC overexpression potentiated IGH rearrangements and Sμ-3'RRrec, even in the absence of AID for the latter.

5B / 5

Exploiting the dark matter of single-cell transcriptomes to encompass suppressive myeloid cell differentiation in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is an inflammation-associated cancer caused by factors such as viral infections, alcohol abuse or obesity. Despite a significant therapeutic advance in the treatment of HCC, ~75% of patients do not respond to immunotherapies for unclear reasons. To gain a better understanding of this resistance, it is important to consider the expansion of suppressive myeloid cells, a hallmark of chronic inflammation and cancer. However, their heterogeneity and their differentiation process are not fully resolved, potentially underlying immunotherapy resistance. Depicting a detailed range of transcriptomic programs, single cell technologies enable the improvement of our knowledge of cell state granularity. The main objectives of this study are to 1) to refine monocyte state annotation and 2) to reach a deep overview of the regulatory programs driving immunosuppression in HCC by exploring protein-coding transcripts and long non-coding RNAs (lncRNAs) together. Indeed, lncRNAs are a class of non-coding RNAs known as key regulators of functions and cell fate having a strong tissue- and cell-specific expression. Thus, using them conjointly with the mRNAs carries the promise of reaching a thorough and clearcut cell-type and cell-state annotation. Using almost 100 samples from seven single-cell datasets from bone-marrow, blood and liver (healthy and tumoral) mimicking the myelopoiesis lineage, we established important and novel computational approaches to build a robust cell atlas. Unlike most embeddings, we elaborated strategies to integrate protein-coding transcripts together with lncRNAs, to refine myeloid cell annotations. Our results show that the use of both variable mRNAs and lncRNAs helps to redraw cell state boundaries corresponding to specific functional programs. We now aim to establish a list of lncRNAs showing differential expression along the differentiation process, in particular towards suppressive monocytes. The key findings will encompass a curated list of potential lncRNA candidates involved in the acquisition of suppressive functions, paving the way for exploration of their therapeutic potential in RNA-based interventions.

Session 6 – Environmental Stress

6 / 1
Exposome Analytics: Definitions, Challenges and Applications

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The Exposome concept has been developed as a necessary complement to the genome to better understand the determinants of health and of the risk of chronic diseases. The external exposome combines a large range of external stressors (i.e. non-genetic) factors potentially impacting human health from conception onwards. These external exposures (i) are heterogeneous in nature, scale, and variability, (ii) feature complex correlation patterns and (iii) may operate as mixtures. The internal exposome can be defined as the way these exposures are embodied and its exploration relies on the screening and integration of high-resolution molecular data. While methods for omics data analyses are established, their application in an exposome context is raising specific methodological challenges including the analysis of complex and correlated exposures. Furthermore, the isolated exploration of an omic profile offers the possibility to capture stressor-induced biological/biochemical alterations, potentially impacting individual risk profiles, but this may only yield a fractional picture of the complex molecular events involved, therefore limiting our understanding of the effective mechanisms mediating the effect of the exposome.

Taking examples from real-world exposome projects we will illustrate the use of statistical and machine learning techniques to accommodate co-occurring exposures contributing to population stratification, explore the links between these and health outcomes, and investigate the (multi)-omic response to these sets of exposures.

Session 7 – Prestige Conference

7 / 1
Is the patient an extraterrestrial ?

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Session 8A – Immunotherapy

8A / 1

Understanding the impact of the tumor microenvironment on immunotherapies

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8A / 2

The multifaceted tumor-promoting functions of myeloid cells

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8A / 3

T cell Immunotherapy: thinking beyond inhibitory receptors

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Although immune checkpoint blockade (ICB) such as anti-PD-1 has represented a turning point in cancer care, clinical responses are not observed in the majority of cancer patients. The mechanisms underlying this lack of responsiveness are still poorly understood and finding additional signals that regulate $CD8^+$ T cell anti-tumor functions has become a major priority. While most studies focus on inhibitory receptors, signals transmitted through activating receptors also critically impact $CD8^+$ T cell cancer immune surveillance and ICB efficacy. Indeed, we recently discovered that the loss of the activating receptor CD226 restrains CD8+ T cell functions and the therapeutic efficacy of cancer immunotherapy (*Weulersse et al, Immunity. 2020, Braun et al, Immunity. 2020*). More recently, we focused on CD137 (4-1BB) activating receptor, an enigmatic yet, promising target for immunotherapy. Through epigenetic, single cell transcriptomic and functional assays, using CD137 conditional knockout mouse models and agonist antibodies, our study revealed the implication of CD137 (4-1BB) signaling in T cell exhaustion program (*Pichler et al, immunity. 2023*). Understanding the cellular process that drive T cell dysfunction has crucial implications for the treatment of cancer and infectious diseases. Thus, our study, that uncovers the importance of CD226 and CD137 pathways in T cell dysfunction, could have broad applications for immunotherapy.

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Repression of exosome secretion by mutated ß-catenin contributes to immune escape in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the most common primary liver cancer; it represents the 6th most frequent tumor in the world and the 4th leading cause of cancer-related death. Due to the lack of efficient therapies and the difficulty to detect the pathology during early stages, HCC has a poor prognosis. Today, the immunotherapy represents the first-line treatment for advanced HCC with beneficial effect on overall survival. 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, the role of ß-catenin promoting this immune escape and leading to tumor cells trigger immunosuppressive cascades is not yet fully understood. Our project focuses on the role of ß-catenin signaling in tumor/immune cells communication through exosomes.

Using transcriptomic analysis, we showed an alteration of gene expression involved in exosome biogenesis machinery in HepG2 cells upon knock-down of mutated ß-catenin. We also revealed by total internal reflection fluorescence microscopy (TIRF), a decrease of exosome secretion when β-catenin is mutated. We further identified two target genes of the exosomal machinery, SDC4 and RAB27A, whose expression is ß-catenin-dependent. Thus, these results suggest that ß-catenin mutations inhibit exosome formation and/or secretion in liver tumor cells. As exosomes and their contents are main factors for intercellular communications, we hypothesized that the decrease of exosome production could lead to a defective recruitment of leukocytes, making these tumors poor in immune infiltrates and resistant to immunotherapy. To validate our hypothesis we generated spheroids of liver cancer cells and co-cultured with human peripheral blood mononuclear cells (PBMCs) and analyzed immune cells infiltration modulation by flow cytometry. We revealed an immune escape in β-catenin mutated HCC cells and confirmed the involvement of exosomes in this processus.

Our results provide a new knowledge on the impact of ?-catenin mutations on exosome biogenesis. This may allow the development of a new tool from liquid biopsies to stratify HCC patients for immunotherapy response.

Session 8B – Patients Fragiles ou Patients Vulnérables (1)

8B / 1

Inégalités sociales en cancérologie. Approches quantitatives pour éclairer la politique publique

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Depuis plusieurs dizaines d'années, les déterminants sociaux individuels et collectifs sont reconnus comme étant des déterminants majeurs de la santé et la réduction des inégalités sociales de santé est à l'agenda de nombreuses institutions nationales et internationales. Selon l'OMS, cette politique doit s'appuyer sur trois piliers : i) améliorer les conditions de vie quotidiennes, ii) lutter contre les inégalités dans la répartition du pouvoir, de l'argent et des ressources, iii) mesurer le problème, l'analyser et évaluer l'efficacité de l'action. Ainsi le développement des outils, des méthodes et des pratiques permettant l'évaluation quantitatives des inégalités sociales et territoriales en santé et des actions visant à les réduire est un enjeu majeur.

Cette évaluation doit reposer sur des indicateurs fiables, reproductibles, précis autant que possible, et disponible autant que possible

En cancérologie, nous disposons, en France comme à l'étranger, d'indicateur d'incidence, de prise en charge, de survie, et de mortalité plus que pour d'autres pathologies, en particulier grâce aux réseau nationaux et internationaux de registres de cancer.

La nature plurifactorielle de la fragilité sociale et la nécessaire prise en compte des déterminaux distaux ou contextuels rend la question de l'indicateur plus complexe. Au niveau individuel, il n'existe pas d'indice plurifactoriel validé de défavorisation sociale. De plus, la disponibilité des éléments composant la triade essentielle (revenus, éducation, profession) est très limitée et biaise la plupart des études conduites sur des grands échantillons.

C'est pourquoi nombre d'auteurs ont désormais recours à des indices agrégés qui permettent une mesure non biaisée et multidimensionnel de l'environnement social et dont l'utilisation à une échelle suffisamment petite limite le biais écologique. Ces indices doivent être représentatifs, sensibles, valides, évolutif et transparent et permettre des comparaisons entre territoires. En France , l'European Deprivation Index est l'indice le plus validé.

Les études réalisées sur les données représentatives du réseau FRANCIM des registres de cancer ont permis de commencer à démonter les mécanismes de formation des inégalités sociales de mortalité par cancer en étudiant de manière distincte les inégalités sociales d'incidence et les inégalités sociales de survie des personnes atteintes de cancer. Ces études ont montré que, concernant l'incidence, la déprivation sociale était souvent associée à un sur-risque de cancer, notamment pour certains cancers digestifs (foie et estomac) et les cancers liés à la consommation de tabac (poumon, tête et cou) mais qu'elle était aussi parfois liée à une diminution du risque de cancer, notamment pour les mélanomes, les cancers de l'ovaire et les cancers de la prostate. Concernant la survie, en revanche, le pronostic le plus sombre était toujours l'apanage des populations les plus défavorisées. Le gradient social de survie était particulièrement marqué pour les cancers du foie chez l'homme et pour les cancers des voies biliaires chez la femme. Ainsi pour nombre de cancers, les inégalités sociales de mortalité sont plus dues au gradient social de survie qu'au différences d'incidence. Pour des localisations cancéreuses fréquentes comme le sein, le colon rectum ou la prostate, la mortalité plus fréquente dans les populations défavorisées est entièrement due au gradient social de survie puisque le gradient social d'incidence joue dans un sens opposé. Ainsi alors que le discours classique qui accompagne les disparités sociales de santé porte usuellement sur les comportements et les habitudes de vie, invoquant ainsi la responsabilité du malade sans questionner l'équité de notre système de prise en charge, ces résultats, qui font référence, mettent en contraire en avant la responsabilité première de l'accès au dépistage et aux soins et de la détermination sociale et territoriale de la filière et de la qualité des soins.

8B / 2
Accès à l'oncofertilité des femmes atteintes de cancer du sein : de l'observation à l'intervention

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Cette présentation décrira des travaux originaux dont l'objectif était d'analyser le rôle des soignants dans la construction des inégalités sociales face au cancer. Nous nous sommes intéressés à l'oncofertilité. Chez les femmes jeunes atteintes de cancer, une chimiothérapie est très souvent mise en place et peut entrainer une aménorrhée justifiant la mise en place de techniques de conservation. Or nous ne disposions pas de données permettant de savoir si cette offre est bien mise en place pour les femmes en situation d'en bénéficier.
Nous avons ainsi mené une étude dont l'objectif était d'analyser les caractéristiques des femmes (âge, lieu de résidence, défavorisation, lieu de traitement) ayant bénéficié ou non d'une consultation spécialisée sur la fertilité ou/et d'une préservation de la fertilité, à partir d'une étude rétrospective incluant des femmes de moins de 40 ans ayant eu une chimiothérapie pour cancer du sein depuis 2012. Les cas ont été identifiés à partir des RCP du dossier communicant de cancérologie régional, et un croisement a été réalisé avec les dossiers des deux consultations spécialisées d'oncofertilité d'ex Midi-Pyrénées. Les résultats montrent que la fertilité n'a pas été abordée chez 62% des femmes, qu'une consultation d'oncofertilité a été réalisée pour 29% d'entre elles et la préservation effectuée pour 11%. Le fait d'aborder la fertilité est significativement influencé par l'âge, la parité, le fait d'avoir un cancer d'emblée métastatique, le type de structure de la prise en charge et par le fait que l'oncologue soit un homme ou une femme. Ce travail a fait l'objet de deux publications (Martinet-Kosinski F et al. Front Public Health. 2023; Martinet-Kosinski F et al. Sci Rep. 2023).

Au regard de ces résultats, un projet visant à augmenter l'accès à la préservation de la fertilité des femmes en âge de procréer exposées à des traitements gonadotoxiques a été développé, le projet EVAPREF. Il vient d'être financé par l'APP RISP de l'INCa. Ce projet vise à développer une démarche d'amélioration et d'évaluation des outils d'information et de coordination existants développés par les réseaux régionaux de cancérologie des Pays-de-la-Loire et Occitanie. Dans un premier temps, une méthode qualitative itérative centrée sur l'utilisateur et participative est proposée afin d'améliorer les dispositifs actuels et créer des contenus de formation pour les médecins qui utiliseront ces dispositifs. Cette étape en actuellement en cours de réalisation. Dans un second temps, nous mènerons un essai randomisé en paliers (« randomized stepped wedge trial ») auprès de femmes de moins de 40 ans et nouvellement traitées par chimiothérapie pour un cancer du sein, évaluant l'accès à la consultation de préservation de la fertilité avant et après la mise en place des outils améliorés, sur une période de 30 mois allant de mi-2023 à fin 2025. Enfin, dans un troisième temps, nous mènerons une analyse d'implémentation de l'intervention pour fournir des éléments clés pour sa transférabilité à d'autres contextes et en particulier à d'autres réseaux régionaux de cancérologie en France. La présentation sera l'occasion de décrire la méthodologie de ce projet et donner des éléments sur sa mise en place.

8B / 3

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Session 9A – Alternative Models to Animal Testing

9A / 1

Development of a physiological microsystem, from a blood-brain barrier-on-chip to a vascularized glioblastoma-on-chip

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The blood-brain barrier (BBB) limits the transport of drugs and nanocarriers, and hampers the development of innovative therapeutic solutions against neurological disorders. The fight against most common and aggressive brain tumor, the glioblastoma multiforme (GBM), faces too many therapeutic failures, with a survival after diagnosis of 12 to 18 months only. The preclinical screening of innovative drug candidates relies on conventional 2D in vitro models which may be too simplistic, and animal experimentation with ethical issues and interspecies differences. Poor translational results in clinical assays are unfortunately the norm. Tissue engineering and physiological microsystems, as organ-on-chips, promise alternative models that mimic a complex microenvironment in healthy or pathological contexts: the 3D organization of different human cell types, the extracellular matrix, and the chemical gradients and mechanical constraints of the blood flow.

Firstly, a self-organized 3D model of BBB micro-vasculature was optimized in a fibrin and collagen type I hydrogel. Human brain microvascular endothelial cells arranged into capillaries, supported by pericytes and astrocytes, and expressed specific tight-junction proteins membrane transporters and carriers (1-3). A second phase of the project has started, with the design of microchip prototypes as a scaffold for the hydrogel enabling nutritive medium to be flown into a central venule. Assays with GBM cells (U87-MG) are carried out, aiming at understanding the impact of cell-cell communication (including through microvesicles) on the stability of the extracellular matrix and on the BBB. This project will question the possible EPR (enhanced permeability and retention) effect in the glioblastoma. The study will focus on the detection of the transport of, first standardized molecules and nanoparticles to test the BBB within the GBM context, then nanocarriers and drug candidates. Their efficiency and specificity will be assessed by imaging and biomolecular techniques, but also via the instrumentation of the GBM-on-chip with biosensors.

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2. M. Matsusaki, A. Figarol, - Cerebrovascular model and device (2020).
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9A / 2

Mechanical interplays between mitotic spindle orientation of colonic epithelial cells and extracellular matrix deformations

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In the human body, the colonic epithelium creates a physical barrier between the external world and our inner body. From a mechanical point of view, the epithelium is subject to various external constraints, for instance shear stress due to the progression of the alimentary bolus, peristalsis or extracellular matrix rigidity. In addition, the epithelium itself exerts mechanical stresses onto its environment. More specifically, it applies radial and/or orthoradial tensile loadings onto the matrix.

Studying the mechanical interactions between the epithelium and its matrix environment can help understanding their impact during various biological processes, such as tissue regeneration or tumorogenesis. Indeed, the mechanical and geometrical properties of the matrix, notably its rigidity and topology, can have an impact on the behaviour and the fate of the epithelial cells. It has been shown, for example, that matrix rigidity impacts the proliferation of intestinal stem cells. Moreover, under the context of chronic inflammation for instance, epigenetic modifications of stem cells/progenitors in crypts can lead their hyperproliferation, i.e abnormal amount of mitosis, resulting in the appearance of bifid crypts established as an initiating stage of colon cancer. However, the mechanical impact of this hyperproliferation onto the extracellular matrix remains to be explored.

In fact, understanding the interplay between biology and mechanics involved in tissue architecture is challenging, especially regarding the 3D organization of tissue. It requires both an appropriate biological model, enabling multi-scale imaging observations, and well-chosen experimental approaches allowing accessing the morphological alteration undergone by the tissue concomitantly to the alteration of the 3D supporting matrix.

Currently, in vivo, no one has been able to study the dynamic mechanical interactions between the epithelium and its matrix due to the lack of suitable methods and technologies. 3D Human colonic organoids seeded in Matrigel® represent a suitable model for this purpose. In fact, this experimental model features rapid growth from isolated stem cells to the formation of 3D mature polarized structures. In addition, this culture set-up allows to study the extracellular matrix such as its displacements and strains during the various biological processes taking place within the organoid.

The aim of this research is therefore to study the dynamic mechanical relationships between the colonic epithelium and its extracellular matrix, particularly during cell division, to try to provide answers on their role in the development of bifid crypts, and therefore the putative initiation of colon cancer.

For this purpose, organoids were stained with Hoechst and tubulin biotracker to visualize nuclei and mitotic spindles respectively and extract biological information such as nuclear volumes or mitotic spindle orientations. In order to extract mechanical information about matrix displacements, we implemented fluorescent silica beads into the Matrigel®. We then performed a timelapse confocal microscopy approach to follow the evolution of organoids morphology and cell mitosis as well as matrix movements. The spatial displacements of the matrix are studied by Digital Image Correlation, with the specialized software VicVolume®.

Here, we present the different methods that have been implemented to establish correlations between biological information (mitotic orientation) and the associated matrix deformations.

9A / 3

Sensitizing the PDAC tumor microenvironment to immune checkpoint therapies: characterization of a PDAC 3D model to decipher immune infiltration

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal solid tumors, with an extremely unfavorable prognosis. The dense stroma rich in Cancer-Associated Fibroblasts (CAFs) and the immunosuppressive microenvironment confer resistance to current treatments including combination chemotherapies, targeted therapies or immunotherapies. Moreover, PDAC tumors are poorly infiltrated by T cells, and the majority of immune cells present at the tumor site are immunosuppressive. This cellular context leads to the failure of clinical trials using immune checkpoint inhibitors in PDAC. The immune infiltrate within the tumor is dynamic and depends in particular on soluble factors secreted in the tumor microenvironment (cytokines, chemokines etc.).

The objective of this project is to develop a combinatorial approach using a monoclonal antibody that targets the tumor microenvironment (anti-AXL) combined with interleukin-15 (IL15) associated with conventional chemotherapies (Gemcitabine or Folfirinox) in order to increase immune infiltration. In clinical applications IL15 activates T lymphocytes (LT) and Natural Killer (NK) cells and promotes their infiltration into tumors. However, IL15 treatment causes significant side effects with high toxicity reported in patients. Thus, we have generated an anti-AXL antibody fused to IL15 to associate the immunomodulatory properties of IL15, combined with the target specificity of the anti-AXL antibody. We thus developed a PDAC three-dimensional in vitro spheroid model composed of xenograft-derived tumor cells from PDAC patients, CAFs (primary and immortalized), and peripheral blood mononuclear cells (PBMCs) isolated from healthy donors, with the aim of closely reproduce the complex pathophysiological features of the cancer-stroma found in pancreatic TME. Human 3D models were first set up and characterized by cytometry, imaging mass spectrometry and immunohistochemistry.

We showed that in our heterospheroid models, CAFs promote tumor cell growth, improve resistance to chemotherapy and are able to down-regulate immune cell infiltration and modulate the nature of infiltrated immune cells. These CAF-dependent resistance mechanisms, immune suppression and cancer progression also described in patients, are one of the trademarks of pancreatic cancer. In parallel we showed that, upon anti-AXL-IL15 treatment, PBMCs infiltrate cell line-derived heterospheroids, whatever the tumor cell line, kill tumor cells and disrupt the three-dimensional structure. Moreover, immunophenotyping experiments showed a modification of the nature of immune infiltration, with a strong increase of CD4+ and CD8+ T lymphocytes and NK cell populations infiltration. We also obtained combinatorial effects that positively modulated immune infiltration and allowed a control of spheroid growth by combining chemotherapy (gemcitabine) with our anti-AXL-IL15. Thus, the heterotypic spheroids described in our study are a suitable model to both characterize the influence of CAF on therapeutic effects and the mechanisms that drives immune suppressive microenvironment. Next step will be to perform in vivo tests using an orthotopic model to verify whether this approach enhances effector immune cells infiltration in vivo, thereby sensitizing the PDAC microenvironment to anti-PD1 therapies.

9A / 4

Combination of immune checkpoint inhibitors with chemotherapies in new biological models of upper tract urothelial carcinomas

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Upper Tract Urothelial Carcinomas (UTUCs) are aggressive tumor of ureter and renal pelvis, representing 5 to 10% of urothelial cancer. Standard care treatment consists in a platinum-based chemotherapy, combining cisplatin or carboplatin with gemcitabine, but relapse is observed in more than 50% of the patients. Despite UTUCs are mostly classified as "cold" or "infiltrated by non-effective immune cells" tumor, previous work [1, 2, 3] showed that classical UTUC chemotherapies are immunomodulatory. This suggest that the combination with immune checkpoint inhibitor (ICI) may be a good option to improve patient standard care. To this extend, many different clinical trials tried to introduce ICI in combination in urothelial carcinoma (mostly bladder primary tumor), but they failed to show a real benefice on patient survival. Interestingly, in the JAVELIN III phase III clinical trial [4], avelumab as maintenance therapy after chemotherapy treatment succeed to increased significatively patient survival, whereas Imvigor130 [5] or DANUBE [6] clinical trials, respectively testing atezolizumab or durvalumab combined concomitantly with chemotherapy failed to show a benefice. Even if it is impossible to compare those different clinical trials, testing different immunotherapies in different cohort of patients, we decide to study the effect of different therapeutic sequences of the combination of chemotherapy and ICI, and we are currently trying to identify specific biological marker of response. However, in vivo models of this cancer do not exist for now to be able to study immunotherapies.

We developed an in vitro 3D heterotypic model, consisting in a coculture of immune cells from heatlhy donors or from bladder cancer patients, and tumoral cells from bladder or upper tract origin. We analyzed responses using imaging cytometry (Celigo), immunophenotyping by FACS, transcriptome analyses by RNA-seq and RT-qPCR, and proteomic analyses by western-blot.

We showed that avelumab was the more effective ICI in our model (compared to durvalumab, atezolizumab, pembrolizumab and nivolumab) and we found that the sequential administration of chemotherapy and ICI is more effective that the concomitant's one for all ICI tested. With immunophenotyping, we showed that our model has a strong B-cell infiltration. Finally, our preliminary results with patient's immune cells allowed us to identify differences in responses that can be correlated with the clinical response of the patient.
Conclusion: We showed that the 3D heterotypic spheroid model is able to reproduce same tendency as seen in the clinic and allow us to test different sequences of treatment and ICI. The long-term goal is to propose a simple, fast and biologically relevant in vitro way to test immunotherapies with the patient PBMCs, to guide the clinician choice.

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9A / 5

Anti-tumor efficacy of new high-frequency electrical protocols in a 3D in vitro colorectal cancer model

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For more than 20 years, electroporation has been developing in the field of cancer and is becoming increasingly common in the treatment of skin cancers. By increasing the cellular uptake of hydrophilic anti-tumor drugs (bleomycin, cisplatin), electrochemotherapy increases their cytotoxic effects and has already proven its efficiency on tumors in human medicine but also in veterinary practice. However, this treatment requires loco-regional or even general anesthesia, as electrical pulses can be painful and cause muscle contractions. Several publications have demonstrated that application of high frequency pulses (above 5000 Hz) causes much less discomfort to the patient than the 1 Hz protocol used in the clinical practice in the 90's and 2000's despite a slight temperature increase. The use of bipolar pulses can also reduce muscle contractions but the amplitude of the field and the number of pulses need to be increased to reach the same efficacy.

In order to reduce the pain associated with contractions but also to maintain the effectiveness of the treatment, we have developed new protocols using a high-frequency generator associated with a new set of multipolar electrodes. Our ongoing results obtained on a colorectal cancer cells line (HCT-116) cultured both 2D and 3D (spheroids) showed a clear effect on cell death with different molecules (cisplatin, bleomycin, calcium and carboplatin). Using our new protocols on cell suspension, the viability rate were around 10 % at 48h after treatment and less than 5 % at 10 days after treatment. Results on 3D spheroid models show a significant decrease of spheroid size, which allow to conclude on growth impairment after one single treatment. These results were similar to the ESOPE (European standard operating procedures for electrochemotherapy) protocol currently used in clinic. In addition, we observed a weak temperature increase (similar to ESOPE protocol) but without noticeable muscular contraction (tested on human volunteers). Therefore, our painless-high-frequency electroporation protocols appear very promising for the widespreading of electrochemotherapy as an effective cancer treatment.

9A / 6

Xenopus laevis is an attractive model for studying the pigmentary abnormalities in xeroderma pigmentosum type C

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Xeroderma pigmentosum type C (XP-C) is a rare autosomal recessive disorder, characterized by an extreme sensitivity to ultraviolet B rays (UVB), leading to photoaging, macules, and skin cancer. UVB can affect genomic DNA by creating cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4PPs). These lesions are mainly repaired by the nucleotide excision repair (NER) system, however, photolyase enzymes activated by blue light are also known to perform this function in Xenopus. Our main goal was to validate the use of Xenopus laevis as an in vivo model for investigating the impact of UVB, the main cause of XP-C clinical features, on skin physiology. The mRNA expression levels of xpc and six other NER genes and CPD/6-4PP photolyases were found at all stages of embryonic development and in all adult tissues tested. When examining embryos at different time points after UVB irradiation, we observed a gradual decrease in CPD levels, increased number of apoptotic cells, epidermal thickening, and increased dendricity of melanocytes. Quick removal of CPDs when embryos are exposed to blue light versus in the dark has also been witnessed, confirming the efficient activation of photolyases. A decrease in the number of apoptotic cells and an accelerated return to normal proliferation rate was noted in blue light-exposed embryos compared with their control counterparts. This mimics the human skin responses to UVB and support Xenopus as an appropriate and alternative model for such studies. Additionally, XPC deficient Xenopus showed interesting observations related to the clinical features of XP-C.

Session 9B – Patients Fragiles ou Patients Vulnérables (2)

9B / 1
Cancers des personnes avec déficience intellectuelle

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Les personnes avec déficience intellectuelle (PDI) ont un déficit cognitif (QI <70) associé à des troubles adaptatifs apparus à la naissance et jusqu'à l'âge de 18 ans, quelle qu'en soit la cause (définition OMS). Les cancers de ces personnes sont mal connus, malgré leur fréquence équivalente à celle dans la population générale (PG). Ces cancers ont une répartition différente selon les organes en comparaison de la PG, ils sont souvent découverts à un âge plus précoce, à un stade avancé. Leur traitement est difficile. Devant l'absence d'équipes de référence en France et à l'international, un projet d'équipe dédiée a été réfléchi en 2002 qui a pris la forme, en 2012, de l'association ONCODEFI basée à Montpellier.

L'association a pour but de 1) Rassembler les données, les synthétiser pour les rendre accessibles aux: médecins, chercheurs, aidants professionnels, familles, sans oublier les PDI elles-mêmes. 2) Mener des recherches lorsqu'une thématique le nécessite pour les soins. 3) Mener des actions de terrain pour expliquer la prévention et le dépistage des cancers; et accompagner les équipes oncologiques, les institutions médico-sociales et les familles.

L'association a été créée grâce au soutien des Fondations Perce-Neige et Jérôme Lejeune et de l'UNAPEI. Elle a reçu le soutien de l'INCa, l'ARS Occitanie, le Cancéropôle GSO, la CNSA, la CPAM de l'Hérault, et de nombreuses fondations: AG2R La Mondiale, Obélisque, de l'Avenir, Paul Bennetot, Malakoff Humanis Handicap, AFER, Ligue contre le Cancer Comité de l'Hérault.
Les recherches ont visé à:
- Préciser la répartition spéciale des cancers (étude CHAID), sur le profil tumoral de certaines pathologies (trisomie 21, X-fragile, autisme, polyhandicap).
- Mettre en évidence les retards diagnostiques (études COLODI, GYNDI, MELADI).
- Monter les défauts de participation au dépistage organisé des cancers (étude INDEP).
- Déterminer la prévalence cancers au troisième âge (étude ADICAN).
- Documenter les difficultés de soins du cancer chez les PDI (étude OSO).
- Estimer la connaissance et le ressenti des éducateurs sur le cancer pour proposer une formation (étude ACERCA).
- Evaluer les capacités d'apprentissage en santé des PDI (études PAM et CHESMON)
- Améliorer l'accès aux examens de mammographie (étude MANISAF).

Ces recherches nous ont amenés à:
- Proposer des modèles de surveillance pour la trisomie 21 et le polyhandicap.
- Créer des livrets expliquant le trajet de soins et le dépistage aux PDI (traduits en allemand) et un carnet de liaison entre les équipes oncologiques, les institutions et les familles.
Ces recherches ont été conduites en collaboration avec des équipes en France, au Royaume uni, en Suède, en Suisse, au Japon.
Les actions de terrain (dispositif ISCaO) soutenues par l'ARS-Occitanie et portées par deux infirmiers se développent sur 13 départements et informent les éducateurs et les PDI elles-mêmes sur le risque de cancer, sa prévention, son dépistage. Ils accompagnent les équipes des institutions où un résident est touché par un cancer pour les aider à la prise en soins.
Les actions d'Oncodéfi ont été récompensées par le prix «Cancer» de l'Académie Nationale de Médecine.
Valorisation Les recherches et l'expérience acquise sur le terrain ont amené à:
- Organiser 3 congrès, 2 internationaux en 2014 et 2018 (11 et 13 pays des 4 continents) et 1 national en 2022.
- Publier un livre en 2011, quatre chapitres de livres, 38 articles, organiser 7 conférences filmées.

Les cancers des personnes déficientes intellectuelles sont particuliers, difficiles à traiter, et nécessitent une prise en soins adaptée. Oncodéfi contribue depuis 12 ans à approfondir les connaissances, rassembler données, les diffuser et faciliter la prise en soins, à la fois pour les équipes oncologiques et les institutions médico-sociales.

9B / 2
Etude CENTRUM: Impact de la centralisation de la prise en charge des cancers du rectum

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La résection du mésorectum est actuellement la référence en matière de traitement du cancer rectal invasif (CR) localisé. La qualité de cette chirurgie et la réduction du risque de rechute semblent corrélées au niveau d'activité chirurgicale du centre. En France, un seuil de 30 interventions annuelles pour cancer digestif a été fixé pour que les centres soient autorisés à traiter chirurgicalement le CR. La centralisation des soins de CR pourrait bénéficier aux patients mais aurait probablement un coût qui ne serait pas le même pour tous. L'objectif de ce travail est d'étudier l'impact de la centralisation des traitements du CR sur la survie (bénéfice) et la distance de déplacement des patients (coûts) en France métropolitaine et la répartition de ces bénéfices et coûts.

Nous avons étudié un échantillon de cas de CR invasifs non métastatiques diagnostiqués de 2010 à 2015, ayant subi une chirurgie curative, identifiés par les registres de cancer du réseau Francim et la base de données nationale PMSI (Programme de Systèmes d'Information Médicale) pour récupérer le nombre total de chirurgies pour cancer digestif et parmi eux le nombre de CR.

Le lieu de résidence des patients et les centres de traitement ont été géolocalisés pour construire une matrice de distance origine-destination à l'aide du logiciel QGIS.

3 221 patients (âge médian : 69 ans, sex-ratio 1,7) ont été inclus. Un tiers d'entre eux ont consulté le centre de traitement le plus proche, étaient plus âgés que ceux ayant consulté un centre plus éloigné ($p < 0,001$) mais ne différaient pas en termes de désavantage social. Cependant, les patients les plus défavorisés (EDI 4 et 5) ainsi que les plus âgés consultaient plus fréquemment les centres réalisant moins d'interventions sur CR ($p<0,001$).

Nos premiers résultats suggèrent qu'une concentration des soins vers les centres les plus actifs sera plus restrictive pour les patients les plus âgés et les plus défavorisés, même si rien ne prédit le sens de la relation en termes de survie. La prochaine étape de l'étude Centrum se concentrera sur ces analyses.

Posters – Axis 1 “Signaling, Microenvironment and Targeting”

P101

The Hippo pathway controls Cytolethal Distending Toxin-induced nuclear remodeling, DNA damage and increased polyploidy in intestinal epithelial cells

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We are frequently exposed to infection with genotoxin-producing bacteria, such as the cytolethal distending toxin (CDT), a prevalent heterotrimeric toxin among Gram-negative bacteria. CdtB subunit causes severe DNA damage in host cells and impairs DNA-damage response, leading to genomic instability and accumulation of mutations. The Hippo pathway plays a critical role in the protection of genome stability in response to DNA damage. In the present study, we investigated the effect of CDT on the Hippo signaling pathway.

In vitro experiments were performed on human epithelial intestinal cell lines. Microarray data and western-blot analyses showed a CdtB-dependent regulation of the transcripts and proteins of the core of the Hippo pathway, such as MST1/2 and LATS1/2 kinases, and their transcriptional coactivators, YAP1 and TAZ. Infection of epithelial cells with CDT-producing bacteria is associated with an increased transcriptional activity of the TEAD transcription factor, the final nuclear effector of the Hippo pathway. This effect was attributed to CdtB subunit following its ectopic expression in epithelial cells. Verteporfin, an inhibitor of the YAP1/TAZ-TEAD interaction, reduced the CdtB-induced effects, i.e. increased TEAD transcriptional activity, increased nuclear remodeling, as well as increased polyploidy, DNA damage and repair, confirming the involvement of Hippo signaling pathway in CdtB effects. In addition, colibactin, a genotoxic secondary metabolite produced by Escherichia coli, induced similar effects in cell culture. Overall, these data show that infection with genotoxin-producing bacteria modulates the Hippo signaling pathway.

P102

Identification of a translation machinery in all types of invadosomes thanks to the universal marker Tks5

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Invasion of the extracellular matrix is needed for physiological events such as wound healing but it is also required at different stages of the metastatic process. The ability to progress and invade through the extracellular matrix is a characteristic shared by both normal and cancer cells through the formation of structures called invadosomes. These invadosomes are dynamic structures that can adopt different organizations such as rosettes, dots or can be reorganized along the type I collagen into linear invadosomes.

In this study, we used the universal marker of invadosomes Tks5 to identify common features in the different organization. We used Tks5 co-immunoprecipitation coupled with mass spectrometry analysis that allowed us to identify a translation machinery in all types of organization and in particular the protein Eif4b. We showed that Eif4b colocalize with Tks5 in dots, rosettes and linear invadosomes. Thanks to functional tests, we showed for the first time that the translation is involved in the degradation function of all types of invadosomes.

Finally, we also identified the presence of endoplasmic reticulum at the level of rosette that colocalize with Tks5.

In summary, we provided a molecular characterization of the different types of invadosomes, in normal and cancerous cells, thanks to the common marker Tks5. Moreover, we demonstrated for the first time the presence of a translation machinery in linear invadosomes essential to their degradation function. We also identified the colocalization of endoplasmic reticulum with Tks5 in rosette. We identified for the first time a translation machinery in all types of invadosomes thanks to the Tks5 marker. The combination of molecular and structural analysis of the different invadosomes organization allowed us to better understand the composition of these invasive structures.

P103

Tyrosine kinase TAM receptors (TYRO3, AXL, MERTK) and their ligands, PROS1 and GAS6, in the primary tumors and brain metastases from colorectal cancer

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Brain metastases (BM) from colorectal cancer (CRC) are rare, with an incidence of about 3 % of the CRC, but associated with chemoresistance and short overall survival. Moreover, BM is a frequent exclusion criterion in clinical trials, not allowing these patients to benefit from therapeutic advances. The cascade of events inducing the dissemination of cancer cells from the primary tumor to a secondary site is influenced by various factors. PROS1 is the ligand, with the GAS6 protein, of the tyrosine kinase TAM receptors family (TYRO3/AXL/MERTK). To study the involvement of this signaling pathway in these events, we have used two brain metastasis stem cell lines (BM-SC-CRC1/2) and tissue microarrays of primary and secondary tumors from a cohort of colorectal cancer patients with brain metastases. We characterized the expression of the members of the TAM receptors family and their ligands in these tissues and cell lines compared with well-known CRC cell models (HT29, SW480, SW620).

The expression of the TAM receptors and their ligands by the cell lines was assessed at the RNA level by RT-qPCR and at the protein level by immunofluorescence, Western blotting (receptors), and ELISA (ligands in the cell-conditioned medium). It was determined by immunohistochemistry in tissue microarrays, patients' biopsies and resections, and brain metastases in mice injected by BM-SC-CRC1/2 cells. We also analyzed the effect of the PROS1 ligand on cell survival by MTS assay and on cell proliferation by BrdU assay.

Consistently between RNA and protein expression, the three TAM receptors are expressed by the analyzed cell lines but HT29 cells, with high variability between lines. TYRO3 is expressed in all cell lines except HT29. AXL is barely produced by the studied cells, except in SW480 and SW620, with a massive expression by the SW480 cells from a primary CRC tumor. MERTK is predominantly expressed by the two BM-SC-CRC cell lines. PROS1 is secreted by all the studied cell lines and enhances the survival and proliferation in all of them. GAS6 is expressed by all of them but the HT29 cells. The TAM receptors expression profile by BM-SC-CRC1/2 cells *in vivo* in mouse brain is similar to their expression profile *in vitro*. Moreover, this expression profile is also observed in tumor biopsies and resections of the BM-SC-CRC1/2-issued patients over their clinical history. AXL expression is condensed in vascular structures. In that line, it is highly expressed in the lung metastasis cells of the BM-SC-CRC1-issued patient compared to the BM-SC-CRC1 cells and the other tumoral tissues of this patient. TAM receptors do not appear as prognostic factors in the cohort of patients. TYRO3 and AXL are expressed in half of the samples whereas MERTK is expressed in a majority of the samples.

These results indicate that SW480, SW620, and our BM-SC-CRC1/2 cell lines issued from brain metastases of patients express the tyrosine kinase TAM receptors and their two ligands. The expression of TAM receptors and its ligand PROS1 by brain metastasis cells suggest that this system plays an autocrine/paracrine role for these cells. *In vitro*, PROS1 appears as an important cue in the proliferation of colorectal cancer cells. Although non-prognostic, TAM receptors are frequently expressed in colorectal cancer patients with brain metastases.

P104

Tumor-derived cytokine, Upd3, promotes gut atrophy in a Drosophila larvae model of cachexia

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Cachexia, is an acute involuntary weight loss (>5%), associated with different chronic illnesses, and several cancers (pancreas, colon…). It is a global metabolic syndrome triggering adipose tissue and skeletal muscles atrophy, accounting for 30% of cancer patients' deaths. We have developed Drosophila larvae models of cancer-associated cachexia based on localised Notch-driven wing disc overgrowth. In these models, Notch activation leads to hyperplastic overgrowth without muscle or adipose tissue wasting, while combining Notch activation with epithelial polarity impairment results in neoplastic growth and peripheral tissue wasting. Since these two tumour types are similar in size, these results suggest that neoplastic tumours produce specific pro-cachectic factors. Beside the adipose tissue and muscles, we observed that cachectic tumours also promote the atrophy of the larval gut. This is reflected in smaller width of the gut, and a change in its cellular composition. In particular atrophied guts have fewer stem cells, the adult midgut precursors or AMPs, which are normally put aside for metamorphosis, suggesting an untimely usage and depletion. Screening for the factors secreted by the cachectic tumours identified a role for the Jak/Stat pathway ligand Upd3 in mediating the atrophy of the gut and gut stem cell depletion. Preliminary results in mouse models suggest that gut atrophy is also observed in mammals during cachexia and that gut dysfunction might represent an important feature of cachexia.

P105

Impact of chemokines on tumor microenvironment remodeling in β-catenin mutated hepatocellular carcinoma

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The emergence of immunotherapy for the treatment of advanced hepatocellular carcinoma (HCC) has substantially improved overall patient survival. However, accumulating evidence has demonstrated that β-catenin mutated HCC are devoid of immune infiltrates and thus resistant to immunotherapy. The immune escape process characterizing such tumors was first confirmed by the assessment of immune cells infiltration in our 3D *in vitro* model of liver tumor cell spheroids. This localized immunosuppression mediated by mutated β-catenin could be the result of a deregulated production of chemokines, which are key player molecules involved in cancer cells-immune cells communication. In a liver cancer cell model, the depletion of the mutated form of β-catenin induced an increased CCL20 and CXCL16 expression and secretion. Conversely, we observed a diminution of these chemokines following the overactivation of the β-catenin pathway in β-catenin non-mutated HCC cells. In accordance with these results, our global transcriptomic analyses carried out in cohorts of HCC patients revealed that mutated β-catenin represses the expression of *CCL20* and *CXCL16*. Our results identified CCL20 and CXCL16 as novel negative targets of β-catenin and provide new insights into the functioning of HCC tumor microenvironment in a context of β-catenin mutation.

P106

Metabolism-based scoring of cutaneous squamous cell carcinoma to predict tumor features and responses to treatment

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Alteration in metabolic activities is a critical point in cancer progression and myriad of metabolic-based therapy approaches are now proposed for human cancers. However, emerging evidence highlight interpatient metabolic heterogeneity and the importance of metabolic phenotyping in cancer therapy. To investigate metabolic heterogeneity in cutaneous squamous cell carcinoma (cSCC), combined proteomic and bioenergetic analyses were applied on patient samples with various stages of cSCC ranging from precancerous actinic keratosis (AK) to metastatic cSCC. Three subgroups with low-, medium- and high-metabolic scores were detected in all stages of carcinogenesis. To examine the functional impact of the detected metabolic heterogeneities on tumor features and their responses to treatment, we used patient-derived tumor cell (PDC) and patient-derived xenograft (PDX) models by transplantation of tumor cells and freshly resected patient tumors into immunocompromised mice. Results showed that, in both models (PDC and PDX), the sensitivities of tumors to leflunomide, an inhibitor of dihydroorotate dehydrogenase (DHODH), were inversely correlated with their metabolic scores and directly correlated with the protein expression level of DHODH. Moreover, DHODH overexpression in nonresponding groups render them sensitive to leflunomide. These findings demonstrate the pragmatism of metabolic profiling/scoring in designing of therapeutic approaches targeting bioenergetic vulnerabilities of tumors and propose DHODH as a promising therapeutic target in low-metabolic score subgroup of cSCC.

P107

The cell cycle inhibitor p27kip1 controls cell metabolism

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The cell cycle regulator $p27^{kip1}$ (p27), acts as a tumor suppressor by binding and inhibiting cyclin-dependent-kinases (CDKs) that allow cell cycle progression. This role of p27 is linked to its nuclear localization, and in cancer cells p27 is often cytoplasmic, losing its role of cell cycle inhibitor and thus participating to the relentless cell division. Furthermore, p27 regulates other cellular functions, such as cytoskeleton dynamics, transcriptional regulation and autophagy.

We have recently shown that p27 plays a critical role under metabolic stress, such as amino acid or glucose starvation. Indeed, under amino acid starvation p27 promotes autophagy flux and controls mTORC1 signaling. Interestingly, $p27^{+/+}$ cells are more sensitive to an amino acid starvation than $p27^{-/-}$ cells, which survive by maintaining elevated mTORC1 activity. In contrast, $p27^{-/-}$ cells are more sensitive to a glucose starvation than $p27^{+/+}$ cells, which survive by promoting autophagy. Given the role of p27 on autophagy, mTORC1 signaling and transcription regulation, we hypothesized that p27 may participate in rewiring cellular metabolic programs upon stress. Using cell lines with different p27 status, we performed :
I) RNA sequencing to understand how p27 controls gene expression during metabolic stress.
II) Metabolomic studies to investigate whether p27 induces metabolic reprogramming upon metabolomic stress.

Our data clearly indicates that p27 modulates cell metabolism during metabolic stress. Metabolomics analysis show that many metabolites are impacted by the p27 status, such as lipids, tricarboxylic acid (TCA) cycle and glycolysis metabolites.
Transcriptomics analyses reveal that p27 also controls gene expression during metabolic stress and shows that p27 plays a role in mitochondria homeostasis, cell death, autophagy and metabolic processes.
Furthermore, we showed that mitochondria dynamics is modified after metabolic stress depending on p27 status. Indeed, in full medium culture or during amino acid deprivation, mitochondria of $p27^{-/-}$ cells are more fragmented compare to $p27^{+/+}$. Interestingly, fatty acid oxidation and TCA cycle take place in mitochondria, so we hypothesize that p27 can play a role during metabolic stress by acting on mitochondria activity.

p27 status is often deregulated in cancer cells and p27 status confers resistance to cell death during metabolic stresses. Moreover, mitochondria dysfunction is involved in metabolic reprogramming of cancer cells. Thus, we hypothesize that p27 may help couple the relentless division of cancer cells with the metabolic reprogramming required for this division. Finally, this study will determine whether p27 status could be used to sensitize cancer cells to apoptosis by targeting specific metabolic pathways.

P108

Role of a short isoform of Connexin43, GJA1-20k, on cancer prostate cancer cells metastatic abilities

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Advanced prostate cancer is known to metastasize mainly to the bone, and is then treated with palliative approaches. Previous work has linked the metastatic dissemination of prostate cancer (PCa) cells to a transmembrane protein, the Connexin43 (Cx43). Cx43 belongs to a multigenic family of proteins, which oligomerize to form hemichannels, intercellular channels and GAP junctions. Connexins are known to present modified expression levels according to the stage of cancer, like Cx43 in prostate cancer: in early stage, Cx43 expression is strongly decreased, while a re-expression is observed at more advanced stages. We previously demonstrated that Cx43 overexpression was related to aggressiveness of prostate cancer cells with a potentiation of migration ability of PCa cells and an increase of the sensitivity to an osteoblastic conditioned medium (ObCM), used to mimic the metastatic bone niche. For this promigratory effect on PCa cells, plasma membrane localization is required and Cx43 carboxy terminal (CT) domain is necessary and sufficient (Boucher et al., 2020).

We also found that a short Cx43 isoform named GJA1-20k, generated by alternative translation and corresponding to the end part of the fourth transmembrane domain and the entire CT domain, was expressed in LNCaP cell model of PCa, and its expression level was increased in the presence of ObCM. Interestingly, in the cardiac model, GJA1-20k is involved in the export of full-length Cx43 to the plasma membrane, through the stabilization of the actin cytoskeleton. Based on this effect and our results, a link between membranous Cx43, response to bone microenvironment and GJA1-20k expression during PCa progression to bone can therefore be postulated.

To determine the impact of this isoform during prostate cancer dissemination, GJA1-20k overexpression was performed in Human prostate cancer cells PC3 which exhibit a defect in Cx43 exportation to the membrane, and express different levels of the full-length Cx43. We showed that, similarly to the cardiac model, GJA1-20k overexpression increased the presence of full-length Cx43 at the plasma membrane, leading to increased intercellular communication. Phenotypic characterization of the different PC3 clones revealed that GJA1-20k overexpression led to a decrease in migratory and invasive capacities correlated with an increased expression of the epithelial profile marker, E-Cadherin. In the bone metastatic context, the short isoform also increased in vitro PC3 cells adhesion to osteoblastic cells. As GJA1-20k is considered as a stress protein in the cardiac model, we also studied the role of this isoform on PC3 cells subjected to different stress conditions. Overexpression of GJA1-20k in PC3 cells improved their resistance to staurosporin-induced cell apoptosis and anoïkis. Finally, owing to the fact that GJA1-20k promotes protective mitochondrial effects in the cardiac model during cellular stress, cellular quiescence and metabolic activity of PC3 cells are under investigation.

To conclude, our results allowed to reveal the presence of a short Cx43 isoform in prostate cancer cells that might be an important regulatory factor for survival and dormancy in the bone metastatic context.

P109

Repression of exosome secretion by mutated ß-catenin contributes to immune escape in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the most common primary liver cancer; it represents the 6th most frequent tumor in the world and the 4th leading cause of cancer-related death. Due to the lack of efficient therapies and the difficulty to detect the pathology during early stages, HCC has a poor prognosis. Today, the immunotherapy represents the first-line treatment for advanced HCC with beneficial effect on overall survival. 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, the role of ß-catenin promoting this immune escape and leading to tumor cells trigger immunosuppressive cascades is not yet fully understood. Our project focuses on the role of ß-catenin signaling in tumor/immune cells communication through exosomes.

Using transcriptomic analysis, we showed an alteration of gene expression involved in exosome biogenesis machinery in HepG2 cells upon knock-down of mutated ß-catenin. We also revealed by total internal reflection fluorescence microscopy (TIRF), a decrease of exosome secretion when β-catenin is mutated. We further identified two target genes of the exosomal machinery, SDC4 and RAB27A, whose expression is ß-catenin-dependent. Thus, these results suggest that ß-catenin mutations inhibit exosome formation and/or secretion in liver tumor cells. As exosomes and their contents are main factors for intercellular communications, we hypothesized that the decrease of exosome production could lead to a defective recruitment of leukocytes, making these tumors poor in immune infiltrates and resistant to immunotherapy. To validate our hypothesis we generated spheroids of liver cancer cells and co-cultured with human peripheral blood mononuclear cells (PBMCs) and analyzed immune cells infiltration modulation by flow cytometry. We revealed an immune escape in β-catenin mutated HCC cells and confirmed the involvement of exosomes in this processus.

Our results provide a new knowledge on the impact of ?-catenin mutations on exosome biogenesis. This may allow the development of a new tool from liquid biopsies to stratify HCC patients for immunotherapy response.

P110

Energy metabolism rewiring following acute UVB irradiation is largely dependent on nuclear DNA damage

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Solar ultraviolet B (UVB) radiation-induced DNA damage is a well-known initiator of skin carcinomas. UVB-induced DNA damage response (DDR) is a series of signaling cascades that are activated for maintaining cell integrity. Among different biological processes, little is known about the role of energy metabolism in DDR.
We sought to identify whether the UVB-induced nuclear and/or mitochondrial cyclobutane pyrimidine dimers (CPD) alter cellular energy metabolism. To gain insight into this question, we took advantage of keratinocytes expressing nuclear or mitochondrial CPD photolyase.

To obtain a global overview of signaling pathways triggered by a single exposure to UVB irradiation, we performed a quantitative label-free differential proteomic analysis of samples at different time points after UVB irradiation. Ingenuity Computational Pathway Analysis (IPA®) software (www.ingenuity.com) was used to elucidate the global implications of differentially expressed proteins as well as the potentially modified molecular pathways at different time points after irradiation. As expected, there were numerous well-known signaling pathways that classically contribute to the DDR programs, such as EIF2 signaling, integrin signaling, senescence pathway, death receptor signaling, cell cycle control and ATM signaling pathways. Of note, the majority of these signaling pathways were modified in a biphasic manner with an early downregulation which was followed with a late upregulation. Besides those pathways, we found that the metabolic pathways involved in energy metabolism, such as oxidative phosphorylation (OXPHOS), glycolysis, TCA cycle and fatty acid β-oxidation, were also modified in a time dependent manner. We also found that the biphasic alteration in metabolism was largely dependent on the presence of genomic CPD. The increased oxygen consumption rate at 24h after irradiation as well as the mitochondrial structural rearrangements were dependent on both mitochondrial and nuclear CPDs.

Understanding the influence of the nuclear and mitochondrial DNA damage on keratinocyte responses to UVB could enhance current knowledge regarding skin cancer prevention, initiation, and therapy.

P111

In vivo characterization of the impact of p53 mutation on hepatocellular carcinoma

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Alterations of the tumor suppressor gene TP53 are among the most frequent genetic events (40%) in hepatocellular carcinoma (HCC), the main primary liver cancer. Although p53 has been extensively studied, the precise role of its mutants in oncogenesis remains to be clarified, notably within the framework of its pleiotropic and tissue-specific functions. In HCC, p53 inactivation occurs mainly through missense mutations, which alter the p53 DNA binding and exert a dominant negative (DN) effect on the wild-type protein. The mutations may also confer novel activities to the p53 protein, defining a Gain-of-Function (GOF) phenotype. Our study aims to characterize the consequences of the p53 mutational profile on hepatocellular carcinoma development: we selected eight p53 mutants characteristic of HCC and analyzed their pathophysiological effects in a murine model of hepatic carcinogenesis.

We used hydrodynamic gene transfer (HGT) to transfect hepatocytes*in vivo* and assess the oncogenic cooperation between MYC and eight p53 mutants ("p53MUT": V157F, A159P, R175H, R248W, R248Q, R249S, R273C, R273H), or its inactivation by CRISPR/Cas9 as a control. The resulting tumors were analyzed using immunohistochemistry, RT-qPCR, western blotting, and their transcriptomic profiles were determined through RNA-seq.

Expression of MYC, or p53 mutants, alone in C57Bl6J mouse livers did not trigger tumorigenesis. In contrast, combinations of MYC + p53MUT led to the development of numerous hepatocellular carcinomas within 2 to 8 weeks. Macroscopic analysis identified two highly oncogenic mutants (R175H, R249S), others with an intermediate phenotype (R248W, R248Q, R273C), and three that were only slightly oncogenic in this model (A159P, V157F, R273H). Transcriptomic profile analysis indicated that tumors carrying R175H and R249S mutants had a gene expression profile identical to those resulting from p53 inactivation. On the other hand, tumors with R248Q, R273C, and R273H mutants showed a very different transcriptome (>3000 differentially expressed genes; padj <0.05, log2FC>1.5).

These results indicate that p53 mutations are not equivalent to trigger HCC in combination with MYC, and results in two different classes of tumors. Experiments to decipher the mechanisms by which distinct p53 mutations dictate the fate of hepatic tumors are ongoing.

P112

Role of PI3K class III, Vps34, in pancreas carcinogenesis

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Pancreatic ductal adenocarcinoma (PDAC) is a lethal pathology with a poor prognosis. Although the importance of the Kras activating mutation, observed in 95% of these cancers, in initiation is recognized, most of the cellular and molecular mechanisms downstream of Kras controlling the aggressiveness and heterogeneity of this cancer remain unknown. Understanding these mechanisms could lead to the development of urgently needed new diagnostic and therapeutic tools.

PI3Ks are key enzymes in carcinogenesis. The involvement of certain class I isoforms in pancreatic cancer is being studied by several teams, including my host team, but the role of the ubiquitously expressed Vps34 is still poorly understood. It has known functions in autophagy, vesicular trafficking, endocytosis, phagocytosis and probably in mitochondrial metabolism. As autophagy is implicated in pancreatic carcinogenesis and increased by oncogenic Kras, we studied the role for Vps34 in pancreatic cancer development.

To mimic low Vps34 protein activity found in patients with poorer prognosis, we developed a mouse model combining the KrasG12D mutation with heterozygous or homozygous inactivation of Vps34 protein in the pancreas. This conditional genetic targeting was achieved through the expression of a Cre recombinase under the control of the Pdx1 promoter, a transcription factor required for pancreatic organogenesis. The pathology of the pancreas of KrasG12D-mutated mice with heterozygous or homozygous inactivated Vps34 (named KCVps34lox/+ and lox/lox) was compared with that of KrasG12D-mutated mice alone (named KC). Cell lines were derived from these mouse models.

The KCVps34lox/+ mouse cohort showed a drastically reduced prognosis compared with the KC cohort; the first clinical signs of metastatic spread (cachexia) are observed in mice as early as 4 months of age. KCVps34lox/lox mouse cohort showed instead a significantly improved prognosis compared to KC cohort. From a histological point of view, at 6 months of age, in the KCVps34lox/+ model, we observed an earlier appearance of high-grade lesions at high penetrance compared with KC mice, whereas the pancreas of all KC mice presented only precancerous lesions. Moreover, these high-grade lesions (adenocarcinoma) were aggressive, with metastases detected in the lungs and liver. Associated with these cancerous lesions, immune infiltrates were observed and adjacent pancreatic parenchyma showed lipid accumulation in acinar cells. At more than 9 months old, the lesions were also more aggressive in KCVps34lox/+ compared to KC mice. Reversely, there was no lesions in KCVps34lox/lox pancreas, but the reduction and total loss of Vps34 led to the accumulation of lipid vesicles in the acini, as well as to immune infiltration.

We are now aiming to understand the cellular processes and signaling pathways that explain the poor prognosis of KCVps34lox/+ mice. The better prognosis of KCVps34lox/lox mice can be explained by the early depletion of cancer initiating cells (see also abstract from Thibault B. et al). In humans, we also aim to understand how Vps34 activity is altered by investigating a possible association between pesticide exposure and Vps34 autophagy defects. Understanding the pathology of PDAC and the molecules involved in its poor prognosis is essential to improving the management of this pathology.

P113

Characterisation, role and regulation of lipid droplet catabolism in glioblastoma

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Glioblastoma (GB), the most aggressive primary brain tumors, are characterized by intratumor heterogeneity and a high plasticity of GB cells enabling adaptation and survival under microenvironment pressure. We aim to understand the role of metabolic adaptation in GB aggressiveness, especially the catabolism of lipid droplets (LD) leading to the release of free fatty acid that can be used as membrane components, signaling molecules or source of energy.

We show that LD are more present in the core than at the edge of GB in-vitro and in-vivo models. We then hypothesized that degradation of LD is an important mechanism at the border of the tumor. To characterize this catabolic mechanism we inhibited the main processes of LD degradation; lipolysis and lipophagy, by targeting the three lipases: adipose-triglyceride lipase, hormone-sensitive lipase (HSL) and lysosomal acid lipase. This inhibition induces an accumulation of LD, leading to a decrease of GB cell proliferation and migration under basal condition. We show that under nutrient deprivation condition the LD breakdown is higher than in basal condition. Interestingly, under this stress condition, lipases activity is crucial for cell survival and migration, mainly through the activity of HSL. To understand the link between HSL and metabolic adaptation, we show that its inhibition leads to a decrease of mitochondrial activity.

In conclusion, these results highlight the role of LD degradation in GB cell aggressiveness and the potential link with mitochondrial metabolism. This study could lead to the proposition of HSL as a new interesting target in GB.

P114

Characterization of NRF2 Role in the Development and Therapeutic Resistance of Human Glioblastoma

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The nuclear factor erythroid 2-related factor 2 (NRF2) is a master regulator of human antioxidant defenses. However, constitutive NRF2 activation promotes glioblastoma (GB), WHO grade IV glioma, development, and therapeutic resistance. Despite the therapeutic options, glioma stem cells (GSCs) are responsible for the recurrence of GB tumors. In our study, we investigate the role of NRF2 in GB progression using a P3 patient-derived GB 3D model. Our results show that NRF2-KO tends to decrease the P3 sphere-forming capacity, however, the precise mechanisms underlying its influence on stem cell maintenance and self-renewal require further elucidation. Our study demonstrates that the absence of NRF2 has no discernible impact on P3 cell proliferation under varying oxygen concentrations (21%, 1%, and 0.1% O2). However, a noticeable reduction in the invasive potential of P3 NRF2 knockout spheres is observed in comparison to control counterparts at 21% O2, suggesting a pivotal role for NRF2 in mediating the invasion of P3 spheres. Also, our study points to the involvement of NRF2 in P3 cell metabolism which is to be explored further in terms of the implicated metabolic pathways (LDHs, MCTs…) linked to their invasive capacity. Next, we aim to investigate the role of NRF2 in sensitizing the P3 cells to TMZ treatment using crisper Cas-9 NRF2 KO P3 cells. Therefore, this study may provide novel evidence to improve the outcomes of GB cancer therapy through NRF2-targeted silencing strategies.

~~P115~~

P116

Design of B-glucuronidase-sensitive albumin-binding prodrugs with high DAlbR for cancer chemotherapy

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Conventional chemotherapy does not cure the majority of the deadliest cancers. Most of the drugs used clinically exhibit a poor selectivity against tumour cells and also destroy healthy tissues, leading to severe side effects and premature cessation of treatment. The selective delivery of anticancer drugs in tumors is an emerging therapeutic strategy that limits these side effects. Within this framework, we introduced the concept of β-glucuronidase-sensitive albumin-binding[1] prodrugs that target the specificities of the tumor microenvironment[2]. Once in the blood stream, such prodrugs react in with the cysteine-34 residue of circulating albumin[3] through thio-Michael addition. The albumin conjugate then accumulates passively in tumors[4] where extracellular β-glucuronidase[5] triggers the release of the active compound. We evaluated the therapeutic efficacy of a set of β-glucuronidase-sensitive albumin-binding prodrugs of the potent MMAE for the treatment of human breast, pancreatic, lung and colon tumours implanted mice. In each case, the prodrugs led to remarkable anti-cancer activities, demonstrating both the efficacy and versatility of this targeting approach. Recently we have designed a new generation of β-glucuronidase-sensitive albumin-binding prodrugs capable of transporting simultaneously three cytotoxic molecules[6], allowing the increase of the DAlbR (Drug to Albumin Ratio). This trimeric prodrug produced an unprecedented anticancer activity on orthotopic MIA PaCa-2 pancreatic tumors, leading to dramatic reduction or even remission of tumors (3/8 mice) without causing side effects. In addition, the therapeutic efficacy of this prodrug was evaluated for the treatment of pancreatic PDX (patient derived xenograft) and compared to the standard of care. The results of these studies will be presented.

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P117

Evaluation of the Potential Role of Proprotein Convertase Subtilisin/Kexin 9 on the Tumorigenic and Invasive Properties of Cancer Stem Cells in Gastric Adenocarcinoma

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Gastric cancer is the 5th most common malignancy and the 4th leading cause of cancer-related death worldwide (IARC, 2020). Most cases are gastric adenocarcinomas (GC), for which only 30% of cases are suitable for targeted immunotherapy. GC are usually detected during the metastatic stage and, thus, the number of relapses is high, with a five-year survival rate lower than 20%. Increasing evidence suggests that GC bad prognosis is caused by cancer stem cells (CSCs), which are a small tumor cell subpopulation with the capacity of inducing GC initiation, growth, chemo-resistance, relapse and metastasis. CSCs hijack cell signalling pathways for their survival, including the Hippo YAP/TAZ/TEAD signalling, allowing the expression of oncogenic genes. Recent studies have shown that in GC, the expression of the Proprotein Convertase Subtilisin/Kexin 9 (PCSK9), a member of the proprotein convertases (PCs) family, is correlated with cancer progression and poor prognosis, and it seems to have a role in GC cell functions. In our laboratory, it was shown that PCSK9 is highly overexpressed in GC CSCs. Hence, it is suspected that PCSK9 could play a role in the control of GC CSCs properties. In this context, we studied the potential role of PCSK9 on the tumorigenic and invasive properties of CSCs in GC, and on the activation state of the Hippo YAP/TAZ/TEAD pathway. A pharmacological inhibitor of PCSK9 expression called R-IMPP was used to evaluate the impact of PCSK9 inhibition on: 1) GC CSCs stemness and tumorigenic properties in vitro; and 2) on the Hippo YAP/TAZ/TEAD pathway in vitro. PCSK9 inhibition caused a decrease in the tumorsphere formation capacity of GC CSCs, on the protein levels and nuclear expression of Epithelial-to-Mesenchymal Transition (EMT) transcription factors, and on the invasive capacity of GC cells. Moreover, PCSK9 inhibition affected YAP/TAZ protein levels and their nuclear expression on GC cells. The impact on the TEAD transcriptional activity is currently under investigation. All in all, these results suggests that PCSK9 may control CSCs tumorigenic and invasive properties through the EMT and probably the YAP/TAZ pathway, and it could constitute a potentially new therapeutic target in GC.

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Invadosome formation in response to bacterial genotoxins

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We are frequently exposed to bacterial genotoxins, such as Cytolethal Distending Toxin (CDT) and colibactin, produced by bacteria from the microbiota. These genotoxins cause DNA damage and a high degree of ploidy in host cells, well-known risk factors for carcinogenesis, along with stress fiber formation and deep cytoskeleton remodeling. We observed circular F-actin structures following exposure to bacterial genotoxins that may correspond to invadosomes, whose ability to degrade matrices contributes to invasion and metastasis. In this study, we investigated the mechanism of invadosome formation in response to bacterial genotoxins.

In vitro, the staining of invadosomes' markers in hepatic and intestinal cell lines infected with genotoxin-producing bacteria, associated with in-situ zymography assay, allowed the confirmation of functional invadosomes. The increase in invadosomes formation was dependent on the CDT and colibactin, as it was not observed in non infected cells and in response to the corresponding mutant strains invalidated for these toxins. Extracellular matrix (ECM) degradation was increased following exposure to these genotoxins. Similar results were observed when using transgenic cell lines expressing the CdtB catalytic subunit of CDT, as well as with DNA-damaging agents (Etoposide and Streptozocin), suggesting that DNA damage leads to invadosome formation and ECM degradation. In response to CdtB, a global kinase activity assay revealed the activation of Src-family kinases, crucial in invadosome formation, that was corroborated using the Src-family kinases inhibitor PP2.

Overall, these data show that the genotoxic stress induced by bacterial genotoxins leads to invadosomes formation and ECM degradation, suggesting that chronic and/or repeated exposure to genotoxin-producing bacteria is implicated in cancer progression.

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Immunosuppressive Myeloid Cells foster Cancer Stem Cell (CSC) emergence through TGF-β

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Cells of myeloid origin represent major components of the complex immunosuppressive tumor microenvironment. These myeloid cells such as tumor-associated macrophages (TAM), neutrophils and so-called "myeloid-derived suppressor cells" (**MDSC**) among others have been widely described for their immunosuppressive properties and their ability to inhibit anti-tumor immune responses. They thus represent major obstacles for efficient immunotherapeutic approaches. However, beyond this cardinal immunosuppressive function, MDSC are also endowed with a broad array of "non-immunological" tumor-promoting functions1. Indeed, accumulating evidences has been provided that these cells can directly promote primary tumor cell survival and proliferation and promote local tissue invasion among others. Importantly, MDSC play a key role in the preparation of the pre-metastatic niches before the arrival of cancer cells, thus contributing to the preparation of the "soil" for seeding by metastatic tumor cells. Evidence has also emerged that tumor-induced immunosuppressive myeloid cells may impact cancer stem cells (**CSC**), a subpopulation of cancer cells within the tumor, defined by self-renewal, asymmetrical division and differentiation properties, giving rise to more or less differentiated cells composing the tumor mass.

Using 3-D tumorsphere formation assays we demonstrate that human monocyte-derived suppressor cells (HuMoSC, a surrogate in vitro model for MDSC) are endowed with the capability to promote stemness features in **breast** and **NSCLC** cancer cells in a cell-to-cell contact dependent manner. Moreover, our data also demonstrate that different subpopulation of MDSC isolated from breast cancer bearing mice, as well as immunosuppressive myeloid cells isolated for breast cancer patients promote acquisition of stemness properties by cancer cells.

To understand the mechanisms by which these HuMoSC promote stemness of cancer cells, we combined the use of transcriptomic (single-cell RNA sequencing) and proteomic (surface proteomic analysis (surfaceome)) technologies to explore potential candidates involved in the physical interactions between the two cell types. We found that the induction of the stemness phenotype was partly mediated through **TGF-β**.

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Marine fucoidan oligosaccharides as promising multi-tasking formulations in cancer therapy

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While anti-mitotic chemotherapy has brought significant improvements in cancer management, major challenges persist, especially concerning the strong side effects and drug resistance observed in many cases. In response, combined therapeutic approaches or use of multi-tasking molecules emerge as one of the most promising solutions.[1] Sulfated oligosaccharides derived from marine polysaccharides may offer a such new original therapeutic option. Indeed, this original class of biomolecules Marine fucoidan oligosaccharides as promising multi-tasking formulations in cancer therapy can not only display cytotoxic effects on cancer cells but also, as structural mimics of extracellular matrix components, regulate concurrently various biological pathways within the tumor microenvironment, including those associated with invasiveness and immune response. In this presentation, we will highlight this poly-pharmacological feature by exploring the bioactive properties of fucoidan oligosaccharides (Fuc-OS) originating from brown seaweed in the context of two type of cancer, B-lymphomas and triple-negative breast cancer (TNBC).

In details, our previous work on two Fuc-OS fractions emphasized a decrease in proliferation and an increase in apoptosis of cancerous lymphoblastoid cell line. In synergy, these fractions also downregulate the expression of the immuno-inhibitor PD-L1 at both, the transcriptional and protein level (on the cell surface of apoptotic as well as of intact cells), resulting in a possible restoration of effector T-cells immune response. [2] Regarding TNBC, we are evaluating various Fuc-OS with different structural features or their capacity to inhibit heparanase (HPSE), an enzyme known for versatile key roles in tumor progression. Furthermore, their effects were evaluated on MDA-MB-231 cell-based assays. Preliminary results show a decrease of cancer cells viability after 24h at a 100 µg.mL-1 dose, and, on other hand, an 80% inhibition at 25 µg.mL-1 against the HPSE- mediated cell migration. Altogether, these findings strengthen the promises of fucoidan and its derivatives for a potential use in clinical oncology.

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Non canonical STING activity in lung cancer translate in tumor progression and aggressiveness

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Canonical STimulator of INterferon Gene (STING) signaling leads to a potent induction of type I interferon (IFN) and interferon-stimulated genes governing the establishment of a more efficient anti-tumor immune response in various context. However, recent publications suggest that some type of tumors may rely on STING activation to fuel their growth and aggressiveness.

Wanting to unravel STING function in lung cancer, we took advantage of a KrasLSL-G12D/WT; Tp53fl/fl (KP) driven mouse model of lung cancer, from which we have generated cancer cells lines and clones showing different STING expression levels and phenotypes.

We noticed that STING knockout in the cancer cells significantly reduced their ability to engraft and form tumors in vivo, suggesting an intrinsic pro-tumoral activity of the STING pathway. Consistently with these findings, bioinformatics analysis on human lung cancer cells transcription profiles showed EMT as one of the most upregulated gene set and investigation of EMT markers in autochthonous KP tumors derived cancer clones revealed that STING expressing cells have enhanced migration and EMT-like features. Furthermore, STING expression is induced after EMT promoting stimuli, such as TGFβ or the ectopic expression of SNAIL, one of the major EMT transcription factors. Coherently with this unusual STING activity, in vitro dissection of STING signaling in cancer cells revealed an alteration in the canonical signaling cascade translating into the impairment of IFN-induction, while they were able inducing high level of this cytokine upon other stimuli, as TLR3 triggering. On the other hand, STING pathway in our model is associated to mTORC1 inhibition. This last could reflect the induction of metabolism rewiring via autophagy that we presume to be behind STING protumoral effect. We are currently evaluating this hypothesis and we have promising preliminary data.

Our data delineate a complex role of STING signaling in cancer cells, opening new intriguing scenarios to investigate the biological processes linked to STING pathway, in a context where STING agonists are seen as an extremely attractive option to boost immune response in NSCLC patients.

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Unraveling vascular and telocytes remodeling in cutaneous infantile hemangiomas using large-volume 3D imaging

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Infantile hemangioma (IH) is the most frequent tumor in newborns, occurring in up to 1 in 10 births. This benign vascular tumor exhibits a fast-growing phase followed by a slow and gradual involution. Ten years ago, propranolol emerged as a remarkably efficient drug to accelerate its regression. While mechanisms of actions remain largely unknown, previous work of the team has demonstrated the key role of a new stromal cell in the pathogenesis and propranolol response: the telocyte (TC). Given the pivotal role of TCs in cellular communication and organization, we hypothesized that TCs could participate in IH involution by inducing a vascular remodeling process, further potentiated by propranolol. In this study, we aim to investigate the vascular organization of IH through a comprehensive architectural analysis of patient resections, classified according to the tumor state and the treatment. Achieving deep and accurate visualization of remodeling necessitates large volume imaging of tissue. However, working on thick samples require tissue-clearing, a technique initially developed for mouse brains. Nonetheless numerous adaptations are required for skin, a highly pigmented and matrix-dense tissue. Accordingly, we have developed an optimized tissue-clearing protocol named Skin-iDISCO+, specifically tailored for skin clearing, which was previously insufficiently addressed. With Skin-iDISCO+ and compatible antibodies, we successfully identified a limited number of tortuous vessels within tumors. Preliminary results notably showed that the reduction of the tumor vascular burden was correlated with vessel straightening. Additionally, we observed distinctive arrangement of TCs around lesional capillaries. They either form sheets or branching meshwork covering vessels. These arrangements appear as being associated with different tumor states and therefore vessel straightening.

These findings indicate that natural or propranolol-induced IH involution involves a dynamic TCs reorganization, leading to vascular remodeling. A deeper understanding of propranolol mechanisms holds promise to explore therapeutic prospects for other vascular tumors with unmet clinical needs.

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The oncogene Src controls invadosome formation through a spatial modulation of the eIF3 translation complex

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Tumor progression and cell invasiveness require degradation of extracellular matrix (ECM) induced by invadosomes. Src-induced invadosomes are dynamic F-actin based structures enriched with translational machinery including subunits of eucaryotic translation initiation factor 3 (eIF3). The individual depletion of subunit 3H and 3E reduced invadosome formation and degradation activity associated with lower translational level. The inhibition of Src activity reduces translational level by the regulation of eIF3 expression and PI3K/mTOR pathway and eIF4E/eIF4G interaction. We show that eIF4E/eIF4G interaction, initiating translation, is involved in invadosome formation and associated-degradation activity. We also demonstrate that eIF3-regulated mRNAs enriched into invadosomes (Igf2bp2, TKT) are mandatory for their formation and ECM remodeling. Furthermore, the expression of Src, eIF3, Igf2bp2 and TKT are increased in tumor section from hepatocellular carcinoma patients. These findings reveal the molecular mechanism of Src-induced translation leading to invadosome formation leading to tumor progression.

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FGF19 and its analog Aldafermin cooperate with MYC to induce aggressive hepatocarcinogenesis

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Hepatocellular carcinoma (HCC) is the primary liver tumor, and the third cause of cancer-related death worldwide. The number of HCC cases is increasing, with an estimated 60% growth in the next two decades. This rise is due to Non-Alcoholic Fatty Liver Disease (NAFLD), with one third of worldwide population being affected, and a 55% prevalence predicted for 2040. In response to this rising epidemic, researchers are actively trying to develop therapies based on metabolically active proteins.
FGF19 is a post-prandially produced hormone with potent hepato-protective effects and strong metabolic impact. Because of those effects, it has become a promising potential target for the treatment of metabolic-associated liver diseases. Conversely, the FGF19 locus is amplified in 10-15% of Hepatocellular Carcinoma (HCC). It is considered as a driver in hepatic carcinogenesis and its overexpression is linked to poor prognosis. Therefore, a FGF19 analog, Aldafermin (NGM282), was designed to uncouple its oncogenic and metabolic properties and is currently tested in clinical trials Non-Alcoholic Steatohepatitis (NASH).

We assessed the oncogenicity of FGF19 and its analog Aldafermin in HCC pre-clinical mice models, especially their cooperation with oncogenic events commonly found in HCC. Using Hydrodynamic Gene Transfert (HGT), we performed in vivo stable transfection of FGF19 or Aldafermin in a subset of hepatocytes, along with Trp53 deletion, mutant CTNNB1, CCND1, MYC or H-RAS overexpression.

FGF19 alone is weakly tumorigenic, with small tumors developing in a year, but cooperates with other oncogenes such as mutant CTNNB1S33Y, giving rise to tumors in four months. The strongest effect was seen when co-expressing FGF19 and MYC, which led to critical tumoral burden in a span of two weeks. Next, we studied Aldafermin oncogenic properties in the context of MYC overexpression, and found a strong cooperation, with tumors being histologically and transcriptomically identical to the FGF19-driven ones. Furthermore, to simulate circulating levels of Aldafermin found in treated patients, recombinant proteins were injected daily in MYC overexpressing mice, triggering the appearance of tumoral foci after only 4 days of treatment.

Overall, we show that FGF19 cooperates with MYC to trigger aggressive hepatocarcinogenesis. Importantly, we report that FGF19-analog Aldafermin retains oncogenic properties in the context of MYC overexpression, raising concerns in the framework of its current usage in patients with damaged liver.

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Targeting PI3K/AKT/mTORC1 signalling in gastric cancer stem cells

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Gastric cancer (GC) is the 4th leading cause of cancer death worldwide. We identified and characterized cancer stem cells (CSCs) driving tumor initiation and chemoresistance in GC, including a mesenchymal subpopulation of CSCs detected in circulating blood vessels and metastases expressing CD44v3+ as a surface marker. The PI3K/AKT/mTORC1 pathway is an intracellular signalling pathway important in regulating cell growth and cell proliferation, especially in cancer. We have recently identified the upregulation of PI3K/AKT/mTORC1 pathway in CSC sub-populations from GC patient omics data. The aim of this project is to study the role of the PI3K/AKT/mTORC1 signalling in CSCs tumorigenic and invasive properties in GC using two inhibitors of the pathway in combination. The obtained results showed that BKM-120 (PI3K inhibitor) and Rapamycin (mTORC1 inhibitor) have a potential in preventing tumour growth and dissemination on different sub-populations of GCSCs.

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Impairment of Store-Operated Calcium Entry during MAPKi-induced melanoma phenotype switching: driver or passenger?

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UV-induced 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 bear driver-mutations in BRAF 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 work for a limited time only.

Transcriptional reprogramming is one of the major mechanisms associated with the acquisition of MAPKi resistance in melanoma cells. It allows differentiated cells to switch from a proliferative phenotype to an invasive and pharmaco-resistant one through a dedifferentiation process. Calcium signaling is a powerful and multifaceted tool by which cells can achieve specific outcomes or change their fate. By combining well-characterized pairs of isogenic melanoma cell lines, each composed of a drug-resistant/invasive sub-line derived from a drug-sensitive/proliferative parental cell line following chronic MAPKi exposure and functional approaches, we have shown that melanoma drug-induced phenotypic plasticity is specifically associated with changes in the calcium signalling modes of melanoma tumor cells. Our data revealed in particular that resistant/invasive cells have a decreased Store-Operated Calcium Entry (SOCE) compared with sensitive/proliferative cells. The SOCE pathway being a major route of calcium signal generation downstream plasma membrane receptors stimulation. Mechanistically, SOCE inhibition in the drug-resistant cells occur through Wnt5A/PKC-induced inhibitory phosphorylation of the SOCE-meditating Orai1 channel. Moreover, we have demonstrated that reducing SOCE in the drug-sensitive/proliferative parental cells alter the expression of cell differentiation/proliferation markers and promotes cell migration.

Hence, this study shows that melanoma cells undergo therapy-induced cell states transition at least in part by decreasing their calcium entries. Whether this adaptation process favors the emergence of a drug resistances is currently investigated.

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Cancer cells transfer invasive properties through tracks

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Invasion and migration through the extracellular matrix are prerequisites for metastasis, the leading cause of cancer-related deaths. During tumor development, the extracellular matrix is remodeled, including by overexpressing type I collagen that facilitate cancer dissemination. Most studies have focused on events at the leading edge as cells invade. We describe a new event at the trailing edge, as cells detach from the matrix.

We show that small vesicles containing the collagen receptor DDR1 are left behind on collagen fibrils in the migration path. We named these structures attached to the collagen fibers "collagen-tracks". The vesicles are similar in size to exosomes but lack the exosome markers, and they also are different to migrasomes. We show that collagen-track formation is stimulated by DDR1 and by factors that promote adhesion, including collagen cross-linking. We report the protein, mRNA and miRNA content of collagen-tracks. They contain adhesion proteins, suggesting that they form when membrane fragments containing adhesions are torn from the cell as it migrates along collagen fibrils. We show that collagen-tracks are deposited by breast cancer cells in 3D matrices in vitro and in vivo. Collagen-tracks are stable and can be taken up by surrounding cells, promoting epithelial to mesenchymal transition, matrix degradation and invasion, finally leading to increased lung metastasis of breast cancer cells.

In summary, we have identified and characterized a new vesicle entity directly attached to collagen fibrils that plays a role in cell-cell communication and can transfer invasive properties to surrounding cells. We conclude that cancer-related collagen-tracks are a new player acting locally to drive tumor invasion and metastasis.

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The MS275 inhibits AKT and P38 proteins to alter cell proliferation and invasion in lung cancer cells

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Tumorigenesis is divided into three main successive and interconnected stages represented by initiation, promotion and progression. Cancer cells results from normal cells by accumulation of mutations and modifications to acquire distinctive properties specific to malignant cells. Indeed, cells transit from epithelial state to mesenchymal phenotype during an epithelial-mesenchymal transition (EMT). On one side, expression of epithelial markers decreases whereas mesenchymal markers expression increases. These variations are modulated by transcription factors represented by Zeb-1, Snail, Slug and Twist. One the other hand, cytoskeleton is also modified to promote migration through the formation of migratory structures while cells create interactions with the extracellular matrix (ECM) by focal adhesions. Thus, tumor cancer cell proliferation arises mainly from up-regulation of AKT and/or MAPK pathways.

It is actually obvious that cancer progression is based on accumulation of epigenetic mutations. Hence, targeting actors playing a major role in these processes is an attractive strategy for cancer treatments. Within this framework, HDAC inhibitors have emerged as candidates of choice. In the last decade, MS275 (Entinostat) has been evaluated for the treatment of solid and hematological cancers. This synthetic anti-cancer drug reduced cancer cell viability through the induction of apoptosis by promoting class I and IV HDAC enzymes inhibition, in order to stimulate Histones 4 (H4) acetylation.

However, despite advances highlighting the effect of MS275 on tumorigenesis, there is a lack of information regarding biological mechanisms following MS275 action on H4. Nevertheless, it seems that MS275 stimulates wound healing in breast cancer cells and attenuates cell proliferation by inhibiting AKT and P38 proteins in esophageal, melanoma, breast and colorectal cancer cell lines. Concerning cell invasion, it is shown in breast cancer cells that MS275 decreased Snail and Twist nuclear action which reduced N-cadherin and vimentin expressions, while E-cadherin marker is increased.

The aim of this project was to identify signaling pathways and proteins modulated by MS275 associated with cell migration, proliferation and invasion in A549 lung cancer cells.

Our study highlights for the first time mechanisms modulated by MS275 in A549 human lung carcinoma cell line. We first confirm that MS275 altered cell viability by apoptosis through H4 acetylation. Then, we further found that MS275 did not affect wound healing, nor migratory structures or focal adhesion formation. Besides, we showed that MS275 altered cell proliferation through inhibition of AKT phosphorylation on Thr308 and P38 phosphorylated proteins. We also demonstrated that these inhibitions affected invasion properties of cancer cells by reducing Zeb-1, Slug and Twist activation that increased E-cadherin and ZO-1 expressions, whereas N-cadherin decreased.

P129

Myelin as a Key Player in Glioblastoma Progression

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Glioblastoma (GBM) is a rapidly growing and highly lethal brain tumor with an average survival of only 15 months post-diagnosis. GBM exhibits profound infiltration into healthy brain tissue, particularly within the white matter tracts composed of neuronal axons enveloped by myelin sheaths. Oligodendrocytes, specialized glial cells in the central nervous system, produce myelin, a lipid-rich substance crucial for rapid action potential transmission. During early GBM growth, cancer stem cells reconfigure myelin architecture. Conversely, cells within the oligodendrocyte lineage facilitate tumor invasiveness and confer resistance to chemotherapy and radiation therapy. This intricate interplay fosters a symbiotic relationship between GBM cells and oligodendrocytes, culminating in significant membrane reorganization and extensive demyelination at the tumor's invasive front.

Although demyelination in GBM has been observed, it remains inadequately explored.
Starting from *in vivo* evidence, we established an *in vitro* model to investigate GBM-oligodendrocyte interactions, unveiling the tumor cells' capacity to engulf myelin fragments, enhancing their invasive potential. Recent findings indicate that GBM's aggressive phenotype is associated with increased mitochondrial activity and broader cellular metabolism rearrangements. In light of this, we propose a two-step mechanism by which myelin serves as an energy source for glioblastoma, leading in parallel to long-term alterations in its cellular characteristics. This may occur through the modulation of key gene expression regulators, such as CREB, subsequently influencing critical biosynthetic processes like the mevalonate pathway, thereby affecting the trajectory of GBM progression.

In essence, myelin emerges as a source not only of energy substrates but also as a repository of chemical messengers pivotal for sustaining tumor growth and invasion.

P130

Discovery of SOCS7 as a versatile E3 ligase for protein-based degraders

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Targeted protein degradation (TPD) technology hijacks the ubiquitin-proteasome system (UPS). It brings into close proximity a protein of interest (POI) and an E3 ubiquitin ligase, which ultimately induces the POI proteolysis. However, the restricted number of functional E3 ubiquitin ligases and the acquisition of resistance to degraders via mutations on the E3 or the other components of the UPS are limitations of this approach. Therefore, identifying alternative E3s working for TPD is important to build new degraders and overcome potential resistance mechanisms. Using a cell-based screening with a protein-based degrader approach, we describe the discovery and characterisation of SOCS7, an E3 ligase that depletes its targets in several cell lines and within distinct subcellular localisations. We show its utility by building a SOCS7-based KRAS degrader that inhibits mutant KRAS pancreatic cancer cells' proliferation. These data show SOCS7 is a versatile E3 ligase that can be exploited to make potent degraders.

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Oncostatin M modulates cell plasticity in a model of head and neck squamous cell carcinoma

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Despite the decrease in alcohol and tobacco consumption, the incidence of oropharyngeal squamous cell carcinoma (HNSCC) has been increasing over the past 30 years in industrialized countries, notably due to HPV-16 infection. In the context of growing successes in cancer immunotherapies, the study of the impact of the tumour-immune microenvironment (TIME) in the carcinogenesis and the intrinsic aggressiveness of the tumour may allow to identify targets for new immunotherapeutic in HNSCC cancers. As a secondary lymphoid tissue, the pharyngeal mucosa contains large amounts of tumour-infiltrated immune cells that secrete cytokines. Oncostatin M (OSM) is one of them whose pro-tumoral impact in several cancer subtypes has been widely demonstrated. Recently, its role in cutaneous and cervix squamous cell carcinoma has been studied and confirmed a specific pro-aggressive activity.

We first demonstrated by RT-qPCR that one of the OSM receptor subunit (OSM-R) was overexpressed in human tumour samples compared to contralateral normal mucosa. By studying OSM-R expression by immunohistochemistry (IHC) in 74 HNSCC locally advanced patients, we further suggested the detrimental effect of OSM showing a worse prognosis in patients overexpressing OSM-R. In addition, epithelial-mesenchymal transition (EMT) activation seems to be one of the most potent OSM-mediated mechanisms implicated in the aggressiveness of tumour cells as suggested by a recent study of the TCGA registry showing a correlation between the expression of OSM-R and EMT-associated proteins. Thus, by using several HNSCC human cell lines (Cal33, SCC154, SCC90), we demonstrated that OSM activates STAT-3, Akt and ERK signalling pathways, and modifies the expression of EMT-associated proteins such as TGM2, SNAI1, Cytokeratin 13, CD44, E-Cad and Epcam. Cellular features of aggressiveness were also modulated by OSM with a significant increase in cell migration, cell proliferation and clonogenicity associated with a strong increase of SERPIN-B3 expression.

To confirm those results, we will further investigate *ex vivo* tumor biopsies of patients from a prospective ongoing trial led by the university hospital of Poitiers. We will perform spatial transcriptomic and flow cytometry analyses in order to characterize the relations between OSM-R expression and TIME composition.

In conclusion, OSM-R appears as a new prognosis marker in HNSCC. In addition, we report a pro-tumoral role of OSM in HNSCC development, suggesting that a new therapeutic approach targeting this cytokine could be considered.

P134

Effect of Calcium-dependent channels' inhibition on Gastric Cancer Stem Cells Tumorigenic Properties

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Stomach cancer is currently the 4th cause of mortality by cancer in the world. The majority of cases are gastric adenocarcinomas (GC). Diagnosed late most often at advanced stages, the chances of remission are low. This gloomy prognosis is the consequence of the strong aggressiveness of GCs and their resistance to current conventional treatments of chemo and radiotherapy. Recent work by the research team has identified a rare subpopulation of cells within tumors, called cancer stem cells (CSC), responsible for the initiation, progression, chemoresistance and dissemination of GCs. The team showed that the use of a general calcium flux inhibitor, Verapamil, made it possible to sensitize CSCs to current chemotherapy treatments in in vitro culture models. Store-operated calcium channels (SOCs) consist of stromal interaction molecules (STIM1/2 proteins), which act as endoplasmic reticulum (ER) Ca2+ sensors, and Orai1/2/3 proteins, which form the structure of calcium release-activated calcium (CRAC) channels in the plasma membrane (PM). The two main Ca2+ influx channels of non-excitable cells are the ORAI and TRPC families of Ca2+ channels. The objective of this project was to study the impact of SOCs inhibition on the tumorigenic properties of gastric CSCs in vitro.

Subpopulations of epithelial-like and mesenchymal-like CSC from different GC cell lines and patients derived tumors were analysed by transcriptomic analysis to study the expression of calcium channels genes. Different inhibitors targeting different categories of calcium channels were tested on GC lines rich in CSCs (MKN45) or poor in CSCs (MKN74) in vitro : SKF-96365 and YM58483 inhibit both ORAI1 and TRPC channels ; GSK7975A inhibit more specifically ORAI1 channel. Effects of these inhibitors were determined by proliferation assays and immunofluorescence staining on 2D cultures, and on the tumorigenic properties of gastric CSCs by tumorspheres assays in 3D cultures.

Transcriptomic analysis showed that ORAI1 was overexpressed in epithelial-like CSCs and TRPC6 was overexpressed in mesenchymal-like CSCs. Proliferation assays showed a dose dependent effect of SKF inhibitor on both cell lines while only a slight effect was observed with the others inhibitors. Tumorspheres assays showed that preventive treatment of cells with inhibitors decreased the number of tumorspheres formed attesting a decrease of CSCs population. However curative treatment of tumorspheres already formed had no effect on their growth. Immunofluorescence analyses of the consequences of inhibitors on the localization of the calcium channels are on going.

We demonstrate that SOCs inhibitors have an influence mainly on tumorspheres initiation properties of CSC in GC. Inhibition of both ORAI1 and TRPC channels seems to be more efficient than ORAI1 inhibition alone. A CRISPR-Cas9 strategy will be implemented in order to identify the Ca2+ channels involved in CSC tumor initiation properties.

P135

Hippo pathway effectors are associated with glioma patient survival, control cell proliferation and sterol metabolism through TEAD3

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Glioblastomas represent the most common and lethal primary brain tumors in the world. Despite therapeutic advances during the last two decades, the patient prognosis remains very poor. The Hippo signaling pathway effectors YAP/TAZ-TEADs are strongly involved in tumor progression and represent promising therapeutic targets in gliomas. In this study, we identified and investigated the clinical and biological significances of TEAD transcription factors.

Bioinformatic analysis on TCGA glioma dataset and Immunohistochemistry analysis on 133 glioma patient samples from different molecular subtypes were used to assess the prognostic impact of TEADs. Up to 5 different patient-derived glioblastoma stem cell lines were used to decipher the expression and activation status of the core Hippo pathway proteins. Verteporfin (VP) treatment, siRNA approach and RNASeq analysis were used to assess the biological role.

Differential expression, gene ontology and survival analyses revealed TEAD transcription factors as prognostic markers in TCGA glioma cohort. Moreover, we found on glioma samples that TEAD3-4 protein expression was associated with poor patient outcome. In vitro, we confirmed the preferential expression and activation of TEAD3-4 as well as their transcriptional coactivators YAP/TAZ. Pharmacological inhibition of YAP/TAZ-TEAD interaction by VP significantly decreased tumor cell growth whereas specific inhibition of TEAD3 did not impact cell proliferation but affected sterol biosynthetic and metabolic processes.

This study paves the way toward a better understanding of the role of Hippo effectors in glioblastoma pathophysiology. These transcription factors, especially TEAD3, could represent potential therapeutic targets regarding recent data concerning sterol homeostasis in glioblastomas.

P136

Role of MAPK signaling in T-cell mediated anti-tumor immune responses

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Tumorigenic activation of Mitogen-Activated Protein Kinases (MAPKs) is central for translational reprogramming of cancer cells, as it allows rapid cell adaptation to highly dynamic environment by promoting selective mRNA translation. Amongst these MAPKs, we found that alterations of the MEK kinase MAP3K8 (also known as TLP-2/COT) constitute an alternative to BRAF mutations. Accumulation of MAP3K8 protein correlates with MEK/ERK activation and poor patient outcome in high-grade serous ovarian cancers (HGSOC) which are rarely mutated for BRAF. By combining analysis in HGSOC cohorts of patients and relevant cellular and mouse models, we found that constitutive activation of MAP3K8/MEK promotes tumor growth and confers a new translational landscape by regulating the assembly and activity of the translation initiation complex eIF4F. In addition, first analyses show that MAP3K8 expression in tumor-infiltrating T lymphocytes (TILs) suppresses anti-tumoral responses. Proliferation and activation of TILs increase locally in the tumors upon Map3k8 deletion suggesting that MAP3K8 might restrain T cell activation after cancer cell priming by controlling translation of selected mRNA targets. By using knock out (KO) mouse models, we are now uncovering the intrinsic function of MAP3K8 in TILs and assess changes in the MAPK pathway and mRNA translational programs involved in cancer progression. Deciphering the crosstalk of cancer cells and TILs at the level of mRNA translation will be the basis of future combination therapies enhancing immune checkpoint inhibitor efficacy by targeting MAP3K8.

P137

Role of calcium signaling in glioma cancer stem cells in 2D and 3D environments

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Glioblastoma (GBM) is the more frequent and aggressive brain cancer. Current treatment of this cancer is based on radiotherapy, chemotherapy and surgery. Despite combined therapies, the median survival of the patients reaches only 15 months after the diagnosis. In more than 90% of the patients, the tumor recurs which leads to the death of the patients. This relapse has been ascribed to the presence of cancer stems cells within the tumor. This small subpopulation of cells is endowed with stem cell properties, including proliferation and self-renewal, which allow them to build the tumor and reconstitute tumor after therapies. Glioblastoma stem cells (GSC) respond to microenvironment signals. Among them, calcium is a very subtle messenger that regulates many signaling pathways and cellular functions. Previous studies have shown that GSC transcriptome is enriched in calcium signaling genes. Therefore, we undertook this work to study the calcium signals in human GSC and its role in cellular functions.

In non-excitable cells, the main calcium entry is via calcium channels called "store operated channels" (SOC). Molecules from the microenvironment bind to their receptors and induce the activation of cellular signaling pathways leading to the reduction of endoplasmic reticulum calcium concentration that open SOC. Our data show that GSC express functional SOC that support calcium entries whose pharmacological inhibition decrease GSC proliferation, impair GSC self-renewal, and reduce expression of the stem cell marker SOX2. In addition to their stem properties, GSC display important migration capacities that allow them to invade the brain tissue. To investigate the impact of calcium signaling in GSC, tridimensional environments have been used to mimic the different substrates that GSC will find in the brain. Biocompatible polyacrylonitrile matrixes made of nanofibers have been developed in Institut Européen des Membranes with two nanofibers organizations (aligned and criss-cross) and two stiffnesses (3 kPa and 166 kPa). GSC (isolated cells or sphere) have been laid down on the different nanofibers to study the impact of stiffness and organization on calcium influx as well as proliferation and migration. Our data suggest that specific configurations of the matrixes impact calcium signaling and specific cellular functions.

To conclude, calcium signaling plays a role in GSC and could represent an interesting therapeutical target.

P138

Contributions of mitochondrial Cancer/Testis Antigens (CTA-mt) to cancer metabolism rewiring

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Mitochondria play a central role in cell metabolism homeostasis. In cancer, this function is often altered (Shift to aerobic glycolysis, excessive ROS production, inhibition of mitochondrial-mediated cell death, appearance of mitochondrial DNA mutations…), contributing to tumor development and progression. Therefore, identifying and understanding the functions of actors involved in these metabolic alterations are essential to improve cancer treatment strategies.

Cancer/Testis Antigens (CTAs) are a group of proteins with a unique pattern of expression. Indeed, their expression is normally restricted to the testes, in physiological conditions, but they can be activated or aberrantly expressed in various human cancers. Co-expression of multiple CTAs in different cancers worsens patient's prognosis. This distinctive expression pattern and their contribution to patient outcomes has made CTAs an area of interest in cancer research and immunotherapy (1).

Five mitochondrial CTAs (CTA-mt) were reported acting in mitochondria and we recently reported, using bioinformatic analysis, 147 transcripts encoded by 67 CTAs encoding for CTAs potentially targeted to mitochondria (2). Among them, we identified experimentally, two isoforms encoded by CT55 for whom the function is poorly understood (3). First, we found that patients with tumors expressing CT55 are associated with poor survival. Second, to investigate the role of CT55 on mitochondria, we first show that CT55 is localized to both mitochondria and endoplasmic reticulum (ER) due to the presence of an ambiguous N-terminal targeting signal. Then, we show that CT55 silencing decreases mtDNA copy number and down-regulates aerobic respiration, indicating that CT55 sustains mitochondrial respiration (2). Finally, we show that CT55 silencing decreases dramatically cell proliferation. CT55 is just one out of the 147 transcripts identified by bioinformatic analysis. We are currently investigation a new CTA-mt that plays a role in mitochondrial proteins phosphorylation. This exciting research project focused on Investigating the involvement of CTA-mt in mitochondrial rewiring in cancer could shed light on new mechanisms contributing to cancer hallmarks.

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Posters – Axis 2 “Genome Dynamics and Expression”

P201

Impact of SNPs associated with an increased risk of lung cancers on 3D genome organization in proliferative and senescent contexts

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We have recently shown that numerous genetic cancer-susceptibility sites located at the edges of topological domains (TADs) are likely to influence spatial organization of the genome (Jablonski et al., Human Genomics 16:2, 2022). The effects of the so-called at-risk forms of these variants, which are genetically inherited, generally only become apparent with age. Our working hypothesis is that certain sites of genetic susceptibility contribute through their mutation to changes in spatial organization of the genome and that this effect is amplified during cellular senescence.

We are interested in a region of human chromosome 6 that carries numerous genetic variants, whose high-risk forms predispose to the development of lung cancer. This region, which consists of five TADs, includes a high-risk SNP (rs-13218875) located at the edge of a TAD. Three neighboring SNPs (<25kb) are in strong linkage disequilibrium with it: rs-34788973 and rs-61742093, which are both located in an olfactory receptor gene (OR2B2) and are inducing nonsense mutations, and rs-188015 which is intergenic. A Chromosome Conformation Capture (3C) experiment showed that, in human fetal lung fibroblasts (IMR-90 cells), the border separating TAD2 and TAD3 is precisely located at the OR2B2 gene. In addition, an analysis of ChIP-seq profiles available in international databases indicates that rs-61742093 is located in a region which, in IMR-90 cells, is bound to the CTCF insulator protein. This SNP is therefore the best candidate to explain an increased risk of lung cancer associated with an alteration of this TAD border.

We know that histone genes located in TAD2 are incorporated into Histone Locus Bodies (HLBs). The presence of SNP variants linked to the at-risk form of rs-13218875 could therefore lead indirectly, via increased contact with TAD2, to retention of TAD3 genes and/or their transcripts in HLBs, thereby disrupting regulation of these genes and promoting cancer development. To test this hypothesis, we are using the RD-HRS method, developed in the laboratory (Baudement et al., Genome Research 28:1733-1746, 2018; Lecouvreur et al., in preparation), which determines the rate of association of genomic sequences (HRS-qPCR) and transcripts (HRS RT-qPCR) from a given locus with large RNP complexes. Preliminary RD-HRS experiments carried out in proliferating IMR-90 cells confirm the association of TAD2 histone genes with HLBs and the absence of association of genes located in TAD3.

Our project now consists in inserting (CRISPR/Cas9) into IMR-90 cells the rs-61742093 variant in its form that is in linkage disequilibrium with the at-risk form of the rs-13218875 variant. We will then analyze the alteration in genomic organization around this mutated region (3C and RD-HRS assays) in a proliferative or senescent context, and the consequences for gene expression (RNA-seq).

Our approach should bring new insights to better understand how these sites are linked to the development of cancers.

P202

Role of R-loops in the secondary resistance to EGFR-TKIs in lung cancer

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EGFR tyrosine kinase inhibitors (EGFR-TKIs) are effective targeted therapies for lung cancer with EGFR mutations. However, they are limited by the acquisition of secondary resistances. Few tumour cells with no pre-existing resistance mutations can survive the treatment and become non/slow-proliferating drug-tolerant cells (DTCs). Over time, some of them acquire resistance mutations in the presence of the drug and restore the tumour. To date, the mechanisms by which non-replicating DTCs acquire secondary resistance mutations are unclear. Transcription can be a threat to genome integrity in non-dividing cells, primarily through R-loops, nucleic acid structures consisting of an RNA/DNA hybrid and a displaced single-stranded DNA (ssDNA). Preliminary results show that EGFR-TKI treatment induces R-loop increase in EGFR-mutated lung cancer cell lines. Furthermore, modulation of the R-loop level affects the emergence of resistant cells after prolonged exposure to EGFR-TKIs. Therefore, our preliminary data support a potential role of R-loops as promoters of secondary resistance and our aim is to investigate by which mechanisms.

P203

Rational development of RNA therapeutics against Acute Myeloid Leukemia

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Acute Myeloid Leukemia (AML) is a highly aggressive blood cancer that progresses rapidly and has a low 5-year survival rate of less than 30%. Despite recent advances in cancer therapy, the need for novel and more effective treatments for AML patients remains a matter concern. Recent studies have identified RNA binding motif 39 (RBM39) as a promising target for AML. Through the elucidation of the mode of action of anti-AML drugs called aryl sulfonamides, the essential role of RBM39 in AML cell survival has been pinpointed. Aryl sufonamides, as indisulam, trigger the targeted degradation of RBM39. The drug acts as a molecular glue between RBM39 and DCAF15/E3-ubiquitin ligase [1]. However, when tested on AML patients, this novel therapeutic approach was limited to 30% efficiency which positively correlates with DCAF15 expression level [2]. To overcome this challenge, we have investigated RBM39 gene expression regulation to identify approaches that could selectively induce RBM39 depletion independently of DCAF15. Our research uncovered that RBM39 autoregulates at the mRNA level through a negative feedback loop mechanism that promotes the inclusion of a poison exon (exon 2b) in the mRNA [3]. Based on this mechanism, we aim to develop two distinct RNA therapeutic strategies that should induce RBM39 depletion independently of DCAF15 and, ultimately AML cell death. These approaches could offer the potential for more efficient treatments against AML.

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P204

Transcriptomic analysis as a tool to decipher oxaliplatin induced adaptive response of colorectal cancer cells

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Oxaliplatin (OXA) is a third-generation platinum-based chemotherapy agent used in the treatment of colorectal cancer (CRC). We recently showed that ATR inhibition synergizes with oxaliplatin to induce tumor cell death and antitumor immunity.(1) In order to decipher the molecular mechanisms involved in this synergistic interaction, we performed RNA-Seq analysis.

We performed gene set enrichment analysis (GSEA) of RNA-Seq data obtained from CRC cell line HCT116 treated in vitro for 24 or 48 hours with OXA at 0.5 mM (which correspond to the IC50). We used pathview and SBGNview R packages to perform extensive pathway-based data mapping and visualization. To untangle the fine tuning of these adaptive responses, we performed an in-deep analysis of the subsets of genes (referred to as the leading edge) that contributed the most to the statistical significance of the corresponding gene sets.

Our analysis of mRNA-seq data showed (1) a great negative enrichment of gene sets involved in DNA repair at 24 and 48h hours, including homologous recombination and mismatch repair process, with a concomitant activation of p53 and apoptosis signalling, (2) a widespread positive enrichment of genes sets related to processing of rRNA and ribosome biogenesis and (3) a significant positive enrichment of proteasome related mRNA, among which immunoproteasome subunits are the most upregulated ones. Recent literature confirms the transcriptional inhibition of DNA damage repair (DDR) genes when cells are treated with oxaliplatin and suggest that OXA-induced cell death may depend on ribosomal stress rather than direct DNA damage (2) (3), supporting the results of our study. We then experimentally confirmed the capability of OXA to induce the expression of immunoproteasome β1i and β2i subunits by Western-blot and and proteasome activity quantification.

OXA inhibits transcription of DDR genes and induces ribosome biogenesis stress and expression of immunoproteasome subunits. Immunoproteasome activity may then favors the presentation of new peptides and induce antitumor immunity. Thus, combination of OXA with ATR inhibitor could increase the OXA-induced repression of DDR genes and enhance tumor immunogenicity.

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P205

Mechanisms of regulation of cytokinesis and importance in genetic instability of tumors

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Cytokinesis, an essential step of mitosis, allows the physical separation of cells during cell division. This process must be highly regulated. Indeed cytokinesis defects lead to polyploidy which is a source of genetic instability, a well-established cause of cancer development and progression.

Glioblastoma (GBM) is the most common primary brain tumor with a median survival of 15-18 months. It is thought that resistance to treatments are caused by glioblastoma stem cells (GSC). GSCs are highly prone to mitotic errors, but the causes of these aberrant divisions are still unknown.

Citron Kinase (CitK) was identified as a key regulator of cytokinesis, acting both as a kinase and as a scaffold protein. Its loss of function as well as its overexpression leads to abscission failure, resulting in multinucleated and/or polyploid cells. Its also plays a key role in neural progenitors division and mice lacking Citron Kinase die shortly after birth due to incomplete neurogenesis. In human, CitK mutations cause microcephaly. Globally, CitK regulation is largely unknown.

We have identified that CDK1 phosphorylates CitK *in vitro* and the phosphorylated sites were identified by mass spectrometry. By using phospho-mutants for CDK1, we have shown that one phospho-mutant in particular (named CitK 13PA) leads to an increase of multinucleated cells suggesting cytokinesis failure. Futhermore CitK 13PA interacts more with Borealin, a component of the Chromosomal Passenger Complex and impairs its localization at the end of cytokinesis.

Given the importance of CitK in the division of neural progenitors, we hypothesize that CitK may also be important for GSC cytokinesis and may constitute an attractive target. Our objective is to determine the importance of CitK in GSC division. To do so, we use GSC derived from glioblastoma patients which form spheroids in culture allowing us to work on a 3D model. We choose lestaurtinib to inhibit CitK activity in GSCs. Lestaurtinib is a well-known tyrosine kinase inhibitor that has recently been described as a potent (IC50 of 90nM) inhibitor of CitK activity. Our preliminary data show that lestaurtinib leads to a decrease of GSC spheroids area accompanied with cell death. Further investigations will help us to determine if CitK may be a potential target for glioblastoma.

P207

Relating temporally resolved changes in gene expression and DNA accessibility in an in-vivo model of Acute Myeloid Leukemia chemotherapy resistance using 3D chromatin networks

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Therapy resistance is the main cause of mortality for patients affected by acute myeloid leukemia (AML) however understanding of the molecular and cellular mechanisms underpinning therapy resistance is still incomplete. Alterations in RNA splicing, energy metabolism and the epigenome are hallmarks of AML and more broadly of cancer, and have been associated with the onset of therapy resistance. JE Sarry's team has previously shown that AML chemoresistance phenotype depends on the acquisition of an enhanced mitochondrial metabolism and the activation of an ATF4-dependent pro-survival mitochondrial stress response.

As part of an investigation of the processes involved in the onset of resistance in a mouse model of AML, we have recently produced gene expression and DNA accessibility time courses detailing the different phases in the development of resistance to chemotherapy.

U937 AML cells transfected with an ATF4-GFP reporter vector were subjected to Cytarabine (AraC) treatment and leukemic bulk as well as CD39/ATF4 high or low cells were retrieved 2, 8 and 12 days post AraC treatment to generate RNAseq and ATACseq datasets.

We exploit our recently developed methods to represent chromatin structure as a network, which can be used to integrate different types of epigenomic data in the context of 3D nuclear organization. We build on available 3D chromatin structures for myeloid cells that are thought to be close to the cell of origin of AML to relate changes in accessibility in regulatory regions with target genes, integrating datasets across the time course.

More specifically, we used TCseq to identify gene modules that are altered at different time points, defining different processes involved in early and late stages of resistance. Analysis of the corresponding ATACseq data highlights regions that are altered in these different stages that can be related to specific genes or to more general rearrangements of the epigenome 3D organisation.

P208

Impact of 11p15.5 LOH in oncogenesis: modelling using the CRISPR/cas9 system

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Loss of heterozygosity (LOH) occurs whereby regions of the genome normally heterozygous for polymorphic loci in a particular individual appear to become homozygous. LOH constitute major genetic alterations in many types of cancers. Despite their frequencies, the scientific community still debates about the chronology of LOH appearance and whether they are a cause or a consequence of oncogenesis. They could be primary events, leading to tumor initiation by unmasking, for example, a recessive mutation in a tumor suppressor gene, or secondary events resulting from genomic instability, a hallmark of cancer cells. Modelling LOHs at different loci in normal cells could clarify their respective roles in the tumorigenic process. However, it was, so far, not possible to model them, due to technical limitations, and thus to study their pathogenic contribution. Recent studies of our team on the use of the CRISPR-Cas9 system as a tool for gene therapy for red blood cell disorders have shown that this system induces, in addition to its desired knock-out or knock-in abilities, megabase-scale LOHs from the CRISPR-Cas9 cut-site to the telomere, by deletion or mitotic recombination. For gene therapy applications, these chromosomal events represent a carcinogenic risk and should be prevented. However, if we can generate, on purpose, in non-cancerous cells, LOH at given loci, it would constitute a unique way for understanding their contribution to oncogenesis. We are particularly interested by the 11p15.5 locus, on the distal juxta-telomeric part of the short arm of chromosome 11. This locus includes several imprinted genes and is subjected to frequent LOHs in different types of cancers (breast cancers, Wilms tumours, sarcomas…) and in genetic disorders such as the Beckwith-Wiedemann syndrome (BWS), the most common overgrowth syndrome which is associated with tumour predisposition. This project should enable us to better understand the oncogenic consequences of a genetic alteration such as a loss of heterozygosity, here at the 11p15 locus, and its impact on the oncogenic process, with particular interest in studying the role of genes subject to parental genomic imprinting in this process.

P209
Characterizing new mediators of cell competition

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Intratumoural heterogeneity is found in most solid cancers and describes the differences among cells in the same tumour. Interactions between these cells promote the diversification of malignant and non-malignant clones, and are likely to be involved in therapy resistance and tumour relapse. These interactions seem to be related to the cell quality selection process in growing epithelial tissues to maintain genetic identity and tissue integrity. Cell Competition is a conserved cellular phenomenon contributing to tissue development, homeostasis, and disease. Individual cells within a tissue or organ compete for survival and resources by constantly checking their status regarding metabolism, growth, or genetic identity to favour the strongest cells at the expense of the weakest. Interestingly, the deletion of tumour suppressor genes such as *scribble* or *discs large 1* makes the cells behave as losers in a heterotypic context. These data suggest that cell competition may be involved at multiple levels during tumorigenesis. Comparing mRNA levels in wild-type flies and flies inactivated for *scrib*, in which cell competition is activated, highlighted genes potentially involved in cell competition. We selected the most modulated genes in *scrib* mutant cells and performed a small-scale genetic screen through tissue-specific RNAi-mediated invalidation or overexpression to unveil new players of polarity loss cell competition. Surprisingly, one of our candidates, an uncharacterized factor up-regulated in *scrib* mutant cells, resembles the protein sequence of an already known cell competition mediator. The lab demonstrated that while our candidate cannot promote cell competition alone and is dispensable for normal development, it is indeed required for polarity-loss-mediated cell competition to implement the loser program. I further characterized this protein via mass spectrometry and biochemistry as a general stress protein.

P210

Interplay between enhancers and G4s in transcription regulation and disease

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Enhancers are essential for cellular identity and gene expression regulation. This regulation relies on the recruitment of specific factors, chromatin accessibility and physical contacts with target promoter(s). Besides these features, a significant subset of enhancers are also enriched in G-quadruplexes (G4s) which are G-rich single-stranded secondary DNA structures that have been shown to be associated with open chromatin and gene activation (Esnault et al., 2023). Interestingly, some studies have pointed out to the role of G4s in chromatin loops formation (Hou et al. 2019, Yuan et al, 2023) but also the ability of RNA G4s to impede PcG binding and contributing to chromatin opening (Beltran et al., 2019). Moreover G4s are known to be hotspots for DNA breaks and mutations (Bossaert et al., 2021) often leading to various diseases including cancer.

In our work, we aim to decipher this link using datasets from human and murine cell lines, by comparing them according to their differential G4 content, binding of a large set of chromatin-associated factors, STARR-seq activity and nearby gene expression. As perspectives, we will also detail upcoming strategies for functional in vivo testing, involving knock-in of enhancers with varying G4 content.

P211

Cytoplasmic RNA granules are key modulators of post-transcriptional regulation in normal and cancer germinal centre B cells

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Diffuse large B-cell lymphoma (DLBCL) is the commonest non-Hodgkin's lymphoma accounting for 30-40% of total cases. DLBCLs are classified into germinal centre (GC)-derived B cell lymphomas and activated B cell lymphomas that differ in their origin and mutagenic load. Cytoplasmic RNA granules have been involved in the development and progression of cancers by mediating the expression of oncogenes and controlling mutagenic- associated stress responses. In this study, we characterize the dynamic of cytoplasmic RNA granules in normal and GC-derived cancer B cells (GC B cells). We show that activation of B cells in-vivo and in-vitro is associated with an increase in the condensation of cytoplasmic RNA granules associated with a highly proliferative cellular profile. Using primary cells from paediatric tonsils and cancer cell lines, we identify dynamic modulation of key proteins for cytoplasmic RNA granule assembly such as G3BP1, DDX6, TIA1 and TIAL1 and, by using conditional knockout mouse models, we confirm the essential roles of some of these proteins for GC and antibody responses. In the future, identification of cytoplasmic RNA granules content and its dynamic modulation in GC and cancer cells might reveal novel avenues for better understand the origin and expansion of DLBCL cells and new strategies for therapeutic treatment.

P212

Modelling gene regulation for drug target identification

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Cellular adaptive responses play an important role in cancer cell resistance to chemotherapy agents. They help the tumor cells to deal with different types of insults and contribute to improve cell survival in the setting of pharmacological perturbations. Untangling this response provides precious information for the selection of targeted treatments to complement chemotherapy. This requires the identification of the signaling pathways that drive the response of the cell to the treatment. For this, we hypothesize that we are observing the result of a stereotyped cellular response to chemotherapy (common to any stress induced by pharmacological perturbation) alongside a more specific response. In this context, we propose a strategy aimed at identifying the transcription factors (TF) targeted by the signaling cascades specific to chemotherapy response and responsible for the observed change in gene expression.

We developed a machine learning approach which investigates the link between regulatory sequences (promoters, enhancers) and gene expression. Our model predicts whether a gene is differentially expressed in response to a particular treatment on the basis of a vector of scores reflecting the affinity of TFs (JASPAR Position Weight Matrices database) for its regulatory sequences. Moreover, it has been previously shown (Crow et al. 2019) that certain genes are frequently differentially expressed, regardless of the specific biological question of interest. Our model integrates this stereotyped response in the form of the differential prior score provided by (Crow et al. 2009).

One model is trained for each signaling pathway of the KEGG database, using the PWMs associated with the TFs targeted by the pathway as predictive features, along with the differential prior score. We use a logistic regression with a Lasso penalty (Tibshirani, 1996) to identify key PWMs by minimizing the number of predictive variables. By measuring and comparing the accuracy of the different models, we can identify the most likely pathway(s) used by the cell in response to the chemotherapy.

The method was applied on RNA-seq data performed on HCT116 cells treated or not for 24h with oxaliplatin. Our results show that a large part of the cell response to oxaliplatin belongs to the stereotyped response, but another part is specific to the drug. In this specific response, the signaling pathways linked to the P53 TF family, which have been shown to control specific cell death programmes in response to acute DNA damage, appear as important actors, along with the signaling pathways linked to the TEAD, FOX and JUN TF families.

In conclusion, we propose an *in silico* interpretable approach which enables us to identify the regulatory elements that have the strongest association with the differentially expressed genes and that constitute the targets of the identified signaling pathways driving the cell-specific response to chemotherapy.

Posters – Axis 3 “Therapeutic Innovation and Biomarkers”

P301

The CRISPR/CAS Technology for the detection of rare KRAS mutant alleles

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The diagnosis of pancreatic ductal adenocarcinoma necessitates tumor biopsy by endoscopic ultrasound fine needle aspiration (EUS-FNA), which shows insufficient negative predictive value. As mutations in the KRAS oncogene are very common in PDAC, their detection from circulating tumor material may provide a powerful diagnostic tool for PDAC formal diagnosis but this requires a level of sensitivity challenging the available molecular tools. We assessed the emerging CRISPR/Cas13a SHERLOCK technology promising the high discrimination of genetic alterations, from large intragenic rearrangement to single nucleotide polymorphism.

Guides hybridizing various positions on the KRAS target were designed to detect G12V, G12C and G12C mutant alleles. Mismatches around the single mutation of interest, as well as hairpin spacer sequences were tested to prevent hybridizing the wild-type allele. Mutant allele detection was tested on matrix containing known concentrations and compared to Q-PCR, allele-specific PCR and ddPCR. The sensitivity of a large intragenic rearrangement and a 15nt deletion of the EGFR were compared to that of single KRAS mutation. The combination of an allele specific PCR and a CRISPR/Cas detection (AS-SHERLOCK) of the related products was finally set-up and tested with pancreatic cancer and non-small cell lung cancer liquid biopsy patients' samples.

The position of RNA guide affected the ability of Cas13a to detect KRAS alleles and the possibility to discriminate between different alleles. We observed efficiency variations between mutations, possibly related secondary structures of the matrices and the nature of the mismatches between the guide and the matrix. Hairpin spacer strategy only slightly improved specificity for KRASG12D mutant detection. The detection of a large intragenic rearrangement and a 15 nt deletion mutation in the EGFR gene reached a total specificity and a sensitivity similar to the one of ddPCR. Moreover, AS-SHERLOCK reached performance similar to ddPCR for KRASG12D mutant detection. Finally, AS-SHERLOCK technology allowed the efficient detection of KRAS and EGFR mutant in patients' samples.

As other sensitive tools, CRISPR/Cas13a technology is challenged to detect mutant variants outnumbered by WT alleles. However, the use of highly discriminant guides reached or outperformed the gold standard ddPCR for the detection of rare alleles. It is implemented with a simple workflow, without expensive equipment, and applicable to patients' samples analysis. Efforts are still needed to increase the specific detection of single mutations to avoid initial allele-specific PCR.

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P302

Development of pH-modulated antibodies, that recognize immune system checkpoints

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Targeting and blocking the immune system checkpoints using antibodies is revolutionizing cancer therapy, especially in those that generate many mutations such as melanoma. Since these checkpoint targets are not tumor specific, it is common to generate toxicities like autoimmune syndromes. Interestingly, some approved anti cytotoxic T-lymphocyte associated protein 4 (CTLA4) antibodies showed increased life expectancy of patients with melanoma. Nonetheless, the systemic autoimmune-related adverse effects due to T-Regulator lymphocyte depletion outside the tumor do not allow most patients to continue their treatment. Hence, it would be desirable to promote the activation of T-Effector and eliminate the T-Regulators only in the tumor site to reverse cancer progression. Thus, we can rely on the fact that tumor tissues have a more acidic pH than healthy ones due to the metabolic switch stated by the Warburg effect. Therefore, we plan to generate pH-dependent antibodies able to bind and inhibit the CTLA4 checkpoint only at the tumor acidic microenvironment to promote the activation of the immune system, but not at physiological pH in normal tissue. We will obtain anti-CTLA4 antibodies with reduce systemic toxicities by a phage display selection with two different types of synthetic scFvs libraries, one enriched in histidine residues and one without enrichment, to acquire antibodies against different epitopes of CTLA4. Subsequently, we will reformat and produce the selected pH-sensitive scFvs fragments into full IgG formats to characterize them. After having found the pH-dependent anti-CTLA4 scFv antibodies, we will change their format to IgG and perform further tests. Initially analyzing their capacity to fix on human T-lymphocytes positive for CTLA4 with acidic pH conditions. So that afterwards, the high-profile selected antibodies can be tested in "Knock-in" mice for human CTLA4 to see if they are able to deplete the T-Regulatory cells at the tumor site and not in healthy tissues (e.g., Spleen). Finally, we will study their overall tumor distribution and ability to reduce the tumor mass compared to other published and FDA approved controls.

P303

Sensitizing the PDAC tumor microenvironment to immune checkpoint therapies: characterization of a PDAC 3D model to decipher immune infiltration

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal solid tumors, with an extremely unfavorable prognosis. The dense stroma rich in Cancer-Associated Fibroblasts (CAFs) and the immunosuppressive microenvironment confer resistance to current treatments including combination chemotherapies, targeted therapies or immunotherapies. Moreover, PDAC tumors are poorly infiltrated by T cells, and the majority of immune cells present at the tumor site are immunosuppressive. This cellular context leads to the failure of clinical trials using immune checkpoint inhibitors in PDAC. The immune infiltrate within the tumor is dynamic and depends in particular on soluble factors secreted in the tumor microenvironment (cytokines, chemokines etc.).

The objective of this project is to develop a combinatorial approach using a monoclonal antibody that targets the tumor microenvironment (anti-AXL) combined with interleukin-15 (IL15) associated with conventional chemotherapies (Gemcitabine or Folfirinox) in order to increase immune infiltration. In clinical applications IL15 activates T lymphocytes (LT) and Natural Killer (NK) cells and promotes their infiltration into tumors. However, IL15 treatment causes significant side effects with high toxicity reported in patients. Thus, we have generated an anti-AXL antibody fused to IL15 to associate the immunomodulatory properties of IL15, combined with the target specificity of the anti-AXL antibody. We thus developed a PDAC three-dimensional in vitro spheroid model composed of xenograft-derived tumor cells from PDAC patients, CAFs (primary and immortalized), and peripheral blood mononuclear cells (PBMCs) isolated from healthy donors, with the aim of closely reproduce the complex pathophysiological features of the cancer-stroma found in pancreatic TME. Human 3D models were first set up and characterized by cytometry, imaging mass spectrometry and immunohistochemistry.

We showed that in our heterospheroid models, CAFs promote tumor cell growth, improve resistance to chemotherapy and are able to down-regulate immune cell infiltration and modulate the nature of infiltrated immune cells. These CAF-dependent resistance mechanisms, immune suppression and cancer progression also described in patients, are one of the trademarks of pancreatic cancer. In parallel we showed that, upon anti-AXL-IL15 treatment, PBMCs infiltrate cell line-derived heterospheroids, whatever the tumor cell line, kill tumor cells and disrupt the three-dimensional structure. Moreover, immunophenotyping experiments showed a modification of the nature of immune infiltration, with a strong increase of CD4+ and CD8+ T lymphocytes and NK cell populations infiltration. We also obtained combinatorial effects that positively modulated immune infiltration and allowed a control of spheroid growth by combining chemotherapy (gemcitabine) with our anti-AXL-IL15. Thus, the heterotypic spheroids described in our study are a suitable model to both characterize the influence of CAF on therapeutic effects and the mechanisms that drives immune suppressive microenvironment. Next step will be to perform in vivo tests using an orthotopic model to verify whether this approach enhances effector immune cells infiltration in vivo, thereby sensitizing the PDAC microenvironment to anti-PD1 therapies.

P304

A proteomic prognostic signature of resectable pancreatic ductal adenocarcinoma

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Early identification of patients with resectable PDAC at high risk of early mortality has strong clinical and prognostic value. The aim of this work is to compare proteomic expression in tumor tissues from good-prognosis (GP) and poor-prognosis (PP) patients in order to determine a proteomic signature that can predict prognosis after CPP for readily resectable PDAC.

During the study period, 9 good-prognosis and 7 poor-prognosis patients were analyzed. The clinical and anatomopathological characteristics of both groups were similar. A comparison of protein abundance between tumor tissue and healthy tissue was performed for each patient.

Among over 1800 proteins analyzed, 47 were differentially expressed between the two groups, including the translation elongation factor eIF5A, which plays a major role in gene expression of interactome proteins. The impact of inhibiting the expression of this protein and exploring the eIF5A-dependent proteomic expression link were studied.

Expression of eIF5A transcripts was significantly lower in the sh eIF5A versus sh LUC cell population (0.19 versus 0.03, p=0.005). At day 20, sh eIF5A population was significantly lower than the control population (79.6±6.7% versus 41.4±1.8%, p=0.004). RPS 13, RPS 20 protein expression was significantly higher in the cell population transduced with the Sh LUC lentivector versus Sh eIF5A (RNA abundance respectively 0.18 versus 0.03; 1.43 versus 0.21; 3.62 versus 0.50; 0.19 versus 0.05, $p<0.05$).

The eIF5A factor has a demonstrated role in the aggressiveness of PDAC and is at the origin of a network of proteins sharing the same functional pathway. Future studies will confirm the validation of this proteomic signature on larger multicenter cohorts, search for this signature in patients' tumor biopsy or blood and evaluate the possibility of exploiting this signature for a targeted therapy inhibiting this eIF5A factor.

P305

Mechanisms of radiation-induced cell death in patient lymphocytes

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Fifty percent of cancers patients will benefit from radiotherapy (RT) as part of their treatment. Approximately 5 to 10% of all patients undergoing RT will develop serious late side effects. Early identification of these patients is therefore essential. The development of the radiation-induced lymphocyte apoptosis (RILA) assay showed that it was possible to predict the intrinsic radiosensitivity of patients suffering from a cancer based on the rates of radiation-induced T lymphocytes apoptosis. A prospective multicenter study involving more than 500 patients showed that patients with a high RILA >16% do not develop breast fibrosis in the long term while patients with a low RILA <12% have a risk of developing a side effect.

This project is dedicated to understanding the mechanisms of individual radiosensitivity of healthy tissues. Until now, no data have been published on the mechanisms that could explain these differences between low and high RILA values among patients. Our hypothesis is that patients' lymphocytes with a low RILA were subjected to a stress which could have activated lymphocyte stimulation causing radiation-resistance.

We first validated on patient samples that stimulated lymphocytes have a lower rate of radiation-induced apoptosis than unstimulated lymphocytes, showing as in the literature that stimulated lymphocytes are more radiation-resistant. We also observed that T lymphocytes after stimulation undergo a decrease in their RILA value compared to the same unstimulated T lymphocytes. Next, we analyzed in patients' samples the activation state of lymphocyte sub-types by flow cytometry. We observed more CD25+ cells in the CD8 patients' lymphocytes with low RILA indicating a greater activation state of T lymphocytes in these patients.

In order to identify biomarkers involved in radiation-induced and radiation-resistant lymphocytes apoptosis signaling pathway, we used a Reverse Protein Phase Array (RPPA) approach. Several proteins over or under-expressed due to lymphocyte stimulation were selected for further validation. One of them, the survivin, an anti-apoptotic factor known to be overexpressed in patients with chronic inflammatory disease happened to be overexpressed as well in stimulated lymphocytes. This result was confirmed by Western Blot and Proteome Profiler.

All together these results showed a correlation between radiation-resistance and lymphocytes activation and the possible involvement of survivin in the radiation-resistance lymphocyte apoptosis. These data will need to be further validated on a larger cohort of patients.

All translational studies will be carried out on samples from healthy individuals from EFS and from patients (cancers patients and patients with chronic inflammatory diseases) from an ancillary study, coordinated by the ICM (PROBA clinical trial, inclusion in 2024). The data from this project will allow us to understand why lymphocytes from different patients react differently to ionizing radiation and thus explain the variations in RILA values (highs and lows). In the longer term, these results will help understand the link between the resistance of lymphocytes to radiation-induced apoptosis and the development of breast fibrosis in these patients.

P306

Metformin Treatment Reduces CRC Aggressiveness in a Glucose-Independent Manner: An In Vitro and Ex Vivo Study

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As per the World Health Organization (Globoccan, 2020) report, colorectal cancer (CRC) ranks as the third most commonly diagnosed cancer in males, following lung and prostate cancer, and as the second most prevalent in females, trailing breast cancer. Despite advancements in diagnostic and screening technologies, CRC remains a formidable adversary, with recent upticks in occurrence among individuals under 50 in high-income nations. Furthermore, CRC frequently reoccurs even when detected at its initial stages. The TNM (Tumor Node Metastasis) classification categorizes CRC into five stages, ranging from 0 to IV. In stage 1, the mucosa is the sole area affected, progressing to stage 2 with involvement of the muscularis, stage 3 with lymph node invasion, and stage 4 with the presence of metastases.

Epithelial-mesenchymal transition (EMT) is chiefly characterized by the loss of epithelial markers like E-cadherin and the activation of cell movement, partly attributed to extracellular matrix modification by metalloproteinases such as MMP2 and MMP9 (1). EMT is a pivotal process promoting the dissemination of cancerous cells, particularly in epithelial cancers like CRC. The reduction of epithelial markers, including E-cadherin, facilitates EMT and is linked to the aggressiveness of CRC (2). **Metformin**, primarily employed in the management of type 2 diabetes due to its actions on mitochondrial metabolism and AMPK, has been investigated for its inhibitory effects on EMT in various cancer types, although its impact on colorectal cancer remains unexplored (3).

This research aims to investigate the influence of metformin on the suppression of EMT-related genes, migration, and invasion in colorectal cancer cell lines (HCT-116 and SW-620), along with a cohort of 23 patients. Special attention is given to its effect on E-cadherin and MMPs. In addition, it was investigated whether this was due to the action of metformin on AMPK. To assess the effect of glucose on metformin-induced EMT inhibition ***in vitro***, all experiments were conducted under **two glucose conditions**, mirroring fasting blood glucose (7.8 mM) and hyperglycemic conditions (17.5 mM). The ***ex vivo*** experiments involve **patients in stage 1, 2, and 3 of CRC**, either with or without diabetes-metformin treatment.

The results indicate that metformin gives favourable results and can prevent the early stages of colorectal cancer. Metformin appears to influence E-cadherin cleavage during EMT, notably by acting on MMP2 and MMP9 through activation of AMPK. Under *ex vivo* conditions, metformin shows promise in the early stages of colorectal cancer. However, further experiments are needed to validate these results.

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P307

Overcoming osimertinib resistance in EGFR-driven-MET amplified lung cancer

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Patients diagnosed with EGFR- mutated lung adenocarcinoma are treated with specific EGFR-inhibitors such as osimertinib. However, inevitably, most patients relapse. A subpopulation of cells called Drug Tolerant Persisters (DTP) seems to be responsible for the tumour recurrence. About 20% of relapse is due to the amplification of the oncogene MET with currently no therapeutic alternative than conventional chemotherapy. Our aim is to establish whether DTPs are associated with the NOTCH pathway, a crucial pathway in lung adenocarcinoma and/or MET.

EGFR-driven cells with or without MET amplification were treated with a combination of EGFR, NOTCH or MET inhibitors. We infected these cells with the FUCCI system to monitor HES1 expression (read-out of Notch pathway activity) during the cell cycle by cytometry upon different drug treatment. Tet-on-EGFRT790M/L858R transgenic mice have been treated with the combination of EGFRi and NOTCHi by oral gavage 5 days per week.

Upon EGFRi treatment, we observe a fluctuation of the NOTCH pathway activity over time. Initially, HES1 drops drastically in DTPs while Notch1 intracellular domain increases significantly. Then, once DTPs are expending, the trend is reversed. In vitro, EGFRi/NOTCHi combination delays the expansion of DTPs compared to EGFRi alone and additionally, Met-amplified osimertinib-resistant cells demonstrate strong sensitivity to EGFRi/METi and EGFRi/NOTCHi combinations.

EGFRi treatment affects the NOTCH pathway activity and targeting NOTCH in combination with EGFR brings therapeutic benefit as it delays the relapse in vitro in EGFR-mutated cell lines. A preclinical in vivo evaluation is currently on going. In the presence of MET amplification, EGFRi/METi and EGFRi/NOTCHi treatments showed moderate therapeutic benefits, what about tritherapies with a NOTCH pathway inhibitor?

P308

Combination of chemotherapy, hormonal therapy and radiotherapy in the treatment oligo-metastatic prostate cancers

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This study aims to optimize combinations including hormonal therapy, taxane-based chemotherapy, and radiotherapy in the treatment of prostate cancer. The objective is to determine whether this triple combination could be used in oligo-metastatic situations for which stereotaxic irradiation of metastases is considered, but for which there are currently no standard treatments. A preliminary in-vitro study on the LNCaP cell line (Lymph Node Carcinoma of the Prostate) allowed us to determine that the most effective triple combination was the one in which docetaxel was administered 24 hours before the radiotherapy plus enzalutamide combination, the triple concomitant association being less effective. In order to identify biomarkers involved in the different response between concomitant and sequential treatment, we used a Reverse Protein Phase Array (RPPA) approach. The first validated results show a differential expression of certain proteins (phosphorylated or not) with a significant increase in □H2AX in the sequential treatment, indicating an increase in DNA damage with this treatment. Furthermore, we observed a difference in the expression of several key proteins from different signaling pathways, in particular an inhibition of Akt1 phosphorylation in the sequential treatment. The in-vivo validation of these results is in process.

The results will allow a better understanding of the mechanisms of action of combination treatments and will be used to identify new biomarkers and/or new targets whose inhibition could improve the tumor response to this triple combination. These data will also be useful to consider using this treatment for oligometastatic prostate cancer in order to improve its effectiveness.

P309

TuLYPPe: Treatment of Burkitt's LYmphoma by Photophoresis coupled with Photodynamic therapy (PDT)

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Anti-cancer therapy using non-conventional, non-invasive and less toxic treatments such as photodynamic therapy (PDT) has been the focus of a great deal of attention for several years now in the study of several so-called 'solid' cancers (skin, colorectal, prostate). PDT is a therapeutic strategy using photosensitisers (PS) that are non-cytotoxic in the absence of photoactivation. Few studies are currently looking at PDT treatment in so-called "circulating" cancers, due to the difficulty of targeting cancer cells. The high incidence of Burkitt's lymphoma worldwide and its aggressive nature (the highest incidence of non-Hodgkin's lymphoma in children aged between 5 and 15), as well as the difficulty in gaining acceptance for treatments (side effects accentuated in children), illustrate the need for new treatment concepts such as PDT to improve the prognosis and quality of life of affected patients. The innovative idea behind this project is to use an extracorporeal photophoresis (ECP) system, which is a therapeutic method that involves irradiating leukocytes previously collected by apheresis and combining it with PDT to reach these circulating tumour cells. Building on the expertise of LABCiS (UR 22722) in the development of new photosensitisers, our innovative strategy will optimise therapeutic targeting while reducing side-effects in the treatment of Burkitt's lymphoma. One of the advantages of this treatment using extracorporeal photophoresis coupled with PDT is that it will ultimately enable a broad spectrum of lymphomas to be targeted.

The emergence of the project is characterised by the Chemistry-Biology-Physics interface, which is the very essence of the LABCiS team and in collaboration with the XLIM UMR CNRS 7252 laboratory. This is an interdisciplinary project: the chemists will design the molecules, the physicists will set up a system reproducing photopheresis and the biologists will set up the in vitro and in vivo models. The project is divided into 3 tasks: (i) creation of a fluidic and optical system recreating the photophoresis/PDT combination, (ii) synthesis and characterisation of vectorised PS and (iii) in vitro and in vivo study of this treatment in Burkitt's lymphoma.

The selected photosensitiser: Purpurin-18 is produced from chlorophyll extracted from the cyanobacterium Spirulina maxima. This molecule was then nanovectorised: this involves reducing the concentration of an already effective molecule without altering its efficacy, to facilitate its entry into cancer cells. Working with the IMP (Imagerie Photonique Moléculaire) team (UMR5255 CNRS), Purpurine-18 was attached to primary amines present on the surface of natural nanoparticles developed in the Bordeaux laboratory. The result is a nanoparticle capable of transporting a PS that produces singlet oxygen in the presence of light. The compound has a very short lifespan and a limited radius of action, which means that its action can be limited to the immediate vicinity of the irradiation site. We began the biological analysis to test the antiproliferative effect, as well as the pro-apoptotic power of this compound on HT29/HTC116 colorectal cancer lines (in control) and RAJI/BL2 Burkitt's lymphoma cell lines. To do this, we performed cell viability tests (MTT), then analysed cell localisation, as well as the apoptotic pathways leading to cell death after treatment.

P310

Evaluation of new purpurin-18-based nanoparticles in anticancer photodynamic therapy

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Colorectal cancer (CRC) is currently the third most commonly diagnosed cancer with persistently high incidence and mortality rates and more than 930,000 deaths annually worldwide. Despite significant advances in CRC treatment, many adverse effects are often observed. Thus, new targeted therapeutic approaches remain necessary to improve patient's quality of life. Photodynamic therapy (PDT), as a non-invasive focal therapy, is designed to destroy tumor cells while sparing the surrounding healthy tissue. It is based on the use of photosensitizers (PS) that induce cell death when photoactivated. Over the last few years, purpurin-18 (Pp-18) has been used as an effective PS in PDT for several types of tumors. In the context of our work, we developed and vectorized Pp-18-based photosensitizers within cellulose nanocrystals, which enhances tumor penetration by EPR (Enhanced Permeability and Retention) and the therapeutic index of Pp-18. To evaluate the *In vitro* anticancer effects of nano-vectorized Pp-18 against colorectal cancer, two human colorectal cancer lines were used: HCT116 and HT-29. For this purpose, we assessed the cytotoxic and phototoxic effects after irradiation, molecular localization, apoptosis induction through the molecular markers, and DNA fragmentation, as well as ROS production. Our preliminary results show that photoactivation of nano-vectorized Pp-18 induces significant photocytotoxicity in both cell lines, making these innovative new nanoparticles promising candidates for PDT in colorectal cancer.

P311

Fascin-1 inhibitors decrease hepatoblastoma cells tumorigenicity via YAP 1

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Hepatoblastoma (HB) is a liver tumor that arises in children. It's a sporadic malignancy that is often very aggressive. The current treatment consists of chemotherapy. However, chemotherapy in young patients has disastrous and long-term side effects such as ototoxicity, cardiomyopathy and infertility. Thus, alternative strategies are needed. One hint is to target the most common mutations in HB. It has been demonstrated that 90% of HB tumors are mutated for the Wnt pathway effector ß-catenin. This mutation leads to an aberrant constitutive activation of Wnt/ß-catenin signaling. Here, we investigate one of ß-catenin transcriptional targets, Fascin-1 that is found up-regulated in many tumors. Fascin1 affects actin organization into bundles and this leads to cell migration and invasion. Whereas Fascin-1 is absent from normal hepatocytes, we found its expression associated to the poor prognosis C2 subtype of HB. In both human and murine HB samples, Fascin-1 is associated to undifferentiated tumor cells. We further demonstrated that Fascin-1 expression modulates tumor hepatocyte differentiation status through gene expression. In this study, we investigate how Fascin-1 is able to regulate tumor cell plasticity and whether Fascin-1 is a druggable target in HB tumors.

We use two classical HB model cells Huh6 and HepG2 and 3 Patient-Derived-Xenograft cell lines. We explore the effect of Fascin-1 actin-bundling activity impairment by using inhibitors NPG2044 and BDP13176, on invasion and migration using Trans-well and wound-healing assays. We follow proliferation and cell death by Flow cytometry and investigate gene expression by PCR and reporter assay. We investigate Fascin modulation of the kinome with PamGene technology.

We show that the inhibition of Fascin actin-binding activity decreases cell invasion and migration as well as proliferation. We show an increase of cell death in Huh6/HepG2 cells but not in the PDX models. Differentiation genes are overexpressed and EMT genes are repressed. Yap expression, is downregulated; Yap promoter activity is downregulated and Yap is found translocated into the cytoplasm upon Fascin-1 inhibition. These data suggest that Fascin inhibition effects on cells are mediated via the Hippo pathway.

Fascin-1 is an interesting target in hepatoblastoma, commercialized phase-2 drugs are available and this study will confirm the potential use of those drugs in HB treatment and elucidate by which mechanism Fascin-1 inhibition impacts tumors.

P312

Tools for analysing spatial data in the context of immuno-oncology

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Immunotherapies are imposing themselves as a revolution in cancer treatment, giving hope to many previously incurable patients. They aim to restore the natural defences mounted by the body against cancerous cells, such as lymphocytes, which are often disarmed by the tumour. Despite the promising potential, around 30% of patients do not benefit at all from immunotherapy. Better characterising the inter-cellular interactions in the tumour microenvironment (TME) will be key to propose new kinds of immunotherapy.

Given the recent discoveries about the importance of specific interactions between immune and cancer cells, understanding the spatial properties of tumors at single-cell resolution becomes crucial. We have recently developed computational tools to describe spatial patterns and clustering of specific cell types in tissues using network theory. These approaches allow us to extract statistical properties from imaging or spatial-omics datasets that can be used as biomarkers.

In particular, we have developed tools to extract information from biological images in the form of cellular networks, in which cells are nodes and edges are present if the cells are close to each other in the tissue. Our tysserand python package (tysserand, Coullomb and Pancaldi 2021, https://github.com/VeraPancaldiLab/tysserand) is better and faster at reconstructing these cellular networks than previous approaches allows us to perform a quantitative analysis of the topology of these networks.

Briefly, the nodes of this network represent single cells and can be associated to a cellular phenotype, which can be either a cell type, as can be easily achieved using single markers for specific cell types in the image) or a cell state, which can be defined based on multiple markers. So far we have developed methods to quantify the spatial localization of different immune and cancer cells within the tumour microenvironment as identified, for example, by multiplex Immuno-fluorescence experiments (a few markers per panel).

Making these networks can be useful to compare samples, extract biomarkers, and also make simulations of the tissues with realistic statistical distributions.
We also developed mosna (https://doi.org/10.1101/2023.03.16.532947), a Python package that exploits these networks to analyze spatially resolved experiments and discover patterns of cellular organization.

We apply concepts from network theory (assortativity) to measure the extent to which cells of a particular type/state cluster with each other and with cells of different cell types/states (mixing patterns). We are thus able to spot whether the presence of T-cells in a tumour involves close contacts between them and the cancer cells or if other cells (for example macrophages or myeloid suppressive cells) are preventing this direct contact. Published approaches mostly rely on distance between cell types as measure on the images to predict survival or response to therapies, or sometimes ratios of distances. We have show on public data that the cell type assortativities can be more predictive.
The detection of preferential interactions between specific cell types can also be used to populate tissue simulations for in-silico cancer models.
We have tested the method on different types of spatial proteomics and transcriptomics and have used public data from cancer patient samples annotated with clinical response to immunotherapy. *mosna* can identify a number of features describing cellular composition and spatial distribution that can provide biological hypotheses regarding factors that affect response to therapies.
Finally, *mosna* uses the Neighborhood Aggregation Statistics method to assign a feature vector to each cell, describing the composition of its neighborhood with different statistics. Applying clustering of the cells based on these neighborhood features, we can identify niches, specific subsets of interacting cells, that can also be used to predict response to immunotherapy.

P313

Evaluation of tumor heterogeneity in colorectal cancer and its implication in metastatic process

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Colorectal cancer (CRC) remains the 3rd most diagnosed cancer in Europe. Liver represents the most frequent metastatic site of colorectal cancers and about 50% of patients with colorectal cancer develop this kind of metastases, despite therapeutic advances and new medico-surgical strategies. Consequently, better understanding of the cellular and molecular biology of colon cancer and liver colorectal metastasis are urgently needed. This study was designed to determine the key process and proteins associated to metastatic dissemination in CRC, in order to identify potential biomarkers for accurate prognosis and establishment of personalized therapies. Thereby, transcriptomic (RNAseq) analysis were first performed on 60 pairs of primary colorectal tumors and liver metastases from the same patients and the more significant markers were validated using RT-qPCR and immunostaining. Some of the identified genes were previously reported linked to cancers but never linked to colon cancer. Others genes were specifically dysregulated in metastatic tissues are with unknown function, never reported in cancer. The functional validation of the identified genes in the metastatic process will help not only for patient stratification and prediction of CRC patients that will develop colorectal liver metastasis but also in the potential development of pertinent therapeutic options for each patient.

P314

Development of a novel technology based on surface plasmonic resonance for detecting tumor circulating biomarkers in Glioblastoma

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Glioblastoma (GBM) is the most common and most aggressive brain tumor. Despite advances in diagnosis, median survival remains very poor due to tumor heterogeneity and the emergence of cells resistant to radio- and chemotherapeutic treatments. However, tumor biopsies do not allow intra-tumor heterogeneity to be visualised, making it difficult to monitor patients longitudinally in order to adapt management and prevent the risk of recurrence. In this context, the identification and detection of circulating biomarkers by liquid biopsy are of prime importance to improve diagnosis or prognosis. Extracellular vesicles (EVs) are considered a major source of circulating biomarkers. In GBM, the truncated EGFR variant (EGFRvIII) expressed in 45% of GBMs is present in EVs and affords a target of choice. However, its detection in liquid biopsy-derived EVs with current methods is not convenient with routine clinical applications, due to their lack of sensitivity for detecting biomarkers at low concentrations in patients' blood. Our goal is to develop an innovating technology based on surface plasmon resonance (SPR) within a fibre for ultrasensitive detection of circulating tumor biomarkers in GBM.

P315

Magnetic hyperthermia tumor ablation and tumor microenvironment modulation monitored by optical imaging

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Magnetic hyperthermia (MHT) represents a promising tool for cancer therapies but requires fine control of the thermal power dissipated by magnetic nanoparticles (MNPs) to achieve the complete tumor ablation while preserving surrounding healthy organs. A better control would also make it possible to sensitize its microenvironment by mild hyperthermia in order to potentiate the efficiency of therapeutic agents. The present work reports the real-time monitoring by optical imaging of local MHT at different thermal power used to induce cancer cell death and microenvironment sensitization in vivo.

MNPs are multifunctional hybrid nanoparticles composed of iron oxide nanoflower surrounded by a near-IR fluorescent silica shell. These MNPs are functionalized with quaternary ammoniums enabling them to expose permanent positive charges in order to improve residence time in extracellular matrix of tumors and promote endocytosis. Mouse prostate cancer (RM1) cells were modified for luciferase firefly (Fluc) or nanoluciferase (Nluc) constitutive expression. RM1-Fluc were implanted into WT mice and RM1-Nluc into transgenic thermo-sensitive mice by subcutaneous injection. Thermo-sensitive mice express Fluc under a thermo-sensitive promoter Hsp70. After intratumoral injection of MNPs, magnetic hyperthermia was generated using an in vivo setup and monitored by optical imaging.

RM1-Fluc tumors injected with MNPs were submitted to magnetic induction and the viability was followed by bioluminescence imaging (BLI). The results showed a significant decrease of BLI signal attesting of a partial or total tumor thermo-ablation correlated to a measured dissipated thermal power. RM1-Nluc tumors were implanted into transgenice mice expressing Fluc under thermo-sensitive promoter. Tumors were injected with MNPs and submitted to magnetic hyperthermia. Viability of tumor and tumor microenvironment activation were followed by BLI. Results showed a decrease of BLI signal into the tumor attesting a thermos-ablation. In contrast, we observed apparition of BLI signal from the transgenic mice attesting of a microenvironment activation. Results obtain in vivo show that, by injecting a dose of iron oxide ten times lower than that used for clinical trials, core tumor thermoablation can occur while inducing thermo-sensitive gene expression in the peripheral microenvironment.

We demonstrate that in vivo optical imaging is a powerful method to monitor MHT in preclinical studies by using both BLI models and fluorescent MNPs. In particular, we found conditions of dissipated thermal power allowing both the core tumor thermo-ablation and microenvironment sensitization. Once controlled, MHT would enable to potentiate conventional therapies by combining of thermos-ablation and mild hyperthermia.

P316

Targeting the TNBC tumour microenvironment with Exatecan-conjugated anti-Cathepsin-D ADC

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Triple Negative Breast Cancers (TNBC), which account for 15% of Breast Cancer (BC) patients (Estrogen Receptor-negative, Progesterone Receptor-negative, HER2 non-amplified), are mainly treated with chemotherapy and urgently require new targeted therapies. The aspartic protease Cathepsin D (Cath-D), a poor prognosis marker in BC including TNBC, is overexpressed by BC cells and hypersecreted in the tumour microenvironment. In BC extracellular Cath-D displays protumour activities by proteolysis at acidic pH and also acting as a ligand on stromal fibroblasts. Cath-D is an eligible target for ADC (Antibody Drug Conjugate) therapy in TNBC because it is associated with the tumor cell membrane in 85.7% of 147 TNBC samples analyzed (Ashraf*, Mansouri* et al, JITC, 2019). In addition, a previous study showed that extracellular Cath-D bound to anti-Cath-D monoclonal antibodys (mAbs) is endocytosed together with the mAb by TNBC and also by stromal fibroblasts (Laurent-Matha et al, J Cell Sci, 1998). Therefore, anti-Cath-D ADCs should allow the intracellular release of the payload in all cell components of the tumor (e.g., TNBC and stromal cells). The anti-Cath-D F1M1-187 was conjugated to Exatecan (topoisomerase I inhibitor).

It binds specifically to secreted Cath-D (EC50 = 2.4nM), internalizes within 3h and induces a 2D cytotoxic effect in vitro on cell lines recapitulating different subtypes of TNBC. ADC F1M1-187 could be a new therapeutic option for the treatment of TNBC patients.

P317

Transcriptomic Profiling of the Non-Small Cell Lung Cancer (NSCLC) Microenvironment Identifies a Duality in Natural Killer Cell Behavior

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Cancer is the second leading cause of death globally. Lung cancer is the leading cause of cancer death worldwide, with a survival rate of 7 (small cell lung cancer) to 28% (non-small cell lung cancer) after 5 years. Our current understanding of the complex processes defining cancer is insufficient to treat a majority of patients effectively. In a clinical setting, bulk transcriptomics is often the assay of choice to screen and characterize groups of patients quickly and effectively in a cost-effective fashion. Here, we applied a computational immunology approach involving differential expression and pathway analyses, immune cell proportion quantification by deconvolution, transcription factor activity inference and immune score estimation in order to better characterize bulk RNAseq dataset of lung adenocarcinoma (LUAD) samples. This analysis allowed us to identify biomarkers of disease progression and potential immune infiltration patterns across disease stages. Through our methodology and novel feature integration pipeline, we identified a duality in the behavior of natural killer (NK) cells in the tumor microenvironment (TME), suggesting a potential association with immune response or dysfunctional states. We validate these findings in an independent LUAD cohort with bulk and scRNAseq samples, allowing us to further characterize the NK cell subsets into dysfunctional (reduced cytotoxic potential), peripheral and tissue-resident NK populations.

P318

NSCP1 is involved in nucleolar stress resistance and represents a promising anti-cancer therapeutic target

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Cancer cells are addicted to high levels of ribosome production to sustain their high proliferation rate. Impairment of ribosome production is sensed by cells as a "nucleolar stress" (NS) that triggers p53-dependent and independent response pathways leading to cell cycle arrest and apoptosis. Therefore, specific inhibitors (CX-5461; BMH-21) that target ribosome production are being tested in clinical trials. Furthermore, other chemotherapies (5-FU, Doxorubicin) not originally developed to target ribosome synthesis also induce NS response, that plays a role in their therapeutic benefits. However, mechanisms of NS resistance have been observed in cancers, without their origin being identified.

The 5S particle, constituted by the association of 5S rRNA to ribosomal proteins L5 and L11, is instrumental to NS response. After ribosome assembly defects, the 5S particles accumulates as a free form outside the ribosome. This free form is able to sequester Mdm2 and inhibits its function, that promotes p53 stabilization following NS. In order to study how cancer cells could resist to NS, we purified free-5S particles and identified a new uncharacterized partner called NSCP1 'Nucleolar stress Counteracting Protein 1'.

Here, we will present our data showing that NSCP1 depletion increases the sensitivity of U2OS and HepG2 cell lines to NS, while its overexpression promotes their resistance to the latter. Furthermore, in some cancers (HCC, Head and neck and adrenocortical carcinomas), high expression of NSCP1 correlates with poor overall survival in patients expressing TP53WT. In adrenocortical carcinomas, a rare pediatric disease with poor prognosis, high level of NSCP1 expression significantly correlates with poor overall survival compared to low expression of NSCP1 (p adj value= 0.01). This indicates that NSCP1 is a prognostic marker for certain cancer types that could be targeted to increase the sensitivity of cancer cells to NS (chemotherapies). In order to determine how to target NSCP1, we carried out a structural and functional characterization and were able to show that NSCP1 competes with MDM2 for free-5S binding. This ability most likely hijacked by cancer cells to resist to nucleolar stress.

P319

Improving the TA99 immunomodulatory effect with combination therapies using a new controlled delivery technology in oncology

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Melanoma is the most aggressive form of skin cancer at metastatic stages, the overall survival rate is 32% (American Cancer Society). The first line treatments for the unresectable and metastatic melanoma are anti-PD1 immunotherapies or anti-BRAF and anti-MEK targeted therapies for BRAF mutated patients. While the patient response rate to immunotherapies is often low (around 40%) and unpredictable, the resistance to targeted therapies is prevalent at almost 100% occurrence post-treatment. Combined therapeutic approaches are more effective, however the associated increased drug toxicity and the severe side effects often cause the suspension of the treatment. Our main objective is to overcome these treatment burdens by exploring new combined therapeutic approaches using a biodegradable delivery system, which is able to locally deliver therapeutic molecules. In addition to the improved efficiency, drugs will be released in a controlled manner thereby aiming to avoid systemic drug toxicity.

We previously demonstrated in the B16F10 subcutaneous melanoma that intraperitoneal administration of TA99, a monoclonal antibody (mAb) targeting melanoma cell specific surface antigen TYRP1, was able to induce partial tumor protection trough immunomodulatory mechanisms (They et al, Oncoimmunology, 2017). In this new study, we used the B16F10 wild type melanoma syngeneic mouse model to study how an immunomodulator currently used in clinic, may synergize with TA99, to generate a sustained and protective antitumoral immune response. Combined therapy was compared to monotherapies by evaluation of safety, tumor growth and survival rate. Tumor challenge consisting of B16F10 cells graft in the opposite flank of the survival mice was performed to evaluate anti-tumoral immune response.

Next steps will consist of understanding the anti-tumor mechanisms and how anti-tumoral immune response is impacted following this therapeutic protocol.

P320

Combination of immune checkpoint inhibitors with chemotherapies in new biological models of upper tract urothelial carcinomas

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Upper Tract Urothelial Carcinomas (UTUCs) are aggressive tumor of ureter and renal pelvis, representing 5 to 10% of urothelial cancer. Standard care treatment consists in a platinum-based chemotherapy, combining cisplatin or carboplatin with gemcitabine, but relapse is observed in more than 50% of the patients. Despite UTUCs are mostly classified as "cold" or "infiltrated by non-effective immune cells" tumor, previous work [1, 2, 3] showed that classical UTUC chemotherapies are immunomodulatory. This suggest that the combination with immune checkpoint inhibitor (ICI) may be a good option to improve patient standard care. To this extend, many different clinical trials tried to introduce ICI in combination in urothelial carcinoma (mostly bladder primary tumor), but they failed to show a real benefice on patient survival. Interestingly, in the JAVELIN III phase III clinical trial [4], avelumab as maintenance therapy after chemotherapy treatment succeed to increased significatively patient survival, whereas Imvigor130 [5] or DANUBE [6] clinical trials, respectively testing atezolizumab or durvalumab combined concomitantly with chemotherapy failed to show a benefice. Even if it is impossible to compare those different clinical trials, testing different immunotherapies in different cohort of patients, we decide to study the effect of different therapeutic sequences of the combination of chemotherapy and ICI, and we are currently trying to identify specific biological marker of response. However, in vivo models of this cancer do not exist for now to be able to study immunotherapies.

We developed an in vitro 3D heterotypic model, consisting in a coculture of immune cells from heatlhy donors or from bladder cancer patients, and tumoral cells from bladder or upper tract origin. We analyzed responses using imaging cytometry (Celigo), immunophenotyping by FACS, transcriptome analyses by RNA-seq and RT-qPCR, and proteomic analyses by western-blot.

We showed that avelumab was the more effective ICI in our model (compared to durvalumab, atezolizumab, pembrolizumab and nivolumab) and we found that the sequential administration of chemotherapy and ICI is more effective that the concomitant's one for all ICI tested. With immunophenotyping, we showed that our model has a strong B-cell infiltration. Finally, our preliminary results with patient's immune cells allowed us to identify differences in responses that can be correlated with the clinical response of the patient.
Conclusion : We showed that the 3D heterotypic spheroid model is able to reproduce same tendency as seen in the clinic and allow us to test different sequences of treatment and ICI. The long-term goal is to propose a simple, fast and biologically relevant in vitro way to test immunotherapies with the patient PBMCs, to guide the clinician choice.

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Study of irreversible electroporation-induced cell death in spheroids derived from a murine hepatoma cell line

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Liver cancer, and predominantly hepatocellular carcinoma, is the third leading cause of cancer death. The most common ablation method, namely radiofrequency ablation, is a thermal method. When the tumor is located close to a vital structure, like the portal vein, the risk of damage is a major drawback thus more selective methods should be used. In this context, Irreversible Electroporation (IRE) has emerged as a novel non-thermal ablation method. Electroporation is the phenomenon of cell membrane permeabilization, caused by the application of short and intense electric fields, resulting in cell death in the case of IRE. IRE has proven its effectiveness and safety by eliminating tumors while preserving surrounding tissues, yet cases of relapse among certain patients are still an issue. One potential reason for that is the heterogeneity of the electric field effectively covering the tumor. Additionally, on its own, IRE may not elicit an immune response substantial enough to achieve complete disease remission. Combining IRE with a chemotherapeutic or immunotherapeutic agent could kill remaining cancerous cells. In addition, finding the optimal time frame at which damage associated molecules or proteins are released, could improve the efficacy of immunotherapy. In this regard, we explored IRE-induced cell death, its amplification by chemotherapy (bleomycin) and its immunogenicity. Unlike classical experiments, which rely on 2D in vitro cellular models, our experiments were made on 3D multicellular spheroids, which are morphologically closer to small avascular tumors.

In this work, we have applied pulses of electric fields to spheroids composed of murine hepatoma-derived cells (Hepa 1-6), stably expressing the green fluorescent protein (GFP). Through fluorescent imaging, we have analyzed the growth of spheroids over a period of 4 days after the treatment (80 monopolar pulses of 100 µs duration, applied at a pulse repetition rate of 1000 Hz with various electric field intensities). Immediately after the treatment, the spheroids undergo a quick and transient swelling. We observe a complete permanent loss of GFP fluorescence at 2000 V/cm and above. Remarkably, at 1500 V/cm, the spheroids transiently lose almost all fluorescence, but a subset of cells survives and is capable of proliferating rapidly. In this condition, the addition of bleomycin, an anticancer drug, completely inhibits cellular growth.

To understand the immunogenic potential of IRE-induced cell death, we studied the release of major known Damage Associated Molecular Patterns: ATP and HMGB1. A strong ATP release was observed right after the IRE treatment, while HMGB1 was detectable in the medium after 3 to 6 hours, depending on the level of electric field applied. This disparity of events in time was also observed when we looked at the activation of the caspases 3/7, a hallmark of apoptosis. Indeed, in our experiments, we observed a peak of caspase activation 3 and 6 hours after treatment, at 2500 and 1500 V/cm respectively.

IRE is a complex treatment that requires the careful insertion of multiple needle-shaped electrodes around the tumor. In this context, it can be difficult to reach a sufficient intensity of electric field covering the entire tumor. As our findings revealed, a sub-therapeutic electric field leads to a strong relapse. In our experiments, the addition of bleomycin was able to compensate for the inefficacy of the electric field, killing residual cancer cells. Furthermore, we have observed the release of ATP and HMGB1, two DAMPs capable of stimulating an immune response. These results should be considered in the future for the optimization of the timing of combined therapy involving IRE and immunotherapeutic agents. Further in vivo studies performed by our group will validate these results in more complex models (hetero- and orthotopic tumors).

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Persistence of netosis in resected colon cancer patient and impact on circulating DNA applications and post-surgery colon cancer management care

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Photodynamic therapy in colorectal cancer using photosensitizers functionalized by arene-ruthenium complexes

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Colorectal cancer (CRC) is the third most common cancer and the second most common cancer cause of death globally, accounting for roughly 1,2 million new cases and 600 000 deaths per year. CRC mainly originates from a benign tumor or adenomatous polyp that evolves into a dangerous malignant tumor. The metastatic stage is the most dangerous when the cancer cells have acquired the ability to detach from the initial tumor and invade other organs, through the blood or lymph, and create secondary tumors. Recently, new therapies have been developed to improve existing treatments by increasing their effectiveness, reducing side effects for patients by better targeting tumor cells, and targeting CRC resistant to conventional treatments. Several therapies were enrolled to this content in a way to find the best solutions for this disease. Nanomedicine and laser therapy for focal treatment such as photodynamic therapy (PDT) are one of the effective ways to treat CRC. PDT using photosensitizers (PS) presents itself as an original innovative therapeutic strategy that strongly limits the undesirable side effects of conventional cancer treatment. Its strength lies on the principle of destroying tumor cells while preserving surrounding healthy tissue. PDT has been approved for the treatment of certain cancers through the generation of cytotoxic reactive oxygen species (ROS) only after the photoactivation of PS. However, low physiological solubility and a lack of selectivity of PS towards tumor sites are the principal restrictions of their current clinical use. To overcome the problem of solubility and stability of PS such as porphyrins and their derivatives, metallic assemblies based on arene-ruthenium units have begun to attract considerable attention as PS delivery systems. Indeed, the conjugation of porphyrins to peripheral metal moieties is an interesting strategy for the development of compounds that may combine the cytotoxicity of the metal moiety with the phototoxicity of the porphyrin chromophore for additive antitumor effects. Thus, targeted drug delivery systems is an imperative evolution in cancer therapy.

The purpose of this study was to demonstrate firstly the interest in the vectorization of tetrapyridylporphin arene-ruthenium (TPyP-arene-Ru) and Zn-tetrapyridylporphin arene-ruthenium (Zn-TPyP-arene-Ru) metallacages to increase their solubility in biological media and then, consequently their anticancer efficacy in PDT. Secondly, to elucidate the anticancer mechanisms, as well as identify the cell death process mediated by these new vectorized PS-arene-Ru associated to PDT. The results showed that the two PS-arene-Ru have a strong photocytotoxic effect after photoactivation on two human CRC cell lines, HCT116 and HT-29 (IC_{50} of the nanomolar range). TPyP-arene-Ru-PDT induced a remarkable cytotoxicity when compared to the Zn-TPyP-arene-Ru analogue. The two PS-arene-Ru showed no significant effect on proliferation in the dark on the two human CRC cell lines. Consistent with other PDT studies, PS-arene-Ru complexes generated cellular ROS production once photoactivated by PDT. In addition, our results demonstrated that under light, these PS-arene-Ru induced phosphatidylserines externalization, caspase-3 activation, poly-ADP ribose polymerase (PARP) cleavage, an appearance of sub-G1 peak and DNA fragmentation. All these data confirmed that cell death induced by PS-arene-Ru-PDT is dependent on an apoptotic process.

P324

Role of CD39 on γδT cells as a positive marker of anti-CD20-induced ADCC of non-Hodgkins lymphoma cells

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T lymphocytes play a critical role in anti-tumor immunity. γδ T cells have emerged as a key immune cell type in cancer biology, representing very attractive and promising candidates for cancer immunotherapy[1,2]. They recognize tumor cells independently of the major histocompatibility complex and have the ability to eradicate them. γδ T cells can express the Fc receptor CD16 that enable antibody-dependent cell cytotoxicity (ADCC) and through their particular properties be used in allogeneic condition. This represents a major interest especially in non-Hodgkin's lymphomas (NHL) in which γδ T cells and especially vγ9vδ2 T lymphocytes participate in the anti-cancer response[3]. γδ T cells expressed CD39[4,5], an ectonucleotidase playing an important role in the adenosine pathway[6], that is involved in immuno-escape[7] and antibody-dependent cell phagocytosis mechanisms in NHL[8]. However, the role of CD39 in ADCC induced by an anti-CD20 therapeutic antibody has never been assessed. Here, we endeavored to fully characterize vγ9 T cells during their culture and evaluate the role of CD39 in their anti-tumoral activity in presence of anti-CD20 antibody.

We examined 12 blood samples from healthy donors during the culture after activation by BrHPP in presence of IL2. We determined vγ9 T cell phenotypes and evaluated the expression of CD16 and different immune checkpoints whose CD39. In parallel, we measured their ability to promote ADCC in NHL co-cultures in the presence of an anti-CD20 antibody. All these experiments were carried out with fresh and thawed PBMC, in order to compare their properties for future *in vitro* use.

PBMC were followed and characterized from day 0 to day 30 of culture. We observed that:
-vγ9 T cell lines can be established if their basal percentage in the PMBC was superior to 0.7%
-Highest percentage of vγ9 T lymphocytes was reached after 13 days of culture
-CD16 expression varies between donors (from 0 to 55%)
-TIM-3, BTLA and LAG-3 were highly expressed compared to PD-1, CD39 and TIGIT

Functional experiments allow to demonstrate that ADCC (B cell depletion) of NHL cells co-cultured with vγ9 T cells is enhanced by anti-CD20, and is positively correlated with CD39 expression and LAG-3.

We showed that activation of either thawed or fresh PBMC with BrHPP/Il2 enables the establishment of functional vγ9 T cell lines. Moreover, with the full characterization of ICP expression, it appears clearer how vγ9 T cell lines can be used for *in vitro* preclinical studies dedicated to immunotherapy screening. Finally, we identified CD39 as a positive marker in ADCC of NHL cell lines. Obviously, its role needs to be clarified by using specific inhibitory strategies to open new therapeutic perspectives.

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P325

Helicobacter pylori induces pancreatic lesions in a mouse model of gastric carcinogenesis

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Gastric cancer, the 4th cause of cancer mortality worldwide, is mainly caused by a chronic infection with the bacterium *Helicobacter pylori*, which colonizes the stomach lifelong. It induces chronic gastritis, evolving in some cases to intestinal metaplasia, dysplasia and adenocarcinoma. Many studies have tried to correlate Helicobacter infection with disease in extra-gastric digestive organs like the pancreas. It has been reported in *H. pylori* infected patients that this infection could affect the physiology of the pancreas without colonising it directly.

In this study, we evaluated the consequences of mice infection with different strains of gastric Helicobacters on the histopathology of their pancreas. We performed histopathological analysis of HES-stained paraffin-embedded pancreas tissue sections to evaluate fibrosis, inflammation and other lesions.

Preliminary results suggest that mice infected with *H. pylori* for 12 months developed chronic pancreatitis and fibrosis, known precursor lesions of pancreas cancer. Understanding the impact of *H. pylori* infection on lesions of extra-gastric organs could help *in fine* prevent the emergence of other digestive-track related diseases.

P326

Role of Exosomes in the Brain Dissemination of Tumor Cells derived from Breast Cancer (Locoregional and Distant)

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Breast cancer is the most common cancer in women, with approximately 2.3 million new cases worldwide each year. Currently, due to extended disease control through therapeutic advancements, an increase in the incidence of brain metastases is observed (5 to 30% of patients depending on the country). The development of brain metastasis is a poor prognostic factor, and at present, the mechanisms explaining the cerebral tropism of breast cancer remain poorly understood.

It is now known that exosomes, small extracellular vesicles secreted in large quantities by cells, including cancer cells, are involved in the establishment and maintenance of the tumor niche. In this context, tumor-derived exosomes (TDEs) are particularly interesting because their content (mRNA, microRNA (miRNA), proteins, and lipids) is specific to the secreting cell. Recently, it has been demonstrated that cancer stem cells (CSCs) are capable of secreting exosomes. These exosomes enter the bloodstream and, due to their content, contribute to the formation of pre-metastatic niches in secondary organs. Migrating cancer stem cells can thus establish themselves in these secondary targets and form metastases. To date, few studies have examined the role of breast CSC-derived exosomes in tumor progression and the formation of brain metastases.

To carry out this project, we will first study the stem cell characteristics of cells obtained from brain metastasis samples of breast cancer patients. These samples are collected at the University Hospital of Poitiers, in the neurosurgery department. Cell lines are then established, and their in vitro characterization is performed. In parallel, the concentration of exosomes in the samples will be determined using the ZetaView by Particle Metrix (Nanoparticle Tracking Analysis), as well as the presence of exosomal and/or stem cell markers using a double fluorescence colocalization module (488/660 nm).

In the second phase, the exosomal content will be described for proteins, RNAs, and lipids. We hope to obtain an exosomal signature (biomarker) in patients that can subsequently be searched for directly in exosomes isolated from blood samples of patients with different types of breast cancer (hormone receptor status, HER2 status).

This potential new biomarker may enable long-term monitoring of patients during chemotherapy treatments and in the remission phase of the disease to address possible recurrence or therapeutic resistance. Finally, understanding the mechanisms involved in the brain dissemination of cancer stem cells from breast cancer will be necessary for the development of effective targeted therapies for patients.

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

P401

Prise en charge du cancer de la prostate localisé chez les sujets âgés de 75 ans et plus dans le département de l'Hérault

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Du fait de l'accélération du vieillissement de la population, la prise en charge du cancer de la prostate localisé (CaPL) chez le sujet âgé est un problème difficile : il faut éviter les sous et surtraitements et les traitements curatifs sont rarement réalisés.

L'objectif de cette étude est d'analyser les modalités de la prise en charge du cancer de prostate localisé (CaPL) (T1-4, N0-X, M0-X) chez les sujets de 75 ans et plus dans un département français (Hérault) et de leurs conséquences sur leur survie.

Nous avons fait une étude de cohorte rétrospective du 01/01/2017 au 31/12/2020 des données d'un registre français spécialisé en onco-urologie (RHESOU : Registre Hérault Spécialisé en Onco Urologie) chez les patients de 75 ans et plus présentant un CaPL. Les patients ont été divisés en 2 groupes : le groupe 1 pour les patients âgés de 75 à 79 ans et le groupe 2 pour les patients de 80 ans et plus. L'inclusion a concerné tous les CaPL sans biais de sélection : les diagnostics fortuits sur pièces de cystoprostatectomie (CPT), après chirurgie de l'hypertrophie bénigne de la prostate (HBP), les cas sans preuve histologique (code 8000/3 des registres) et les diagnostics sur biopsies prostatiques (PBP).

Une analyse univariée selon les deux groupes d'âge a été réalisée. Les tests du Khi-2 et de Fisher ont été utilisés pour comparer les caractéristiques des patients, de leur tumeur et de leur prise en charge.
La survie relative a été modélisée à partir d'un modèle de Cox et ajustée sur les caractéristiques de ces patients.

1 077 patients ont été inclus, 606 dans le groupe 1 et 471 dans le groupe 2. Pour les 2 groupes, le diagnostic a été fortuit dans 243 cas et sur découvert sur PBP dans 807 cas. Dans 27 cas, il n'y avait pas d'histologie. Pour les diagnostics sur PBP, 410 (85.1%) patients du groupe 1 ont eu un traitement curatif contre 118 (36.3%) dans le groupe 2. La majorité des traitements curatifs ont été une radiothérapie, 64.7% dans le groupe 1 et 33.6% dans le groupe 2. Dans le groupe 2 il y a eu 48.9% de primo hormonothérapies. Il y avait dans ce groupe plus de comorbidités et le CaP était de plus haut risque que dans le groupe 1.

La survie en fonction des traitements réalisés a été ajustée sur l'âge et le facteur de risque de d'Amico. Les patients traités par hormonothérapie isolée ont une survie proche des patients mis en surveillance simple (survie à 5 ans : 83.9% [76.1 ;92.5] et 82.0% [74.5 ;90.1] respectivement).

Les patients âgés de 80 ans et plus ont un CaPL de plus haut risque. Ils ont eu peu de traitement local et l'hormonothérapie a été surutilisée sans améliorer la survie par rapport à une surveillance simple (contrairement aux recommandations des sociétés savantes). Les traitements de ces patients ne doivent pas se faire sur l'âge chronologique mais sur le pronostic du cancer et l'espérance de vie.

P402

Améliorer les connaissances et la participation des personnes avec une déficience intellectuelle au dépistage organisé des cancers dans les départements du Gard, de l'Aude et l'Hérault (France) : Une étude Interventionnelle de l'Association Oncodéfi

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Les personnes avec une déficience intellectuelle (PDI), c'est-à-dire un trouble cognitif et adaptatif, quelle qu'en soit la cause, apparu avant l'âge de 18 ans font autant de cancers que les personnes de la population générale. Cependant, elles participent peu aux dépistages organisés des cancers. Chez les PDI, les cancers du côlon et du sein sont souvent diagnostiqués à des stades avancés. En complément des actions de sensibilisation au dépistage des cancers auprès des éducateurs, cette étude a pour objectif d'évaluer l'efficacité et l'impact d'une information sur le dépistage des cancers directement auprès des PDI.

Cet essai contrôlé randomisé en cluster à deux groupes parallèles, en ouvert, compare l'effet d'une intervention de sensibilisation, directement auprès des PDI pour l'acquisition et le maintien des connaissances sur le dépistage des cancers. Le groupe testé reçoit une sensibilisation sur le dépistage, et le groupe contrôle sur l'hygiène bucco-dentaire. Le questionnaire (18 questions) a été élaboré avec l'aide d'une professeure de pédagogie spécialisée dans l'apprentissage des PDI (Université de Fribourg en Suisse). Ce questionnaire est complété quatre fois par les participants : quinze jours avant l'intervention, le jour de l'intervention, 3 mois après et un an après. Les connaissances sur le dépistage (score 1) et le comportement à adopter face au dépistage (score 2) sont comparées aux différentes périodes. La sensibilisation menée auprès de personnes avec DI légère (QI 50 à 70) et moyenne (QI 35 à 50) est animée par une infirmière pour chaque groupe d'une dizaine de personnes. Elle s'appuie sur des outils (diaporama, livret de dépistage, film, ateliers) en langage adapté facile à lire et à comprendre (FALC) créés par l'association en collaboration avec des PDI. Un groupe de PDI a participé aux différentes étapes de l'élaboration et de la conduite de l'étude.

L'étude a été menée dans 38 établissements médico-sociaux des départements de l'Hérault, du Gard et de l'Aude, incluant 620 PDI. Elle montre qu'une sensibilisation au dépistage des cancers directement auprès de PDI est réalisable. Les PDI ont montré un grand intérêt pour l'étude en maintenant leur participation à la deuxième et troisième étape. Le faible taux d'attrition inférieur à 13% (pour 30% acceptable selon la méthodologie) et des résidents qui avaient initialement refusé de participer à l'étude et souhaitent ensuite la rejoindre en témoignent. Les premiers résultats de l'analyse des questionnaires à trois mois (c'est-à-dire l'objectif principal de l'étude) seront connus et présentés lors du congrès.

La première étude (à notre connaissance) sur les capacités d'apprentissage des PDI sur le dépistage du cancer, montre sa faisabilité. Elle révèle aussi l'intérêt et la forte implication des PDI pour leur santé. Le caractère interactif du mode de présentation du message qui comportait des ateliers avec jeux et d'importants temps de discussion ont été des éléments déterminants pour le succès de sa transmission. L'évaluation du questionnaire à trois mois et à un an permettra de voir si les acquisitions ont été faites, sont maintenues et lesquelles. Il est prévu de suivre l'évolution de la participation au dépistage du cancer chez les PDI dans une recherche complémentaire.

L'étude PAM est soutenue par un financement de l'Institut National du Cancer (INCa) et un soutien de la Caisse Primaire d'Assurance Maladie de l'Hérault.

P403

ACERCA : connaissances et ressenti des éducateurs sur le cancer. Analyse de 933 questionnaires

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Les cancers des personnes déficientes intellectuelles (PDI) sont mal connus des aidants professionnels éducateurs des établissements médico-sociaux (EMS). Pourtant, ils sont aussi fréquents que dans la population générale. Il en résulte des diagnostics souvent tardifs et des traitements difficiles. Pour réduire ces retards il est important 1) d'expliquer les facteurs de risque de cancers afin de favoriser la prévention, 2) d'encourager le dépistage et 3) le repérage des symptômes pouvant révéler un cancer. Cela nécessite la participation des éducateurs qui ont une position privilégiée puisqu'ils accompagnent au quotidien les résidents. L'étude ACERCA (Aidants Connaissances Et Ressentis du Cancer) évalue les connaissances et le ressenti des aidants professionnels sur le cancer. Elle sert de base pour élaborer et délivrer, en tenant compte de leur ressenti sur cette maladie, une formation adaptée sur la prévention et le dépistage des PDI aux éducateurs et autres professionnels des EMS qui n'ont pas reçu d'enseignement sanitaire.

ACERCA est la première étude française menée auprès des aidants professionnels dans 11 départements (Nord, Côtes d'Armor, Île-de-France, Bas-Rhin, Loire-Atlantique, Loire, Gironde, Lozère, Gard, Aude et Hérault). Elle utilise un questionnaire anonymisé comportant 12 questions (testé auparavant pour une étude au Royaume-Uni par une des co-auteurs DW) pour évaluer les connaissances. Les questions portent sur l'âge d'apparition, la fréquence, les différents types de cancers, les moyens de réduire les risques, les signes précoces qui nécessitent une consultation médicale et les contrôles mis en place par les services de santé pour dépister les cancers. Un score évalue le niveau de connaissances. En complément, 6 groupes d'expression (tenus à Paris, Bordeaux, Nice, Strasbourg, Cambrais et Montpellier) ont permis de mieux connaitre les freins psychologiques des éducateurs sur le cancer. Les informations recueillies lors des groupes d'expression ont complété les données fournies par l'analyse des réponses sur le ressenti dans le questionnaire.

Sur 1205 questionnaires complétés par des aidants professionnels, 933 ont été retenus dans 65 établissements médico-sociaux de France métropolitaine pour analyse. Des connaissances insuffisantes ont été révélées concernant : l'âge de survenue des cancers (20% de réponses justes), les moyens de réduire le risque de cancer (35% de réponses suffisantes), les signes précoces qui doivent faire consulter un médecin (45% de réponses suffisantes). Il est significatif que seulement moins d'un quart (24%) des professionnels testés connaissait les trois dépistages organisés des cancers. Dans les six groupes d'expression nous avons constaté, un manque de connaissance minimale sur la façon de prévenir, dépister et repérer les cancers d'une part, et d'autre part une peur de la maladie. Nous faisons l'hypothèse que cette peur pourrait expliquer le défaut en connaissance, et que le manque de connaissance sur le cancer renforce la peur de cette maladie. En tout cas, une augmentation d'information permettrait d'augmenter l'efficacité des éducateurs dans la prévention et le repérage précoce.

Cette enquête, la première menée en France, révèle des connaissances incomplètes sur le cancer des éducateurs, ce qui rend difficile un bon suivi des résidents. Les professionnels ont souligné leur besoin de formation et d'information claires et pertinentes sur : les traitements, les spécialistes médicaux concernés, le parcours de soins du cancer chez les PDI. Ainsi, les supports actuels de communication sur le cancer pour les éducateurs peuvent être complétés par des moyens tenant précisément compte des lacunes en connaissances et des freins psychologiques des aidants professionnels.

L'étude ACERCA est soutenue par un financement d'AG2R la mondiale, du Comité National Coordination Action Handicap (CCAH) et par la Caisse Nationale de Solidarité pour l'Autonomie (CNSA)

P404

ISCaO : soutien à la prise en charge des cancers chez les personnes vivant avec un trouble du développement intellectuel, vers ISCaF

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Les cancers des personnes vivant avec un trouble du développement intellectuel (PDI) (2.5% de la population) sont aussi fréquents qu'en population générale, mal connus, découverts tardivement et difficiles à traiter. Les professionnels du secteur médico-social sont très peu familiarisés avec les problèmes médicaux et particulièrement le cancer, et sont souvent désorientés pour accompagner un patient en soin. Le dispositif ISCaO de l'association ONCODEFI a été mis en place pour alerter les éducateurs des institutions médico-sociales sur le risque de cancer et réduire les retards diagnostiques. Le dispositif a aussi pour but de soutenir et guider les équipes médico-sociales lorsqu'un résident est confronté à un cancer.

Historique : Oncodéfi a conduit dans le département de l'Hérault l'étude pilote ARII (Action-Recherche Infirmière-Institutions) en 2016-2017 soutenue par Malakoff-Médéric Handicap. Cette étude, qui répondait aux demandes des équipes médico-sociales confrontées à des résidents touchés par un cancer, a révélé des besoins pour la prise en soins des résidents. L'action ARII s'est prolongée par la création du dispositif ISCaO (Infirmiers de Soutien à la prise en soins des PDI atteintes de Cancer en Occitanie), créé en fin 2018 et financé depuis par l'ARS Occitanie. ISCaO poursuit l'action mené par ARII et l'étend aux 12 autres départements de la région. Elle mène aussi des sensibilisations sur le dépistage et la prise en soins des cancers chez les PDI auprès 1) des professionnels du secteur du médico-social, 2) des étudiants des secteurs sanitaire et social, et 3) des PDI elles-mêmes. Fonctionnement : Actuellement, ISCaO comporte trois infirmiers pour les treize départements d'Occitanie. Les actions se font, sauf empêchement exceptionnel, par déplacement sur site.

Les sensibilisations auprès de 1212 personnes, 205 éducateurs, 374 PDI, et 623 étudiants en IFSI et IFAS ont été menées en 2022. L'analyse des questionnaires avant et après sensibilisation indiquent un gain de connaissances des professionnels et une évolution des intentions de pratique. Une amélioration du score avant et après formation est également relevée pour les personnes avec DI légère et moyenne. Les enseignements dispensés aux étudiants sont reconduits chaque année, et les nouvelles demandes montrent l'intérêt des enseignants dans les IFSI et IFAS. Une enquête montre que les interventions ISCaO (entre 10 et 15 nouveaux accompagnements par an) ont aidé à mieux comprendre la maladie, l'état du patient, la gestion de la douleur et à prendre les décisions dans l'intérêt du résident. Elles ont permis de réduire les tensions psychologiques, au sein des équipes et avec les autres résidents, lors de la découverte d'un cancer et les soins qu'il nécessite.

Après cinq années d'activité ISCaO, ONCODEFI propose, en réponse aux demandes, de développer le même modèle dans deux autres territoires. Le projet de diffusion ISCaF (pour France) doit débuter en région Grand-Est début janvier 2024. Les nouvelles équipes seront composées de deux infirmiers et d'un médecin. Le programme de formation pratique et théorique prévu sur neuf mois inclut une période d'immersion à Oncodéfi, suivie d'une mise en place sur site. Un audit externe évaluera l'efficacité des dispositifs reproduits.

ONCODEFI anime en Occitanie dispositif ISCaO pour encourager le dépistage, développer la prévention ; et soutenir les équipes médico-sociales souvent démunies lorsqu'un résident est touché par un cancer. Ce dispositif (ISCaF) va être reproduit pour les établissements médico-sociaux de deux nouvelles régions en 2024-2025.

Le dispositif ISCaO est soutenu par l'ARS Occitanie. Le projet ISCaF est soutenu par la fondation Malakoff-Humanis et la Ligue Nationale contre le Cancer.

P405

Cancer des personnes avec trisomie 21, fréquence, répartition et pronostic

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Les données accumulées depuis deux décennies indiquent que les personnes avec trisomie 21 (T21) ont un profil tumoral particulier (Satgé et al 1998), remarquable par un excès de leucémies et une réduction marquée de l'incidence des tumeurs solides. La précision de ce profil permettra de proposer un suivi médical adapté et la participation de ces personnes au dépistage des cancers. Il a été proposé de ne pas pratiquer de mammographie de dépistage vu la rareté des cancers du sein et, par précaution, de maintenir la participation au dépistage du cancer colique devant l'incertitude de l'incidence exacte de ces cancers dans la T21 (Rethoré et al 2020). Nous présentons les tumeurs de l'adulte et de l'enfant T21 et leur évolution sur une période de 25 années dans le département de l'Hérault. Pour la première fois les données oncologiques des personnes T21 sont confrontées à celles des personnes avec une déficience intellectuelle (PDI) toutes causes confondues.

L'étude CHAID (pour Cancer Hérault Adulte Déficience Intellectuelle) étudie les cancers des PDI diagnostiqués à l'âge adulte ≥ 20 ans observés dans le département de l'Hérault et recensés par le Registre des Tumeurs de l'Hérault (RTH). Les tumeurs des personnes T21 pour les années 2008-2023 seront comparées avec celles des personnes avec déficience intellectuelle, non trisomiques, qui ont développé un cancer durant la même période. Les cancers des enfants et adolescents (≤ 20 ans) sont recueillis à partir des données du service d'Oncologie pédiatrique du CHU de Montpellier depuis 2008.

Pour les adultes T21, 11 cancers ont été signalés : 4 cancers du testicule et un de chaque du système nerveux central, colon, rein, sein, de l'ovaire, une maladie de Hodgkin et un mélanome malin cutané. Chez les adultes avec DI d'une autre cause, 226 cancers ont touché 216 personnes parmi lesquels 32 cancers du sein et 19 cancers du côlon-rectum. Les cancers ont été diagnostiqués à un stade avancé ou très avancé et 5 patients sont rapidement décédés de leur cancer. Pour les enfants, 4 leucémies ont été recensées, l'un d'eux est décédé des suites de sa maladie.

Les PDI développent autant de cancers que les personnes dans la population générale (PG). Alors qu'on estime que les personnes T21 représentent à peu près 10% du groupe total des PDI, leurs cancers ne comptent dans ce recueil que pour 4.6%. Cette valeur (11 trouvés, 22 attendus) est en concordance avec l'incidence réduite de moitié des tumeurs de l'adulte trisomique (Hasle et al 2016). Les cancers du testicule des hommes T21 représentent dans cette série la moitié des tumeurs testiculaires de tous les hommes DI de l'étude CHAID. Chez les enfants les 4 cas rapportés, pour 0,2 attendus sur la base des données du RTH, suggèrent une incidence augmentée (x 20). La découverte tardive des cancers chez les adultes incite à instaurer une surveillance régulière, particulièrement des testicules chez l'adolescent et le jeune homme trisomique 21. Les propositions de dépistage et suivi médical pour les personnes T21 (Réthoré et al 2020) sont confortées par ces données.

L'étude CHAID et l'expérience d'un service d'Oncologie pédiatrique retrouvent le profil tumoral des cancers chez l'enfant et l'adulte trisomiques 21, avec une augmentation d'incidence des leucémies et une augmentation moindre des tumeurs du testicule ainsi qu'une forte réduction des cancers solides de l'adulte. La trisomie 21 exerce une protection contre les cancers solides. Néanmoins, une vigilance particulière est nécessaire pour diagnostiquer les tumeurs à un stade précoce.

L'étude des cancers chez les personnes trisomiques 21 est soutenue par la Fondation Jérôme Lejeune. L'Institut National du Cancer soutient l'étude CHAID.

P406

Cancers des personnes avec polyhandicap: données actuelles

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Les personnes polyhandicapées (PPH) ont un retard mental sévère (QI<35) ou modéré (QI<50) pour le polyhandicap élargi, associé à un handicap moteur important. On estime à environ 30 000 le nombre les PPH en situation de dépendance complète en France. Cette pathologie individualisée en 1965 reste mal connue. A la suite d'observations de cancers diagnostiqués à un stade avancé chez ces personnes, il est nécessaire de déterminer leur risque tumoral pour afin de proposer une surveillance médicale adaptée, notamment une participation (ou non) au dépistage. Pour contribuer à la connaissance de ce risque nous avons réalisé une revue de la littérature et ajoutons une série de cas observés en Hérault extraits de l'étude CHAID (Cancer Hérault Adultes avec Déficience Intellectuelle).

La recherche bibliographique a utilisé le terme ''profound intellectual and multiple disability'' (PIMD ou PMD) croisé avec le mot ''cancer'' et les types principaux selon les organes et le type histologique (carcinome, sarcome, leucémie, lymphome…) Nous avons inclus des données japonaises (Pr Motoi Nishi) car dans ce pays depuis 1999 une politique nationale de santé a été instaurée pour les PPH. La recherche des cas dans la série de cancers des personnes avec déficience intellectuelle en Hérault a inclus les observations de PPH au sens strict et de polyhandicap élargi pour la période 2005 à 2022.

La revue de la littérature a permis de recenser 135 cancers chez 133 PPH au Japon. Ils avaient une répartition particulière avec un excès de tumeurs digestives (80/135), urinaires (12) et gynécologiques (10). Ils ont été diagnostiqués à un âge plus jeune (39 ans pour les cancers de l'œsophage vs 71 en population générale et 48 vs 71 pour le colon), mais à un stade souvent très avancé. Dans une série pédiatrique italienne il n'y avait pas de différence significative de pronostic en comparaison des enfants sans déficience. Dans une institution au Japon où le dépistage organisé des cancers était systématique, 7 des 9 cancers (surtout digestifs) ont pu être traités avec de bons résultats.
En Hérault 10 PPH (3 de cause génétique, 4 liés à des évènements périnataux, 2 à des encéphalites et 1 à un accident de la voie publique) ont présenté 11 cancers ; colon (2), ovaire (2), endomètre (2), amygdale (1), prostate (1), testicule (1), voies urinaires (1) et myélome (1). Chez 5 patients les tumeurs ont été diagnostiquées à un stade avancé et tous sont décédés de leur cancer. Pour quatre autres le cancer a été trouvé à un stade curable et l'évolution est restée inconnue pour un patient.

L'espérance de vie auparavant très réduite des PPH avait masqué la pathologie cancéreuse qui à présent révèle avec l'avancée en âge de ces personnes. La répartition des cancers est différente de celle de la population générale et de celle des personnes avec déficience intellectuelle, ce qui peut s'expliquer par des facteurs de risque spécifiques : pas de surpoids, pas d'exposition au tabac et à l'alcool.
Dans la littérature et dans notre série de 11 cancers, qui est la plus importante rapportée à ce jour, le pronostic est fortement dépendant du stade au moment du diagnostic.

L'incidence des cancers chez les PPH reste à établir afin d'instaurer des politiques de suivi et éventuellement les inclure dans le dépistage de masse en tenant compte de la survie réduite et de la survenue plus précoce des tumeurs. Chez ces personnes, le profil tumoral montre, dans l'état actuel des connaissances, un excès de tumeurs digestives qu'il convient de surveiller à un âge précoce. Ces patients peuvent être traités avec succès quand les tumeurs sont découvertes suffisamment tôt. Les modalités de surveillance restent à établir.
Un soutien de l'INCa, de la Ligue contre le cancer de l'Hérault, De la Fondation Obélisque ont permis ce travail. La Fondation Jérôme Lejeune soutien DS dans l'étude des cancers des personnes avec déficience intellectuelle

P407

Cancer de l'ovaire chez les femmes ayant une déficience intellectuelle : données actuelles

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À l'échelle mondiale, les cancers sont au moins aussi fréquents chez les personnes ayant une déficience intellectuelle (DI) que dans la population générale. Mais les spécificités des cancers de l'ovaire (CO) chez les femmes atteintes de DI sont très peu documentées. Cette étude est la première qui fait la synthèse bibliographique sur ce sujet, nous l'avons complétée par des données locales non publiées.

Nous avons effectué une recherche documentaire et inclus des données provenant d'études épidémiologiques sur l'incidence et la mortalité du cancer de l'ovaire ainsi que sur les expériences institutionnelles. Nous avons également utilisé les données d'une étude que nous avons réalisée dans le département de l'Hérault (étude CHAID) sur la répartition et les caractéristiques des cancers chez les personnes atteintes de DI.

Nous avons sélectionné 62 études répondant aux critères d'inclusion. Les données montrent que les CO sont au moins aussi fréquents chez les femmes ayant une DI que chez les femmes de la population générale. Notre revue de la littérature comprenait 72 % de tumeurs germinales, principalement chez les filles et les jeunes femmes, et seulement 9 % de carcinomes de l'ovaire. En revanche, les CO chez les adultes participant à l'étude CHAID étaient pour la plupart des carcinomes. Les symptômes révélateurs de CO chez les femmes atteintes de DI ne différaient pas par rapport aux CO dans la population générale, mais le diagnostic est souvent difficile à poser chez ces patientes car elles ne communiquent pas facilement et peuvent exprimer leur douleur de manière inhabituelle, souvent par le biais de changements de comportement.

Cette première revue des CO chez les femmes atteintes de DI suggère que ces tumeurs sont aussi fréquentes que chez les femmes de la population générale. Des études supplémentaires sont nécessaires pour mieux déterminer l'incidence, l'âge au moment du diagnostic, les modes de révélation, les types histologiques, le traitement et l'évolution. Il est important de développer des compétences de communication adaptées à ces personnes et de garder à l'esprit que le CO doit être détecté le plus tôt possible pour permettre le meilleur résultat dans la prise en charge de ces patientes.

Cette étude a été financée par La Ligue Nationale contre le Cancer, comité de l'Hérault et l'Institut National du cancer.

P408

Spécificités des cancers gériatriques dans l'Hérault et évolution dans le temps : étude de population entre 1987 et 2020

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La population française est vieillissante, or l'incidence des cancers augmente avec l'âge. Désormais, plus de la moitié des cas sont diagnostiqués après 70 ans. Le cancer chez les personnes âgées est devenu un problème de santé publique. L'objectif de cette étude est d'analyser les spécificités des cancers gériatriques en se concentrant sur l'incidence, la vérification histologique du diagnostic, les prises en charge thérapeutique et les évolutions au cours du temps.

Tous les nouveaux cas de cancers (sauf les cancers de la peau hors mélanome) diagnostiqués entre le 01/01/1987 et le 31/12/2020 chez les personnes âgées de plus de 70 ans ont été extraits de la base de données du registre des tumeurs de l'Hérault.
L'âge est regroupé en 5 catégories: 70-74, 75-79, 80-84, 85-89 et les 90 ans et plus.
La base du diagnostic est définie en deux catégories: vérification histologique (histologie sur la tumeur ou sur métastase, cytologie); diagnostic clinique (examen clinique, imagerie, examen biologique).
Le traitement «actif» est défini par la mise en place d'au moins un des traitements suivant: chimiothérapie; chirurgie; radiothérapie; thérapie ciblée ou hormonothérapie.
Les taux moyens d'évolution annuel (AAPC) et leurs intervalles de confiance ont été estimés par des modèles de régression joinpoint par sexe, par tranche d'âge.

Sur la période 1987-2020, 77178 nouveaux cas de cancer chez les plus de 70 ans ont été inclus dans l'analyse dont 59,1% d'hommes.
En 34 ans, le nombre de nouveaux cas a augmenté de manière significative de +4.3%/ an pour les plus de 70 ans, passant de 1 053 cas en 1987 à 3 591 cas en 2020. L' augmentation était plus importante pour les 85-89 ans (+4.9%/an) et pour les 90 et plus (+7.5/an). Les taux bruts augmentaient de manière significative pour toutes les tranches d'âges mais de manière plus rapide chez les 85-89 ans (+2.1% / an) et chez les 90 et plus (+2.8/ an).

Durant les dernières années d'étude (2018-2020), 92,7% des cancers chez les plus de 70 ans ont bénéficié d'une vérification histologique. Toutefois, ce pourcentage de cancers diagnostiqués par histologie diminuait de manière significative avec l'âge passant de 97% chez les 70-74 ans à 68% chez les plus de 90 ans.

Pour les tumeurs solides, il y avait un effet de l'âge et de la période sur la mise en place d'un traitement «actif». En 2018-2020, le taux était de 83% chez les 70-74 ans alors qu'il était de 44% chez les plus de 90 ans. En parallèle, le pourcentage de traitement «actif» augmentait en fonction des années de manière significative ($p < 0.001$) surtout pour les 80-84 ans, passant de 63% en 2006-2008 à 71% en 2018-2020.
Pour les hémopathies malignes, la mise en place d'un traitement «actif» diminuait de manière réguière avec l'âge. Sur la période 2018-2020, le taux était de 55% chez les 70-74 ans alors qu'il était de 26% chez les plus de 90 ans. Aucun effet période n'est retrouvé.

Cette étude de population fournit des caractéristiques de l'incidence des cancers chez les personnes âgées de plus de 70 ans dans l'Hérault: evolution rapide du nombre de nouveaux cas , surtout chez les plus de 85 ans; moins de vérification histologique du diagnostic, surtout pour les âges extrêmes; augmentation des traitements «actifs» au cours au temps, surtout pour la tranche d'âge 80-84 ans; diminution des traitements «actifs» avec l'âge, mais près de 40% des 90 ans et plus en bénéficient ces dernières années.
La prise en charge diagnostique et thérapeutique des personnes âgées est complexe, associant une approche médicale, familiale, financière et sociale. Cette étude montre que le poids du cancer chez les personnes âges est de plus en plus fort, notamment chez les très âgées. La mise en place de moyens pour les prendre en charge dans les meilleures conditions devient une «urgence» de santé publique.

Posters – Axis 5 “Health Technologies”

P501

Study of cellular communication processes induced by artificial cell-cell interactions for the development of novel cell-based therapy

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Cancer represents a leading cause of death worldwide.Many novel strategies are explored constantly in order to fight this disease. Therefore, scientists are currently investigating more selective therapeutic approaches with the aim to limit side effects caused by existing treatments such as chemotherapy.

Within this framework, the design of innovative cell membranes engineering strategies is of prime interest to develop novel cell-based therapies in the field of cancer. over the past decade, genetic engineering was the most utilized approach for the reshuffle of cell membranes, leading recently to the approval of chimeric antigen receptor (CAR) T cells for the therapy of B cell malignancies. Based on the incorporation of an artificial receptor at the surface of T cells, thereby allowing them to recognize and kill cancer cells, this approach sheds the light on the potential brought by the control of intercellular interactions for the development of new cancer treatments. However, genetic engineering techniques are time consuming and produce variable results often with unpredictable efficiency.

Recently, we explored a different approach based on the use of fully artificial cell surface markers that can be introduced by bioorthogonal chemistry on the membrane of cells, previously functionalized by metabolic glycan labelling. In this case, cell-cell interactions are driven by a pair of complementary molecular recognition partners allowing the selective adhesion of different type of cells through non-covalent click chemistry As proof of principle, we coated tumor cells (A549) and T lymphocytes (Jurkat) with complementary surface markers and we demonstrated that their forced interaction activated the natural killer (NK) cells to kill tumor cells.

In the present study, we aim to visualize the appearance of a possible intercellular exchange resulting from this artificial association of Jurkat T and A549 tumor cells. Thus, we studied the impact of this non-natural binding on the proliferative, invasive and migratory properties of tumor cells.

The results of our investigations in this field will be presented in this communication.

P502

Therapeutic targeting of pro-tumoral Tumor-Associated Macrophage by vectorized anti-folate receptor beta magnetic nanoparticles

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Tumor-associated macrophages (TAM) are well known as protecting tumor cells against apoptosis, immune attacks and therapies. Elimination of these pro-tumoral TAM remains a challenge in cancer therapies. Several ways of TAM targeting exist such as the receptor of CSF-1 or the use of bisphosphonates. However these strategies are not specific to pro-tumoral TAM, potentially leading to serious adverse effects.

We produced and patented a monoclonal antibody called 6-25 capable to specifically recognize the nurse-like cells (NLC), TAM of the chronic lymphocytic leukemia (CLL), and TAM from different solid cancers. We showed that the 6-25 antibody recognizes the folate receptor beta (FRβ) at the surface of these cells and is internalize in these cells without inducing any toxicity. The FRβ is also expressed by the M2 monocytes-derived macrophages (M2M) but not by the M1 monocytes-derived macrophages (M1M) or other myeloid cells.

The goal of the project is to produce a tool that specifically targets and kills pro-tumoral TAM in the tumor in order to sensitize cancer cells to chemo- or immunotherapies.

In cancer treatment, magnetic hyperthermia represents an emerging approach with promising therapeutic potential. The magnetic hyperthermia induces an increase of the temperature of the tumor containing magnetic nanoparticles (MNPs), leading to cell death, in response to a high frequency alternating magnetic field (AMF) application. Iron oxide MNPs are highly biocompatible and non-toxic (rapid degradation and iron cations recycling), which allows their combination with conventional therapies.

Thus, we develop a magnetic nanoparticle based on a PEGylated iron oxide MNP functionalized with the 6-25 mAb (MNP-6-25) as a specific tool to target pro-tumoral TAM expressing the FRβ or IgG control as a negative control thanks to a Michael reaction, and a fluorophore, the Cyanine 5, allowing its detection.

For this study, two cellular models were used: M2M as expressing FRβ at their surface, and M1M as negative control without FRβ at their surface. M2M and M1M were obtained by the culture of monocytes from healthy donors in the presence of appropriate cytokines cocktails.

First, we showed that MNP-6-25 were not toxic toward M1M and M2M up to 64 µg Fe_2O_3/mL after 72h incubation. Then MNP-6-25 binds specifically M2M but not M1M, with a maximum of binding at 48h of incubation at 8 µg/mL Fe_2O_3/mL. Finally, confocal microscopy imaging showed that MNP-6-25 accumulated in the lysosomes of M2M.

Secondly, we performed an alternative model to study the penetration and the specificity of MNP-6-25 in a 3D model. We realized 3D co-cultures with M2M and A549 (lung cancer cell line) using the technic of ultra-low-attachment plate for the formation of spheroids. We showed that co-culture with M2M favored proliferation of the cancer cells.

In the perspective, we plan to evaluate the efficacy and the specificity of MNP-6-25 to target and kill M2M in this 3D model upon application of magnetic field and then the in vivo targeting of macrophages in a murine model of non-small cell lung cancer with MNP-6-25.

P503

Implantable theranostic device for in vivo real-time NMR evaluation of drug impact: application to brain tumors

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Evaluating the efficacy of a drug is a fundamental step in the development of new treatments or in personalized therapeutic strategies and patient management. Ideally, this evaluation should be fast, possibly in real time, simple to implement and reliable. In this study, we present a device designed to meet these objectives for assessing therapeutic response. This device is based on the use of MRI and NMR for the diagnostic aspect, and on the application of the CED (Convection-Enhanced Delivery)[1]technique for the therapeutic aspect. The miniaturized device is implantable and can be used in vivo in target tissue. In this study, the device was applied to rodent glioma models with localized administration of a choline kinase inhibitor and acquisition of magnetic resonance images and spectra.

The device comprises an NMR microcoil used for image and spectrum acquisition and a capillary for localized drug delivery.
Six Wistar rats were used as an animal model of glioma. The brain tumor model was obtained by stereotactic intracerebral injection of 10^6 C6 glioma cells into the cortex.
RSM-932A[2], an anticancer molecule that inhibits the choline kinase activity (CHKα), was administered intratumorally (2 mM at 0.4 µL/min) using the device. MRI and MRS acquisitions were performed on a preclinical 7T MRI magnet. NMR spectra were processed using Lcmodel software for metabolite identification and quantification of concentration.

Statistically significant changes in the amplitude of total choline (tCho), N-acetylaspartate (NAA) and lactate/lipids NMR peaks were observed one hour after the start of the CED of RSM-932A solution.The relative tCho concentration decreased by an average of 80%, with a standard deviation (SD) of 53%. The NAA concentration also decreased by 31% ± 17% SD, while the Lac/lipids concentration increased by 83% ± 62% SD.

The aim of this study was to demonstrate the feasibility and potential of the implantable device for the delivery of a therapeutic molecule and for the local assessment of its impact using MRI and MRS. The differences observed in the NMR spectra confirmed that changes in the concentration of metabolites can be assessed within the 1 µL detection volume of the implanted microcoil with acquisition times compatible with in vivo conditions.The device could be used for screening and evaluation of drugs under development in animal models and could also benefit patients by offering the possibility of adjusting and evaluating the efficacy of locally administered treatments.

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P504

Dissecting cellular communication through gene regulatory network inference

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Understanding cancer development and progression relies closely on gene regulatory networks (GRNs) and mathematical modeling. These tools are essential for unraveling intricate regulatory mechanisms, identifying critical genes and pathways, and uncovering potential therapeutic targets in cancer. Yet, building GRNs remains a challenging task, due to the different scales of regulation involved, their context- and system-specific nature, the difficulty in including experimental data, as well as the complexity emerging from the structure of these networks. In this context, transcriptomics time-courses allow studying the gene regulatory networks and interactions in temporal scales, to identify novel molecular interactions and potential drug targets. Building on the power of transcriptomics to shed light into the intracellular mechanisms, several data-driven GRN inference methods have been developed, incorporating advanced Machine Learning algorithms, and providing an unbiased approach to studying gene regulation, and furthermore, system behavior.

Within the tumor microenvironment (TME), the dynamic interplay between immune and cancer cells triggers a series of regulatory events at various levels. These interactions shape the behavior of cancer cells and their reactions to external stimuli. Studies have shown that when cancer cells are present, several immune cell types, including macrophages, neutrophils, and T cells, can undergo transitions toward pro-tumoral phenotypes or exhaustion. A similar ecology appears also in Chronic Lymphocytic Leukemia (CLL), a blood disease characterized by malignant and highly proliferative B cells. While there is abundant research on the reprogramming and state changes of immune cells, we still lack a comprehensive molecular understanding of how cancer cells behave and transition between states in response to their interactions with immune cells. To address this question, we performed in vitro experiments in three conditions: monoculture of CLL cells, co-culture of monocyte-CLL cells, and cultures of Peripheral Blood Mononuclear Cell (PBMC) of CLL patients, and obtained bulk RNAseq time courses of CLL cells over 14 days.

We then perform data-driven gene regulatory network (GRN) inference for each experimental condition, highlighting important structural and functional differences between the GRNs in the three conditions, and indicating significant differences in the CLL cell response in presence of macrophages and other immune cells. To build the gene regulatory networks of CLL cells for each experimental condition we use dynGENIE3 inference method, which employs non-linear modeling and random forest algorithms to infer putative gene regulations. Furthermore, in order to provide more biological insights, the TCseq package was used on each experimental condition, highlighting the changes in pathway enrichment throughout the evolution of the culture, and outlining processes like stress response, regulation of cytokine production, response to external stimuli, and metabolic shifts. Furthermore, gene clustering analysis gives important information on the cyclic processes (e.g. stress response) and pathways activated due to the presence of immune cells in the culture (e.g. regulation of cytokine production). These results were afterwards analyzed and validated to check the robustness of the findings.

With this data-driven approach, we aim at a deeper understanding of the cancer cells' behavior, their interactions with the immune cells, and hope to identify the molecular targets we need to disrupt in order to maximize the action of immune cells. Moreover, the reconstructed GRN enables the application of various types of mathematical models, thus allowing the study of the temporal behavior of the system, with possible implications on patients' response to therapy.

P505

MRI Study of the effect of Magnetic Fluid Hyperthermia on the onset of lung metastases

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Magnetic Fluid Hyperthermia (MFH) can be used to treat tumors, using iron oxide particles (IOPs) to target and enhance the heat at the tumor site. It is interesting to note that most studies focus on one heating dose. Yet, the dose of heating can be a factor of the therapy efficiency [1]. While hyperthermia is commonly used for primary tumors ablation, it is well established that metastasis is the major cause of cancer-related deaths. To our knowledge, no work has studied the impact of the hyperthermia intensity on the development of metastases.

In order to obtain such information, 4T1 breast cancer cells were implanted in 18 Balb/c mice. Two weeks later, commercial IOP (Synomag®-D, 70nm, Micromod) were injected into the subsequent primary tumor (stock solution at a dose of 30-40% volume compared to the tumor volume), either coated with Poly-L-Ornithine (PLO) or not. Five sessions of MFH treatment, one per day, were performed using a DM3 commercial applicator (NanoScale Biomagnetics) at 473.5 kHz during 1 hour. Magnetic Resonance Imaging (MRI) was used to assess every 3-4 days the presence of pulmonary metastases, using a specific 3D imaging sequence (balanced Steady State Free Precession (bSSFP) sequence) that was modified in order to decrease its sensitivity to respiration motion and susceptibilities at 7T [2]. Each image was then analyzed using DeepMeta U-Net network [3] to automatically segment and measure the volume of the lung metastases.

Preliminary in vitro studies demonstrated that the coating of the nanoparticles with PLO was necessary to efficiently generate a high cell internalization within only 30min. On the contrary, no Synomag particle was internalized into cells if they were not previously coated with PLO.
After the injection of Synomag (coated or not with PLO) into the primary tumors, two intensities of heat were measured depending on the PLO coating. The absence of PLO enabled to reach a mean temperature of 46°C +/- 1°C inside the primary tumor during the first session. Whereas, at this same session, with the coating, the IOP generated a mild hyperthermia of 40°C +/- 2°C (called mild-hyperthermia therafter). Then, the highest tumor temperatures decreased progressively at each MFH session.
Using MRI, The bSSFP sequence enabled to clearly detect pulmonary metastases as small as 0.02 mm3. This enabled to deeply assess the delay for the onset of the pulmonary metastases after the MFH treatment. When mild hyperthermia was applied, the metastases appeared 12 days +/- 1 day earlier than the non-treated mice. In parallel, when a higher temperature was reached inside the tumors, the delay of the onset was 19 days +/- 4 days post-MFH. Conversely, metastases appeared 24 days +/- 3 days post-MFH in untreated mice.

This study highlights the potential adverse side effect of MFH anti-cancer therapy when used as a unique treatment. It would be interesting to evaluate if the combination of MFH with radiotherapy or chemotherapy promotes or reduces tumor burden.

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Contribution of UHF-Dielectrophoresis Microfluidic Lab-on-a-Chip to predict Tumor Mutational Burden of tumor cells

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Tumor Mutation Burden (TMB) represents the total amount of mutations harbored by cancer cells and reflects the burden of neo-epitopes, making it an emerging biomarker of Immune Checkpoint Inhibitor (ICI) sensitivity. However, prohibitive costs of molecular analysis reduce patients' eligibility for ICI treatment, underscoring the need of new predictive tools. We previously identified subcellular populations of malignant cancer cells by using **UHF-Dielectrophoresis (UHF-DEP) Microfluidic Lab-on-a-Chip** to determine **electromagnetic signature** (EMS). In light of this, as TMB reflects the mutational index of cancer cells, we speculate that EMS could provide information on TMB cancer cell status.

To secure this new hypothesis, we performed retrospective analysis of previously established EMS of cell lines from different types of cancer. We identified a correlation between EMS and TMB values found in databases. To confirm this, we are currently determining both the TMB and EMS of cell lines cultivating under comparable conditions. We use a **next-generation sequencing** (NGS) method designed for tumor profiling by annotating cancer-driving variants and providing a precise TMB evaluation.

Thus far, we observed a significant correlation between the expected TMB of solid cancer model cell lines and their validated EMS, with **EMS increasing proportionally to the TMB index**. However, we currently do not observe a correlation for liquid cancer model cell lines. To validate this finding, we are currently developing two solid cancer cellular models that allow us to manipulate TMB levels and accurately monitor the progression of EMS. Interestingly, preliminary results show a simultaneous increase in EMS with the rise in the number of mutations.

In conclusion, our results suggest that EMS is a promising **biophysical marker** that reflects the TMB status of solid cancer cells. Here, we propose a valuable potential therapeutic tool for **immunotherapy deliverance** to cancer patients.

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3D cell models to study liver pathophysiology: From healthy liver to NASH and HCC disorders

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The liver is a central organ involved in critical functions, among them metabolism, lipid homeostasis or detoxification. Hepatocellular carcinoma (HCC) is the most common liver cancer and a major public health problem worldwide. With an increasing incidence linked to obesity and diabetes epidemic, NASH is the fastest growing etiology of HCC and is about to become the leading cause of HCC worldwide. However, there are currently no early or predictive markers of HCC development and little is known about NASH carcinogenesis.

Through proteomic analysis of patients samples, we obtained promising results that now require functional validation. Therefore, we need a model of NASH disease and HCC carcinogenesis, that will be crucial to better understand the underlying molecular pathways and find new therapeutic targets. In accordance with the 3R strategy and to model the complexity of hepatic pathophysiology, we focused on 3D cellular models. These models have seen a great breakthrough since twenty years, making it possible to model 3D interaction with a better cellular differentiation and function. Existing models may recapitulate NASH disease but no 3D model is currently available and easy to manipulate with a fairly long viability to study HCC carcinogenesis on NASH.

Our goal is to set up a new 3D cell model for each step of the disease progression : from healthy liver to NASH and HCC development in order to allow functional and molecular investigation of carcinogenesis.

To address this challenge, we chose HepaRG® cell line that is known as the most similar to primary human hepatocytes (PHH). This cell line makes it possible to overcome inter-donor PHH variability, their low availability and high costs. We sat up a spheroid model of healthy liver and NASH liver by growing cells either in normal medium or in a NASH culture medium enriched in fatty acids, LPS and glucose. Finally, we obtained a healthy liver model and a NASH model that maintain main hepatocytes functions and which recapitulates main NASH features for the latter. For the last step of NASH carcinogenesis, model set up is ongoing by testing the addition of carcinogenesis triggers into our NASH model.

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Gut-in-caps: exploring the biophysical cues that drive self-organization of Caco2 cells into patho-physiological models of the intestinal epithelium

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Over the last decade, the development of *in vitro*intestinal models has open new possibilities for preclinical drug testing and disease modeling. Using Caco2 colorectal cancer cells, microphysiological systems evolved from flat epithelial barriers to complex gut-on-chips[1]. These biomimetic models replicate the invaginated crypt-villi organization of intestinal epithelium, which maintain stem cells in niches and differentiated cells close to the lumen. Advances in 3D culture of patient-derived cells also led to self-organization of primary intestinal organoids. The latter has enabled to uncover mechanisms of crypt-villus morphogenesis[2] and paved the way for drug screening and personalized medicine. But when it comes to modeling tumors, self-organization of mutated intestinal cells often results in disorganized 3D tumoroids with less known morphogenetic processes[3,4]. Yet, Caco2 colorectal cancer cells have been extensively used to design gut-on-chips reliably mimicking the intestinal crypt-villi architecture. Using a new method to culture Caco2 cells in 3D, we propose a new organoid-like model to investigate the (patho)physiological self-organization of intestinal carcinoma cells *in vitro*.

Using the Cellular Capsule Technology (CCT)[5], we harness microfluidic-based biofabrication to culture Caco2 cells in a 3D alginate shell. Briefly, a microfluidic chip is used to coextrude an alginate solution around cells suspended in a Basement Membrane Extract (Cultrex®). The external solution of alginate gels in a calcium bath, forming an elastic nutrient-permeable shell around the cellular environment. These capsules can then be handled for imaging or sorting for further characterization of the cells they contain. Over a week of culture inside capsules, Caco2 self-organize into either hollow enteroid-like cysts or crowded tumor-like spheroids. We explore the parameters that can trigger one organization or the other, such as the initial number of cells, the matrix, or the mechanical confinement. In parallel, we aim to characterize the cellular phenotypes associated with these 3D organizations, using markers of cancer stemness or intestinal differentiation in RTqPCR and immunofluorescence imaging. Finally, we monitor the aggregate growth rates with medium throughput live-imaging[6], and we investigate their respective mechanisms of growth.

By correlating the 3D cellular self-organizations with the cell phenotypes, we aim to decipher some key-features of *in vitro* intestinal morphogenesis in healthy or tumoral states. In the future, such *in vitro* models could help better understand the biophysical cues that favor intestinal epithelium homeostasis or pathological developments such as in colorectal cancers.

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P509

Transcriptional regulatory networks unravel cell states from immune cell type deconvolution and uncovers cell niches predictive of cancer progression

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The tumor microenvironment (TME) can influence and modulate the physiopathological process of cancer development. Despite great progress to describe this complex system, we do not know yet why some patients react well to cancer therapies while others do not and undergo recurrence. Current technologies such as single-cell approaches have allowed us to estimate cell type proportions inside the TME. However, these methods have a high cost and are complex to perform. Computational algorithms to perform cell type deconvolution from bulk RNAseq have been developed, but the quantity of features and high variability between them hinders their potential use in patient stratification.

Based on the fact that gene regulation is heavily dependent on the action of transcription factors (TFs) and that transition and maintenance of biological states is usually controlled by these regulators, this project aims to propose a novel framework to characterize immune patient profiles by the construction of transcriptional regulatory networks (TRN) based on inferred protein activity and cell type immune deconvolution using bulk RNAseq data to capture multiple possible phenotypes/states of immune cells in patient samples. We performed this analysis on a NSCLC cohort of 76 Early stage samples from Vanderbilt university, another from IUCT-Oncopole with 80 NSCLC patients at varying stages and three public melanoma datasets totaling 180 patients with immunotherapy response. We applied algorithms to estimate TF activity from gene expression data and construct different subnetworks of highly correlated TFs along with the results of different published deconvolution algorithms and signatures that estimated immune cell proportions based on bulk and single cell data.

Data integration captures molecularly distinct cellular subpopulations sharing similar TF activity across patients. We identified specific TF modules which separate certain cell types. Patients were split using the high and low TF activity identified in these modules. Differential expression and gene set enrichment analysis reveal sets of patients with high infiltration of NK and myeloid cells associated with an immune-active behavior and another group of NK cells, cancer and CAF cells associated with an immune-suppressive behavior in NSLSC samples. Single-cell RNAseq data from the Vanderbilt cohort were used to characterize the two NK cell populations. In parallel, two publicly available melanoma datasets (with clinical response) were used to determine whether TF activity can split responders from nonresponders. Results in these datasets show two sets of patients where responders present a high infiltration in groups of CD4, CD8 and B cells highly associated with JAK.STAT pathway ($r = 0.82$, $pval = 1e\text{-}15$) and TFs enriched in interferon and interleukin signaling, while non responders have a high infiltration in NKT, NK resting, cancer and neutrophils cells highly associated with TGFb ($r = 0.43$, $pval = 6e\text{-}4$). These immune cell groups along with their TFs in responders are being used to train machine learning models and potentially predict immunotherapy response in other melanoma datasets.

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3D Models for colorectal cancer treatment and personalized medicine

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By 2020, colorectal cancer (CRC) will be the leading digestive cancer and the second leading cause of cancer-related death. Treatment of CRC is based on curative excision and chemotherapy for advanced stages (> stage 2). Currently, chemotherapy treatments, administered systemically, don't take into account the specific needs of each patient, and lead to disease recurrence within two years of treatment. This is due to the residual presence of clusters of specific cells with a high capacity for quiescence and proliferation. These characteristics correspond to Tumor Initiating Cells (TICs) or Cancer Stem Cells (CSCs), which are responsible for the therapeutic escape and mortality observed in post-surgical CRC. Specific targeting of CSCs would therefore make it possible to avoid any risk of recurrence.

The aim of this project is to develop a model of 3D multi-cellular tumoral spheroids (MCTS) initiated from isolated CSCs, to enable chemoresistance testing. In the future, the use of these models could enable the development of personalized treatment for colorectal cancer patients. Indeed, identifying the appropriate treatment on these in vitro models will ensure that the patient eliminates all residual cancer cells, thus avoiding any risk of recurrence.

Sedimentation field flow fractionation (SdFFF) has been shown to be a label-free technology capable of sorting CSCs according to physical criteria (size, density and deformability). However, it is still necessary to characterize these cells post-sorting to validate the quality of CSCs isolation.

To simplify its clinical application, we have coupled SdFFF with a DEP HF (High Frequency DiElectrophoresis) detector to characterize different cells populations as a function of transition frequency. This label-free technique will automate the isolation and characterization of cells post-sorting.The effectiveness of this coupling has already been demonstrated in glioblastoma, where CSCs isolated by SdFFF had specific DEP signatures.

This coupling requires the replacement of conventional SdFFF mobile phases (phosphate buffers) by a medium with low ionic conductivity, compatible with the operation of the DEP detector. Initial results from this coupling show that the ability of SdFFF to isolate CSCs is preserved, and further experiments are underway to validate this conclusion. In parallel, other preliminary results tend to prove that the DEP detector can obtain a specific signature of CCR CSCs.

In addition, we have demonstrated that it is possible to obtain tumor spheroids by growing SdFFF-isolated CSCs in a synthetic hydrogel.

In the future, we aim to validate the reproducibility and robustness of our model in the face of chemoresistance tests. The development of this model has been carried out on CRC cell lines, and in the future, we aim to use primary cultured cells from patients.

P511

Thermal and mechanical stresses generated by magnetic nanoparticles upon magnetic field exposure induce immunogenic cell death in pancreatic adenocarcinoma

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Pancreatic adenocarcinoma (PDAC) is a cancer with a very poor prognosis since the 5-year survival rate is less than 10%, with more than 220,000 deaths each year worldwide; it is predicted to be the second leading cause of cancer death within a few years. PDAC is 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). CAFs promote proliferation and progression of the tumor, secrete extracellular matrix proteins forming a physical barrier that limits not only the penetration and the diffusion of therapeutic drug but also the infiltration of immune cells and so an efficient anti-tumoral immune response. Consequently, immunotherapy turned out to be disappointing against PDAC and PDAC was classified as a "cold" tumor or a "immune desert" cancer.

Thanks to their physico-chemical properties, iron oxide magnetic nanoparticles (IONPs) are innovative tool notably as theranostic agent for cancer diagnosis and therapy. IONPs exposed to high frequency magnetic field (AMF) can heat and induce thermal related damages, leading to cell death and tumor regression. A clinical trial was conducted in 2011 to treat high-grade brain tumors and one is currently realized on prostate cancer, combining magnetic hyperthermia with radiotherapy. On the other hand, IONPs exposed to low frequency rotating magnetic field (RMF) can generate mechanical forces that induce mechanical related damages, leading to cell death.

The aim of the study was to investigate whether local thermal (magnetic hyperthermia) or mechanical forces released by IONPs upon AMF or RMF application, respectively, can stimulate immunogenic cell death and improve anti-tumoral response, in the PDAC model. We developed IONPs that specifically target pancreatic cancer cells and CAF expressing the CCK2 (MiaPaca2-CCK2 and CAF-CCK2) receptor to optimize their accumulation in the lysosomes of these cells. We showed that these IONPs vectorized with gastrin, called NF@Gastrin, bind, internalize and accumulate in the lysosomes of MiaPaca2-CCK2 and CAF-CCK2 cells. We demonstrated that AMF or RMF application kills specifically cancer cells and CAFs having internalized NF@Gastrin and slows down their proliferation without affecting cells lacking the nanoparticles, on 2D culture and 3D MiaPaca2-CCK2/CAF-CCK2 spheroid models. Then, we demonstrated that magnetic hyperthermia as well as mechanical forces generated by NF@Gastrin upon AMF or RMF exposure increase the expression of calreticulin and HSP70 proteins, known as Damage-Associated Molecular Pattern (DAMP), at the surface of MiaPaca2 cancer cells and CAF. This effect is associated with a raise of phagocytosis of these cells by human THP1 macrophages. Altogether, these results strongly suggest that magnetic hyperthermia or mechanical forces generated by IONPs upon AMF or RMF application are two potential new strategies capable of inducing immunogenic cell death and restoring anti-tumor response within the PDAC.

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Quantification of radiosensitizing gadolinium-based nanoparticle concentration in lung tumors using a routine clinical imaging protocol

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Measuring the concentration of Gd-based nanoparticles (NPs) administered as radiosensitizers prior to radiotherapy sessions in patients with lung tumors is essential for determining the therapeutic efficacy and the safety regarding healthy tissue of these NPs. The objectives of this study were to generate T1 (longitudinal relaxation time) maps, to compute NP concentration from MR images obtained with the Volume Interpolated Breath hold Examination (VIBE) sequence and to evaluate the feasibility of the proposed approach on phantoms and patients with centrally located non-small cell lung cancer (NSCLC) tumors.

AGuIX nanoparticles (1) (NPs) are theranostic nanoparticles combining both diagnostic and therapeutic properties, thanks to the presence of gadolinium atoms. Gadolinium acts both as an MRI contrast agent and as a radiosensitizer. These nanoparticles were injected into patients with centrally located NSCLC tumors included in the Nano-SMART clinical trial (2). T1 values were derived from the analytical expression of the NMR signal amplitude for the VIBE sequence and a known value of T1 in a reference tissue (3). The VIBE T1 measurement method was first compared with a reference method using phantom experiments at 3T. Then, the method was applied in vivo on clinical acquisitions in 4 patients recruited in the Nano-SMART clinical trial. Finally, the concentration of theranostic agent in lung tumors was measured using the known longitudinal relaxivity value of the NP.

The slope of the linear model between T1 values from the VIBE and the reference method obtained on phantom was 0.95 (R2=0.95), indicating an excellent correlation between the two measurements. VIBE-based T1 values measured in lung tumors before and after NP injection were found equal to 1562 ± 175 msec and 878 ± 278 msec, respectively. These values are in line with literature values (4). The pre- and post- values were found statistically different ($p < 0.05$). The mean concentration values of Gd3+ ion, representative of NP concentration, in lung tumors were equal to 96 ± 91 µM.

The T1 values obtained on phantom and in vivo show that the approach and the algorithm proposed in this study are valid and robust. The concentration of MRI-traceable NPs can be assessed in centrally located NSCLC tumors using a VIBE acquisition. For patients treated with radiotherapy, these values will be used to correlate the NP concentration with the magnitude of the therapeutic effect and will offer the possibility of adjusting an external therapeutic modality to the concentration of the radiosensitizing NPs.

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Bioprinting early-stage pancreatic cancer models: a new tool to decipher tumor initiation mechanisms

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Pancreatic cancer remains one of the most aggressive malignancies with late diagnosis, limited therapeutic options and low survival rates [1], emphasizing the critical need for early detection and improved understanding of its initiation mechanisms. In this regard, the application of 3D bioprinting technology presents a compelling approach for developing physiologically relevant cancer models. This innovative technique allows for the replication of various microenvironments and niches during the early stages of pancreatic cancer development, offering valuable insights into its progression.

In this study, we present the development and characterization of a novel bioprinting methodology capable of replicating distinct matrix stiffness gradients that correspond to various stages of pancreatic cancer [2]. Our approach combines inkjet bioprinting, an extracellular matrix-derived bioink, and primary pancreatic cells extracted from wild-type and genetically modified mice to create highly realistic 3D bioprinted pancreatic models.

Rheological assessment showed our ability to finely modulate the properties of the bioinks, enabling us to accurately replicate the matrix stiffness observed in vivo. Image analysis showcase the successful replication of the bioprinted model while maintaining cell viability. Additionally, we show that the model facilitates large-scale image analysis, highlighting its utility in capturing phenotypic changes with high statistical power. Moving forward, our research aims to delve deeper into the dynamic crosstalk between cancer cells and their microenvironment within the 3D model, utilizing advanced techniques such as secretome analysis and multi-omics approaches.

By closely mimicking the in vivo tumor microenvironment, this model offers a valuable platform for investigating the underlying mechanisms involved in cancer initiation and therefore, pancreatic cell tumor phenotype acquisition.

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Characterization of KRAS dynamic and nano-organization in the cell membrane in lung adenocarcinoma

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Lung adenocarcinoma (LUAD) is the leading cause of cancer death worldwide, accounting for more deaths than breast, prostate and colon cancer combined. KRAS mutations are the most frequent oncogenic drivers of LUAD, and are among the most common genetic alterations in all human cancers. However, despite the extensive knowledge on RAS biology accumulated over the last decades, no strategies have yet been developed that effectively target abnormal KRAS signalling in cancer patients. Recent evidence suggests that KRAS forms dimers and higher order KRAS nanoclusters in the cell membrane, resulting in a conformation that is optimal for its interaction with downstream effectors to generate productive signaling. In this project, we have developed imaging approaches to identify and characterize KRAS dimers/nanoclusters. Specifically, we use two complementary independent imaging techniques, the combination of which will provide strong evidence for the existence of KRAS dimerization: single-molecule localization microscopy combined with Voronoï-based segmentation algorithm and FRET/FLIM-based imaging. The obtained results elucidated the dynamic of KRAS and the organization of KRAS as dimers or higher order nanoclusters in the membrane and will be used to study the interaction of KRAS and its putative scaffolding factors implicated in the generation of productive KRAS signalling clusters.

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Development of 3D microphysiological systems to study intestinal stem cell fate and model early steps of tumorigenesis

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With 1 million deaths pear year, colorectal cancer is the world's third deadliest cancer. Over-activation of the Wnt signaling pathway (Wnt/Apc/β-Catenin axis), which initiates hyperproliferation of crypts that contain intestinal stem cells, appears to be the primary driver of this cancer. Conventional therapeutic approaches frequently fail due to tumor heterogeneity and resistance of cancer stem cells to treatments, causing relapse of the disease. Moreover, animal testing is not reliable and over 90% of translation from animal models to clinical trials fail, mainly because of the biological differences between species. It is therefore necessary to have new models that recapitulate human intestinal physiology and complexity more closely. Thus, different in vitro models have been developed,some reproducing mechanical cues such as peristalsis-like movements, others recapitulating the villi and/or crypt topography. However, none of these models have combined both aspects. My project aims at reproducing an intestinal epithelium in microphysiological systems (MPS) by using 3D printing and microfluidic devices. The first part of my project, ongoing, is to develop a photosensitive biomaterial allowing the adhesion and proliferation of primary intestinal cells derived from organoids, that is compatible for 3D printing and deformable to peristaltic movements. We chose a combination of polyethylene glycol diacrylate and gelatin methacrylate for the mechanical properties of the former and the adhesive properties of the latter. We succeeded in printing 3D intestinal structures by using high-resolution stereolithography. The second step will be to integrate these scaffolds into microfluidic devices for the mechanical cues. Thus, these MPS will recapitulate different aspects of in vivo organ such as topography, peristaltic movements and microenvironmental parameters. Based on this model, we aim to reproduce the early stages of tumor transformation in the MPS using cells carrying inducible mutants in the Wnt/Apc pathways. Ultimately, we plan to show that these devices could provide standardized and reproducible in vitro models for the study of stem cell fate and cancer etiology as well as a platform to perform preclinical drug testing.

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Bioactive λ-Carrageenan oligosaccharides coated ferrite nanoparticles as potential anticancer nanodrugs

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Polysaccharides (PS) are widely recognized as valuable coatings or scaffolds for the design of multifunctional nanoparticles (NPs) intended for advanced biomedical applications.[1] In addition to providing high colloidal stability to the NPs and facilitate further functionalisation, they can confer additional advantageous features such as targeting abilities or bioactivities of interest.[2] However, these applications typically focus on specific PS varieties, neglecting numerous other families with untapped promises such as λ-carrageenan (λ-CAR). λ-CAR is a galactan-based PS that displays promising antitumoral effects, but its use in vivo as a bioactive coating for anticancer NPs is strongly restrained due to its high viscosity and adverse proinflammatory and anticoagulant properties.[3] The depolymerization of λ-CAR into oligosaccharides (OS) can overcome these issues, providing new candidates with improved innocuity and biological specificity.[4] Surprisingly, such OS have not yet been included as coatings for any NPs. In this study, we initially prepared a λ-CAR OS candidate through a radical-based depolymerisation method and showed the removal of the adverse properties from its native λ-CAR parent was eliminated while retaining a specific anticancer activity-namely the inhibition of heparanase (HPSE), a key enzyme involved in tumour progression. Subsequently, an innovative microwave-assisted synthesis was optimized for the preparation of Mn-doped iron oxide NPs functionalized with this λ-CAR OS coating. A comprehensive physicochemical description was conducted, including size, electronic microscopy (TEM, SEM), advanced spectroscopy techniques (EDS, XPS, Raman, FTIR), magnetic properties and colloidal stability analysis. Next, we confirmed that the favourable balance of biological properties of the λ-CAR OS is effectively transferred when integrated as an NP coating. Finally, we assessed the antitumor performance of the NPs in cell models and, using a mouse model, the in vivo pharmacokinetic (PK) properties via MRI. Results revealed the coveted combination of a prolonged vascular lifetime and relatively fast hepatobiliary clearance. These findings will be discussed in the context of the current challenges for improving clinical translation of nanotherapeutics.

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3D models for hepatocellular carcinoma research: Investigating the interface between healthy hepatocytes and HCC tumor cells

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Hepatocellular carcinoma (HCC) is a highly heterogeneous cancer that develops over an extended period on a pathological liver. Consequently, the interactions between cancer cells and normal cells constantly evolve throughout the various stages of the disease. These dynamic interfaces represent the battleground where tumor cells encounter the defenses of healthy tissues, including cells and the extracellular matrix. New three-dimensional (3D) models emerge as promising models to study HCC in a more physiopathological context.

Our research project focuses on investigating tumor progression specifically at the tumor/healthy tissue interfaces. To address this, we uses a combination of spatial tissue matrix-assisted laser desorption/ionization (MALDI) imaging and Mass spectrometry (LC-MS/MS) analysis on formalin-fixed paraffin-embedded (FFPE) biopsies obtained from proliferative or non-proliferative HCC patients. By identifying the proteome of these interfaces, we aim to uncover potential molecular targets that contribute to the dynamics of these regions.

In parallel, we develops 3D models using primary human hepatocytes, HepaRG and HCC tumor cells. We uses a high throughout microfluidic device to produce alginate capsules or tubes containing a mix of healthy and tumor cells or spheroids. We aim to recreate the interface between healthy tissues and the tumor in a confine space delimited by alginate boundaries. The next step is to perform MALDI imaging of our 3D model to see if we recapitulate biopsies architecture to confirm that our HCC 3D model is close to patient and tissue scale. Finally, we want to test target proteins identified combining MALDI imaging and mass spectrometry in our 3D model to gain insights into the mechanisms underlying tumor progression at the interface scale between tumor and healthy tissue.

Our long-term objective is to facilitate the testing of potential therapeutics targeting this critical aspect of HCC development.

Posters – Platforms

P601

Bordeaux Bioinformatics Center (CBiB): A Resource for Data-Driven Cancer Research

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The **Bordeaux Bioinformatics Center (CBiB)** is a core facility that supports the experimental design, data analysis, visualization, integration, and reporting needs of all cancer investigators from academic and private sectors, ranging from fundamental research, through translational studies up to clinical applications.

This includes projects that generate multiple types of high-dimensional data, including bulk, single-cell and spatial omics datasets from cell lines, animal specimens and patients. The CBiB leverages the dedicated expertise, analytical tools, and data processing and storage capabilities, which are vetted shared resources that receive support from the National Bioinformatics Infrastructure (IFB). As required, the biostatistics / bioinformatics core develops new analytic strategies, working closely with cancer project investigators.

Thus, the CBiB provides investigators with cutting-edge analytical and interpretative support. Daily, the core's bioinformaticians analyze genomics, transcriptomics, epigenomics, proteomics and metabolomics data and report results to investigators. In addition, we can assist with data mining, learning, integrating, and visualizing large public domain datasets from projects such as e.g. TCGA, ICGC and ACCR Genie to complement data from cancer researchers.

We also implement, maintain and update the bioinformatics infrastructure needed to execute the core's best-in-class analytical software and manage their large computational footprint. Currently, the CBiB is experiencing rapid growth in a few key areas. These include single cell transcriptomics analysis support and spatial omics support.

Our expertise includes:

- Consulting and study design
- Grant preparation and project work
- Omics data analysis and integration
- Public domain cancer data integration
- Methods development

In this presentation we will highlight these activities through specific project examples.

An important role of the CBiB in the academic life on campus is to provide training to scientists and, working in partnership with the CNRS Training, we offer a number of classroom-based training courses in omics data analysis with an emphasis on hands-on, practical-based learning.

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"Organoïdes tumoraux et bioimpression 3D" Development of a new platform in Poitiers

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In response to a scientific and technological need to consolidate expertise and federate research in oncology on the Poitiers site, a new platform dedicated to the genesis of tumor organoids by 3D Bioimpression has been created with the support of the Canceropole GSO and the University of Poitiers. Currently, trials are in progress with different settings of the 3D bioprinter, in particular, to initially study the interface between tumor cells and cells of the microenvironment.

Posters – Young researchers from Spain

P701

SOX11 as a potential driver of de novo metastatic hormone-sensitive prostate cancer

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Metastatic prostate cancer represents around 5-10% of the prostate cancer (PCa) diagnoses, but accounts for 50% of the deaths attributed to PCa. When apparent at diagnostic (de novo), the metastatic PCa has spread beyond the prostate gland to distant organs, but remains sensitive to hormone therapy due to not having received any prior systemic therapy. Therefore, further research into metastatic hormone sensitive PCa (mHSPC) is needed to elucidate the molecular characteristics of this form of the disease, which would answer important clinical questions and could be extrapolated to other aggressive forms of PCa.

An RNAseq analysis performed on primary tumour samples of localized and de novo mHSPC patients revealed dramatically different transcriptomic landscapes between conditions, with more than 5000 differentially expressed genes. In the case of mHSPC, processes linked to neural signalling, development and extracellular matrix organisation appeared enriched, with the POU and SOX families of transcription factors being identified as potential drivers of this phenotype. We identified SOX11, a transcription factor with a role in embryonic neurogenesis and tissue remodelling, as having an increased expression in mHSPC. Here, we demonstrate how SOX11 promotes a more metastatic phenotype in prostate cancer cells both in vitro and in vivo.

Publications of the lab:
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Characterization of metastatic hormone sensitive prostate cancer patients by integrating bulk and single-cell RNA-seq data

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Prostate cancer (PCa) is the most prevalent tumor type in men and the second cause of death by cancer in the gender. The development of metastatic disease is strongly related to the mortality and morbidity of PCa. Although most prostate cancer patients are diagnosed with localized disease, 9-20% of cases (what we refer to as metastatic hormone sensitive PCa or mHSPC) present with metastases at the time of diagnosis. Although this is a small subgroup of patients, it accounts for up to 50% of PCa mortality.

As a first approach in the lab, we performed a bulk RNA-seq analysis on primary tumor samples and mHSPC patients. This analysis showed more than 5000 differentially expressed genes revealing a transcriptional uniqueness of mHSPC primary tumors. In the process of understanding the biology underlying such acute changes in the transcriptomic profile of these phenotypes, we identified stromal and immune processes related to mHSPC patients. Moreover, in silico deconvolution suggested that these patterns could be driven by differential cell-type composition.

To further explore this idea, we processed publicly available single-cell RNA-sequencing datasets from human prostates (Chen et al., PMID: 33420488). This has allowed us to identify that some of the above-mentioned stromal and immune modules are derived from epithelial cells. Given this change in the behaviour of mHSPC-derived epithelial cells, we can conclude that some aggressive features of this patients come from cancer cells that acquire cellular plasticity, causing them to have stromal or immune-like identity.

Publications of the lab:

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