18èmes

Journées Cancéropôle Grand Sud-Ouest

November 30 - December 02, 2022

Congress Center / La Grande-Motte

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 membres du Comité de Pilotage Scientifique,**

pour leur participation et leur implication dans l'élaboration du programme de ces 18èmes Journées et la qualité scientifique des présentations.

Comité de Pilotage Scientifique

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

Comités de pilotage des Axes

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

Axe 2 - Dynamique du Génome et Cancer
JC. Andrau, O. Gadal, **E. Julien**, G. Legube, L. Linarès, D. McCusker, V. Pancaldi, E. Pinaud, H. Seitz, PYJ. Wu

Axe 3 - Recherche translationnelle, de la biologie à la clinique
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, B. Jacques, 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

Bienvenue à la Grande Motte pour cette 18ème édition des Journées Annuelles du Cancéropôle Grand Sud-Ouest.

Les Axes scientifiques du Cancéropôle Grand Sud-Ouest se sont encore une fois, largement impliqués pour vous proposer un programme de très haute qualité, tant au niveau des sessions des Axes que pour les présentations des plénières, je les en remercie sincèrement. Ce programme vous permettra d'écouter de nombreuses interventions, de la part des chercheurs et des cliniciens de notre interrégion, mais également comme chaque année des conférenciers invités de grande envergure, sur des sujets variés. Nos collègues frontaliers présents seront ; Manuel Serrano de Barcelone sur la médecine régénérative, Pedro Friedmann Angeli de Würtzburg, du stress oxydatif à la mort cellulaire, Alessandro Ciulli de Dundee sur la dégradation protéique comme traitement anti-cancéreux, Gregory Hannon de Cambridge, Clemens Schmitt de Linz, sur la sénescence*, Jean Bourhis de Lausanne, sans oublier Andrea Alimonti de* Zurich*. Parmi nos collègues des autres cancéropôles, nous avons le plaisir de recevoir Valérie Lallemand-Breitenbach du collège de France, Céline Vallot et Célio Pouponnot de l'Institut Curie, Géraldine Le Duc de Meylan, dont vous découvrirez les sujets passionnants. Nous aurons également l'honneur de recevoir Bruno Quesnel, notre nouveau directeur de l'INCa.*

Cette année 2022 a été riche en rencontres et discussions avec les chercheurs et cliniciens du Cancéropôle Grand Sud-Ouest, dans le cadre de ma prise de fonction en tant que Directrice et de l'élaboration du plan d'actions pour notre labellisation 2023-2027. Ces échanges, ainsi que l'implication des membres des Axes et du Comité de Pilotage Scientifique, ont permis d'élaborer un programme scientifique ambitieux pour les prochaines années, axé sur la pluridisciplinarité et dont la qualité a été apprécié par le jury d'experts de l'INCa. J'en profite pour remercier vivement pour leur implication tous les chercheurs qui ont participé à ces réunions et qui témoignent du dynamisme de la recherche sur le cancer dans nos deux grandes régions. C'est ce dynamisme que je souhaite renforcer grâce à des perspectives de meilleures interactions entre les disciplines et de nouvelles collaborations. Vous pourrez suivre sur notre site internet, qui va vivre une seconde jeunesse, à la fois les thématiques qui représentent notre Cancéropôle et dans lesquelles, je l'espère, vous pourrez vous retrouver, et les nouvelles actions que nous allons vous proposer.

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

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

Nadine Houédé
Directrice du Cancéropôle Grand Sud-Ouest

LE PROGRAMME DE SOUTIEN A L'EMERGENCE DU CANCEROPOLE GSO

OUVERTURE DEBUT FEVRIER 2023 - 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

EMERGENCE DE COLLABORATIONS AXE 4

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

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

FINANCEMENT 3 k€ maximum

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 2023 AUPRES DE LA DRCI DE L'ETABLISSEMENT PARTENAIRE

LES FORMATIONS DU CANCEROPOLE GRAND SUD-OUEST

LES TRANSLATIONNELLES DU GSO

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

PRECEDENTES EDITIONS :

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

PROCHAINE EDITION CANCER DU PANCREAS 8 ET 9 DECEMBRE 2022 A TOULOUSE

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 2023

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 :

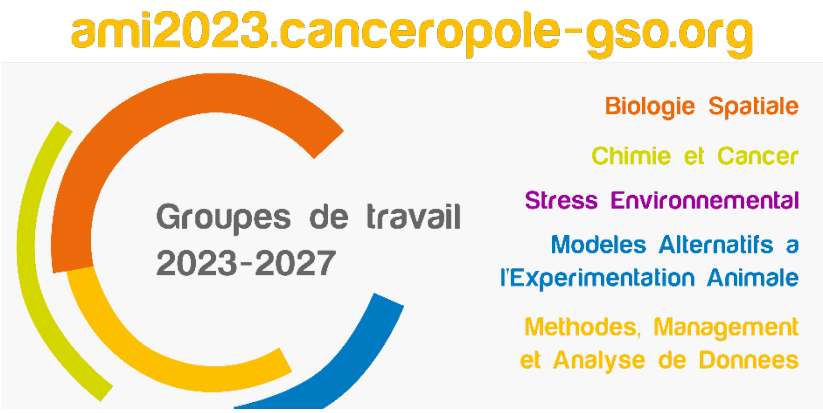
- **2014 : Genomic instability in Cancer**
- **2015 : Signaling in Cancer**
- **2017 : Nanomedicine in Cancer**
- **2017 : Genome dynamics and Cancer**
- **2018 : Signaling in Cancer**
- **2020 : BioFabrication and Cancer**
- **2022 : Génome dynamics and Cancer**

Prochaine édition : Signaling, Microenvironment and Targeting, du 2 au 3 février 2023 à Carcassonne

ACTUELLEMENT

APPEL A MANIFESTATION D'INTERET - CONSTITUTION DE GROUPES DE TRAVAIL

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 met en place **5 groupes de travail sur des thématiques transversales**, en pleine évolution, et incontournables dans un futur proche.

Pour chacune de ces thématiques, des actions seront déclinées dans le cadre des différentes missions que l'INCa attribue aux Cancéropôles (organisation de workshops et de formations, soutien à des plateformes innovantes, fléchage de certains appels à projets). 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**.

Si une ou plusieurs de ces thématiques vous intéresse, nous vous invitons à renseigner le formulaire dédié avant le 9 décembre. Vous pourrez ainsi participer activement à la construction des groupes de travail et proposer des idées d'actions concrètes.

Biologie spatiale

Les outils de génomique et de protéomique permettent aujourd'hui d'accéder aux informations spatiales des évènements cellulaires et moléculaires. L'accessibilité de la chromatine, l'expression des transcrits, la localisation des protéines et des analyses des données 3D multi-échelles permettent d'acquérir un meilleur niveau d'analyse de l'hétérogénéité tumorale et des interactions des cellules cancéreuses entre elles et avec leur microenvironnement. La prise en charge du contexte spatial et de l'architecture des tumeurs est ainsi un domaine actuellement en plein essor dans la recherche en cancérologie.
Associées à de nouvelles approches d'analyses bioinformatiques et d'intelligence artificielle, ces explorations multi-échelles respectant l'architecture des tissus, permettront d'atteindre une compréhension encore plus fine des processus oncologiques et des interactions au sein des tumeurs. Cela représente un pas supplémentaire vers la médecine personnalisée, pour mieux prédire la réponse aux thérapies de chaque patient.

Chimie et Cancer

De la compréhension des processus cancéreux à la proposition de nouvelles molécules pour combattre le cancer, nombreuses sont les applications de recherche dépendantes de la chimie. Avec le repositionnement de molécules existantes et le développement de nouveaux types de molécules (PROTACs, glues moléculaires…), de nouvelles approches de sélection / criblage et de conception de molécules ou de sondes d'intérêt ont émergé.
La chémobiologie, discipline d'interface qui vise à concevoir des outils moléculaires qui vont interagir dans un environnement biologique complexe, permet de mieux appréhender les stratégies thérapeutiques nouvelles ainsi que les outils diagnostiques et prédictifs. Les vecteurs facilitant le ciblage spécifique d'un type de cellules (cancéreuses vs normales), les nouvelles approches de conception d'outils moléculaires ou thérapeutiques, les médicaments utilisés en clinique ou les sondes permettant de caractériser un processus biologique sont autant d'approches d'intérêt que cette thématique se propose de développer.

Stress environnemental

Ce groupe se propose d'explorer le lien entre les facteurs extérieurs aux individus tels que certaines expositions physico-chimiques (pesticides, additifs et contaminants alimentaires, pollution…) et psychosociales (stress chronique, adversité durant la grossesse ou l'enfance, stress au travail…) et les processus biologiques internes aux individus liés au développement et à la progression tumorale.

Des collaborations fortes seront construites, en permettant notamment aux biologistes, toxicologues, physiciens ou chimistes de s'emparer des résultats d'épidémiologie et des sciences humaines et sociales, notamment dans les domaines des systèmes de réponse au stress (inflammatoire et immunitaire), des altérations des fibroblastes et de la matrice extracellulaire, des perturbations endocriniennes ou encore des modifications épigénétiques. Les conséquences de ces altérations restent largement à étudier dans le champ de la cancérologie, en particulier leurs effets à plus ou moins long terme sur le risque de cancérogénèse, la progression tumorale et la résistance aux traitements

Modèles alternatifs à l'expérimentation animale

Depuis de nombreuses années, les modèles animaux représentent le modèle permettant le mieux de récapituler la complexité du développement tumoral in situ. Les avancées récentes, notamment dans la compréhension des interactions cellulaires et tissulaires, le rôle de la matrice extra-cellulaire, ou encore les technologies de bio-impression ont permis de proposer des modèles alternatifs pour la recherche en cancérologie. Des outils comme les organoïdes et les organes sur puces obtenus à partir de patients permettent aux cliniciens d'envisager une évaluation individuelle de l'efficacité du traitement ainsi qu'aux effets secondaires induits.

En s'inscrivant dans la démarche éthique des 3R, de réduction de l'utilisation du nombre d'animaux, et dans l'objectif d'affiner les modèles in vitro disponibles, ces modèles sont devenus incontournables pour les chercheurs et cliniciens/chercheurs. Ce groupe se propose donc de soutenir la structuration inter-régionale autour de cette thématique et plus particulièrement autour des organoïdes, des organes sur puces et des modèles in silico (innovation technologique pour de nouveaux modèles, optimisation et complexification des modèles existants, optimisation des méthodes d'isolement et de préservation des ressources en pré-analytique, éthique des innovations médicales ainsi que le lien entre ces innovations et l'économie de la santé).

Méthodes, Management et Analyses de données

L'analyse et le traitement des données biologiques et de santé constituent des défis majeurs de la recherche en cancérologie. Qu'elles soient issues des cohortes de patients, des séquençages, des analyses haut-débit ou single cell, ces données restent sous-valorisées et trop souvent collectées dans un seul objectif sans se soucier de leur possible réutilisation. Le coût des technologies et la provenance multiple des données complexifie le traitement, de même que l'accès aux données bio-médicales constituent les freins principaux à leurs analyses.

Dans le contexte du développement des big data et de l'Intelligence Artificielle, en complément des biostatisticiens, des compétences existent dans le GSO pour modéliser les systèmes biologiques et utiliser ces nouveaux outils au service des biologistes et des cliniciens. Ce sont tous ces aspects qui seront abordés dans au sein de cette thématique.

Plus d'infos

Répondre à l'appel à manifestation d'intérêt

Date limite de réponse : 9 décembre 2022

Program

Wednesday 30th November

12h30 – 13h45 Welcome lunch

13h45 – 14h00 Opening ceremony

Nadine HOUEDE, Scientific director of Cancéropôle Grand Sud-Ouest

14h00 – 14h40

Opening session 1

Chairs: Pierre CORDELIER & Claude SARDET

Lecture: Manuel SERRANO, *Institute for Research in Biomedicine (Barcelona, Spain)* - **Understanding and manipulating cellular reprogramming in vivo**

14h40 – 16h00

Session 1 - New insights in cell death control 3

Chairs: Pierre CORDELIER & Claude SARDET

Lecture: Pedro FRIEDMANN ANGELI, *Rudolf Virchow Zentrum, University of Würzburg (Germany)* - **Metabolic control of Membrane Redox State: A Matter of Life and Death**

- **Alexandre DAVID**, *Institute of Functional Genomics (IGF), Montpellier* - The aminoglycoside streptomycin triggers mitochondria-dependant ferroptotic cell death of tumor initiating cells

16h00 – 17h00 Poster session & Coffee break

17h00 – 18h30

Session 2A – Nuclear bodies & phase separation in cancer 7

Chairs: Guillaume BOSSIS and Fabian ERDEL

Lecture: Valérie LALLEMAND-BREITENBACH, *Centre Interdisciplinaire de Recherche en Biologie du Collège de France, Paris* - **PML bodies, from leukemia cure to biophysics insight**

- **Fernando MUZZOPAPPA**, *Centre for Integrative Biology of Toulouse (CBI), Toulouse* - Identifying phase separation in nuclear compartmentalization
- **Jihane BASBOUS**, *Institute of Human Genetics (IGH), Montpellier* - TopBP1 condensation: a targetable molecular switch in the ATR signaling pathway
- **Liliana KRASINSKA**, *Institute of Molecular Genetics of Montpellier (IGMM), Montpellier* - The cell cycle as a phase separation cycle controlled by protein phosphorylation

Session 2B – Translational research, from biology to the clinic : Flash Posters 13

Chairs: Marlène DUFRESNE & Fabrice LALLOUE

EMERGENT PROJECT

- **Aurore DANIGO**, *University of Limoges* - Blockade of CCK2R prevents the onset of vincristine-induced sensory neuropathy in mice

FLASH POSTERS

- **Marine BRUCIAMACCHIE**, *Montpellier Cancer Research Institute (IRCM), Montpellier* - Synergistic effect of FOLFIRINOX with an ATR inhibitor on pancreatic tumor cells and its microenvironment
- **Boutaîna CHANDOURI-FAIZE**, *Control of cell Activation in Tumor Progression and Therapeutic Resistance (CAPTuR), Limoges* - Cancer Stem Cell glycosylation markers: A promising biomarker for prognosis and disease progression

- **Jean DESCARPENTRIE**, *Bordeaux Institute of Oncology (BRIC), Bordeaux* - Role of Furin in Colon Cancer Stem Cells Phenotype in KRAS and BRAF-Mutated Colon Tumors
- **Amandine DESETTE**, *Poitiers University* - Biological and molecular characterization of cancer stem cells in brain metastases from colorectal cancer
- **Pénélope DESROYS DU ROURE**, *Montpellier Cancer Research Institute (IRCM), Montpellier* - Therapeutic efficiency of Fc-engineered human anti-cathepsin D antibody in mono and combotherapy in triple negative breast cancer
- **Alexandra FAUVRE**, *Montpellier Cancer Research Institute (IRCM), Montpellier* - Platinum-based chemotherapy induce inflammation via a non-canonical STING pathway independent on cGAS and interferon production
- **Alexia FRANCOIS**, *Bordeaux Institute of Oncology (BRIC), Bordeaux*- Repression of protein maturation inhibits PD-1 expression and enhances tumor clearance and TILS : virtual ligand screening and drug repurposing approach
- **Elsa FRISTOT**, *Structural Biology Center (CBS), Montpellier* - Programming lactic acid bacteria for cancer therapy
- **Tinhinan LAHLOU**, *Institute for Functionals Genomics (IGF), Montpellier* - Unravelling the role of early dissemination in colorectal cancer
- **Chloé PORCHERON**, *Bordeaux Institute of Oncology (BRIC), Bordeaux* - Sensitization of pancreatic cancer to radiotherapy and chemotherapy by proprotein convertase inhibition
- **Zeinab TARHINI**, *Control of cell Activation in Tumor Progression and Therapeutic Resistance (CAPTuR), Limoges* - The Effect of Benzodiazepine and Benzodiazepine-related Drugs on Survival after Surgery for Colorectal Cancer

Session 2C – Health technologies.....27

Chair: Anne-Marie GUE

IMAGING CANCER

- **Elisabeth BELLARD**, *Institute of Pharmacology and Structural Biology (IPBS), Toulouse* - How intravital microscopy helps us to study cancer events
- **Dounia EL HAMRANI**, *IHU Liryc, Bordeaux* - Combination of MRI-guided HIFU, bioluminescence imaging and transgenic mouse model to assess efficiency of noninvasive thermal therapies for solid tumors and their microenvironments

Selected talks for "Ma techno en 180 secondes" (flash posters toutes technologies)

- **Chloé ROYET**, *Institut des "Biomolécules Max Mousseron" (IBMM), Montpellier* - Fluorescent Peptide Biosensors Reporters of Kinase Activities: profiling signatures in human tumour biopsies through a multiplex approach for cancer diagnostics
- **Chloé BESSIERE**, *Cancer Research Center of Toulouse (CRCT), Toulouse* - TranSipedia: a novel framework for large scale RNAseq data analysis with applications in cancer from research to diagnosis
- **Pawan KUMAR**, *Laboratory of Pathogen Host Interactions (LPHI), Montpellier* - Estimating spatial distribution of oxygen and hypoxia in tumor microenvironment: a mechanistic approach
- **Florian COGONI**, *Informatics Research Institute of Toulouse (IRIT), Toulouse* - Involving the biologists in the design of in silico models

18h30 – 19h30 Icebreaker & Poster session

Thursday 1st December

08h00 - 08h30 Welcome coffee

08h30 – 10h00

Session 3 – Heterogeneity & single cell 35
Chair: Julie PANNEQUIN & Mary POUPOT
Lecture: Céline VALLOT, *Curie Institute (Paris)* - **Epigenomic evolution of breast cancers in response to treatment**

- **Emeline BOET,** *Cancer Research Center of Toulouse (CRCT), Toulouse* - Drug persisters arise from transcriptionally and mitochondrially distinct stem cell subpopulations in acute myeloid leukemia
- **Julie GIRAUD**, *University of Bordeaux, CNRS, ImmunoConcEpT, UMR 5164, Bordeaux* - TREM1+ CD163+ myeloid cells are potent immunosuppressive cells and associate with poor survival in human liver cancer
- **Ghita CHABAB**, *Montpellier Cancer Research Institute (IRCM), Montpellier* - Regulatory gamma delta T cells in solid cancer : characterisation, role and ecosystem
- **Juan-Pablo CERAPIO-ARROYO,** *Cancer Research Center of Toulouse (CRCT), Toulouse* - Single-cell transcriptomics for a better understanding of tumor infiltrating heterogeneity

10h00 – 11h00 Poster session & Coffee break

11h00 - 12h45

Session 4 – Innovations in intracellular targetting 41
Chairs: Sébastien BRITTON & Anthony MARTIN
Lecture: Alessandro CIULLI, *School of Life Sciences, University of Dundee (United Kingdom)* - **Targeted Protein Degradation as a Cancer Therapeutic Modality**

- **Eric TRINQUET**, *Perkin Elmer (Codolet)* - The use of the HTRF and AlphaLISA technologies to support compounds identification and characterization in Targeted-Protein Degradation
- **Krzysztof ROGOWSKI**, *Institute of Human Genetics (IGH), Montpellier* - Targeting microtubule detyrosination activity of VASOHIBINS as a new therapeutic approach for cancer and neurodegeneration
- **Francesco CALZAFERRI**, *Institut des Biomolécules Max Mousseron (IBMM), Montpellier* - Novel strategies to study epigenetic mechanisms in cancer
- **Nicolas BERY**, *Cancer Research Center of Toulouse (CRCT), Toulouse* - Targeting hard-to-drug oncoproteins with intracellular antibodies

12h45 – 14h00 Lunch break

14h00 - 14h30
Afternoon opening session 47
Chair: Hervé SEITZ
Lecture: Gregory HANNON, *Cambridge Institute, Cancer Research United Kingdom* - FOXC2 promotes vascular mimicry and resistance to anti-angiogenic therapy

14h30 - 16h00

Session 5A – Genome Dynamics & Cancer 49
Chair: Jean-Christophe ANDRAU & Eric JULIEN

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- **Bérengère PRADET-BALADE**, *Centre for Biochemical and Macromolecular Research (CRBM), Montpellier* - Targeting RUVBL1/2 chaperones in colorectal cancer
- **Cyril ESNAULT**, *Institute of Molecular Genetics of Montpellier (IGMM), Montpellier* - Mechanical regulation of bivalent gene expression via the nuclear lamina
- **Claire VARGAS**, *Cancer Research Center of Toulouse (CRCT), Toulouse* - The E3 ubiquitin ligase TRIP12 induces the formation of heterochromatin altering gene expression and DNA damage repair independently of its catalytic activity

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- **Marie MORILLE**, *Institute Charles Gerhardt (ICGM), Montpellier* - Pharmaceutical development to enhance small Extracellular Vesicles (sEV) therapeutic potential
- **Loubna LAIB**, *Cancer Research Center of Toulouse (CRCT), Toulouse* - Targeted thermal or mechanical nanotherapy of pancreatic adenocarcinoma

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- **Jean-Marc LEMAITRE**, *Institute for Regenerative Medicine and Biotherapy (IRMB), Montpellier* - A single short reprogramming early in life improves fitness and increases lifespan in old age

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- **Olivier CALVAYRAC**, *Cancer Research Center of Toulouse (CRCT), Toulouse* - Farnesyltransferase inhibition overcomes the adaptive resistance to targeted therapy in lung cancer
- **Thomas BESSEDE**, *Montpellier Cancer Research Institute (IRCM), Montpellier* - Sensitizing the tumor microenvironment to immune checkpoint therapy trough monoclonal antibody-based therapeutic combinations in pancreatic cancer
- **Loélia BABIN**, *Cancer Research Center of Toulouse (CRCT), Toulouse* - De novo generation of the NPM-ALK fusion recapitulates the pleiotropic phenotypes of ALK+ ALCL pathogenesis and reveals the ROR2 receptor as target for tumor cells
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- **Jean-Pierre POUGET**, *Montpellier Cancer Research Institute (IRCM), Montpellier* - Role of extracellular vesicles during bystander cytotoxicity and bystander immunity of Targeted radionuclide therapy
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10h45 – 11h15

Institut National du Cancer conference

Bruno QUESNEL, Directeur du pôle recherche et innovation de l'Institut national du cancer et directeur de l'institut multi-organismes cancer d'Aviesan

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- **Philippe POURQUIER**, *Cancer Research Institute of Montpellier (IRCM), Montpellier* - The role of PXR (Pregnane X receptor) and drug metabolism in the resistance of castration-resistant prostate cancers to kinase inhibitors
- **Delphine MILHAS**, *Institute of Pharmacology and Structural Biology (IPBS), Toulouse* - Collagen remodeling leads to inflammation-free expansion of periprostatic adipose tissue and promotes prostate cancer progression
- **Fatima ALHOURANI**, *Montpellier Cancer Research Institute (IRCM), Montpellier* - Molecular characterization of the combined chemotherapy of SUV4-20h epigenetic enzymes inhibitor with Topoisomerase II poisons in metastatic prostate cancer

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Opening session

Understanding and manipulating cellular reprogramming in vivo

Manuel SERRANO

Institute for Research in Biomedicine (IRB), Barcelona, Spain

An emerging theme in response to tissue injury is the acquisition of plasticity and progenitor properties by some cells.

To study cell plasticity *in vivo* in a controllable manner, we have generated "reprogrammable" mice where it is possible to switch on-and-off the Yamanaka factors (Oct4, Sox2, Klf4 and Myc). We are using our "reprogrammable" mice to learn how to control *in vivo* cellular plasticity.

The acquisition of OSKM-driven plasticity and the reversion to the original cell identity is known to reset molecular features of aging. *In vivo*, this process increases the capacity of tissues to repair a subsequent injury. Understanding the basis of this cellular and organismal rejuvenation is a challenge that we are trying to unravel.

We have found that damaged cells secrete factors, like IL6, that strongly promote cellular reprogramming *in vivo*. I will present a novel intervention that greatly improves reprogramming by simply supplementing the diet with a particular vitamin. I will also present data on the identification of reprogramming intermediate states *in vivo*.

Session 1 - New insights in cell death control

1 / 1

Metabolic control of Membrane Redox State: A Matter of Life and Death

Pedro FRIEDMANN ANGELI

Rudolf Virchow Zentrum, Center for Integrative and Translational Bioimaging, University of Würzburg, Germany

1 / 2

The aminoglycoside streptomycin triggers mitochondria-dependant ferroptotic cell death of tumor initiating cells

Hélène GUILLORIT[1], Benjamin ZAGIEL[2], Audrey DI GIORGIO[2], Sébastien RELIER[1], Lucile BANSARD[1], Céline BOUCLIER[1], Xavier MIALHE[3], Armelle CHOQUET[1], Morgan BRISSET[1], Fiona LEBLAY[1], Julie PANNEQUIN[1], Maria DUCA[2], Françoise MACARI[1], **Alexandre DAVID[1,4]**

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[3] BIOCAMPUS MONTPELLIER

[4] PPC - CHU Montpellier

Compelling evidence suggests that tumor initiating cells (TIC) are the roots of current shortcomings in advanced and metastatic cancer treatment. TIC represents a minor subpopulation of tumor cells endowed with self-renewal and multi-lineage differentiation capacity, which can disseminate and seed metastasis in distant organ. For that reason, targeting TIC has become a major goal to design new therapeutic routes that may prevent tumor relapse and metastasis. We focused our attention on streptomycin (SM), a potent bactericidal antibiotic widely used across the globe to prevent cell contamination in cell culture and generally administered for the treatment of individuals with moderate to severe infections such as tuberculosis. Our work identified **SM as a new molecule capable of targeting non-adherent TIC** from colon and breast cancer cell lines by inducing **mitochondria-dependent ferroptosis**. Ferroptosis is a form of cell death characterized by intracellular iron dependence, increased reactive oxygen (ROS) production and aberrant lipid metabolism. SM treatment recapitulates ferroptosis hallmarks and leads to **profound alterations in mitochondrial morphology**, such as swelling and cristae enlargement, coupled with hyperpolarization of mitochondrial membrane potential and production of mitochondrial ROS. At the molecular level, the aldehyde group present on the streptose moiety of SM is essential for this mechanism to occur. As such, the mere **reduction of SM into dihydrostreptomycin abolishes its effect on TICs** while preserving bactericidal activity. In the course of this study, we uncovered a **transcriptional program dedicated to counteract oxidative stress**. Remarkably, **this inducible program was restricted to eight genes** whose upregulation enabled a fistful of TICs to survive IC50 SM concentration and initiate spheres despite severally altered mitochondria. This study reveals a new mechanism of action of SM that could help **comprehend the molecular mechanisms behind TIC adaptation to inhospitable environment** and pave the way for new treatment of advanced cancers, in particular grade three that are susceptible to spread to distant organs.

Session 2A – Nuclear bodies & phase separation in cancer

2A / 1
PML bodies, from leukemia cure to biophysics insight

Valérie LALLEMAND-BREITENBACH

Inserm 1050 - CNRS UMR7241 Cirb, Collège de France, Paris

PML drives assembly of PML Nuclear Bodies (NBs), where it recruits hundreds of unrelated proteins, mainly identified by candidate approaches. PML NBs are required for a large variety of biological processes, such as senescence, apoptosis or metabolism. As for many other membrane-less domains, the question of any specialized biochemical and biological activities remains open. PML NBs are disrupted in Acute Promyelocytic leukemia (APL), driven by the oncogenic PML/RARA protein. Combination therapy - relying on retinoic acid and arsenic trioxide - targets PML/RARA, promoting its degradation and PML NB re-assembly, which ultimately leads to APL cures. Critically, in APL or in non-APL cells, arsenic binds onto normal PML enforcing PML polymerization, NB formation and can exert anticancer effects.

We previously found that PML NBs concentrates UBC9, the sole SUMO-E2, together with PML partner proteins. Accordingly, we proposed that PML NBs may favor partner sumoylation and subsequent poly-ubiquitination by the NB-associated RNF4 SUMO-dependent ubiquitin ligase. We have now established that PML controls partner sumoylation upon stress. We found that SUMO2 conjugation were rapidly induced, in Pml-dependent manner in vivo. Similar results were obtained in mouse Embryonic Stem Cells (mESCs) and upon arsenic-induced PML NB re-organization in APL. Differential in vivo SUMO2 proteomics unraveled several novel PML NB-dependent SUMO2-targets, in particular members of the epigenetic repressive KAP1 complex, as well as the DPPA2 master transcription factor of early embryogenesis. Accordingly, Pml null mESCs re-express transposable elements due to KAP1 sumoylation defects. Moreover, Pml null mESCs display features of 2-cell-like cells, a state regulated by DPPA2, KAP1 and SUMO. Finally, PML is required for adaptive stress responses in mESCs. Collectively, our findings unraveled that PML NBs regulates mESC fate by orchestrating SUMO2-conjugation of key regulators.

We will also report some insights on a PML domain controlling NB assembly dynamics, liquid- to gel-like transition, as well as sumoylation control. Our recent findings decipher structure-dynamics links required for PML NB biogenesis, with new insights in the mechanism by which arsenic enforces PML NB re-organization.

2A / 2
Identifying phase separation in nuclear compartmentalization

Fernando MUZZOPAPPA, Michela ANFOSSI, Fabian ERDEL

Centre de Biologie Intégrative, Toulouse

In order to regulate gene expression and organize chromatin within the cell nucleus, chromatin and nuclear proteins are compartmentalized into different substructures. Some of these compartments have been proposed to form through a process of liquid-liquid phase separation (LLPS); however, this is not the only potential mechanism by which they may form. An alternative model is the binding of proteins to clusters of binding sites on chromatin without phase-separating. One major issue is to distinguish LLPS from other compartmentalization mechanisms. Here, we tested how reliable the identification of LLPS by different methods is. We find that 1,6-hexanediol treatment and classical FRAP fail to distinguish LLPS from the alternative binding scenario. In contrast, the preferential internal mixing seen in half-bleach experiments robustly distinguishes both models. In particular, we show that the decrease of fluorescence in the non-bleached half in a half-bleach experiment is a signature of LLPS. In addition, this decrease is correlated to the energy barrier at the condensate interface that is responsible for preferential internal mixing. By using well-controlled in vitro model systems as a calibration standard, we introduce a workflow termed model-free calibrated half-FRAP (MOCHA-FRAP) to probe the barrier at the interface of condensates in living cells. We use it to study components of heterochromatin foci, nucleoli, stress granules and nuage granules, and show that the height of the interfacial barrier increases in this order. We anticipate that MOCHA-FRAP will help uncover the mechanistic basis of biomolecular condensates in living cells.

2A / 3

TopBP1 condensation: a targetable molecular switch in the ATR signaling pathway

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ATR signaling is crucial to tolerate the intrinsically high level of lesions that block the progression of replication forks in cancer cells. ATR signaling promotes the rescue of stalled replication forks, the regulation of origin firing, the regulation of the nucleotides pool, and allows time for repair via activation of cell cycle checkpoints. Hence, a number of clinical trials are currently evaluating the efficacy of ATR inhibition as an anti-cancer strategy, often in combination with chemotherapeutic drugs. The Gongora's team reported recently that ATR inhibition overcomes resistance of colorectal cancer cells to oxaliplatin (1).

Mechanism of ATR activation: Activation of ATR signaling depends on the sequential and interdepend recruitment of DNA damage response proteins at DNA double-strand breaks and stalled replication forks. The main activator of ATR in S phase is TopBP1, a multifunctional protein scaffold also involved in the initiation of DNA replication, in transcriptional regulation and in apoptotic responses.

TopBP1 biomolecular condensates activate ATR signaling: We found that TopBP1 drives the assembly of biomolecular condensates, defined as subnuclear compartments that concentrate molecules selectively without defined stoichiometry and in absence of a surrounding membrane (2). TopBP1 condensates are induced by phosphorylation and are reversible. Our working model is that upon phosphorylation, TopBP1 forms an extensive protein network via multiple, weak and highly cooperative interactions, which yield reversible compartments with liquid-like properties that appear as foci by conventional microscopy. We use an optogenetic system to probe the functions that arise specifically from the formation of condensates. We found that TopBP1 condensation acts as a molecular switch to amplify ATR activity, enabling the activation of the ATR checkpoint effector protein Chk1 and signal transduction (2).

TopBP1 biomolecular condensates as therapeutic targets: Specific inhibitors of the master checkpoint kinase ATR are under clinical trial. Kinase inhibitors exert a strong selective pressure for the acquisition of drug resistance through kinase mutations. We reasoned that targeting the condensation of TopBP1 with small molecules may provide an alternative strategy to inhibit ATR signaling. To establish a proof of principle, we screened the Prestwick library of FDA drugs approved for human use. This library includes 1520 chemically diverse therapeutic compounds. We identified molecules that inhibit TopBP1 condensation and ATR signaling.

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2A / 4

The cell cycle as a phase separation cycle controlled by protein phosphorylation

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Cell cycle transitions result from global changes in protein phosphorylation states triggered by cyclin-dependent kinases (CDKs), yet the dynamics of these phosphorylation states and the mechanisms by which they promote cellular reorganisation remain largely obscure. Here, we generated a high-resolution map of changing phosphosites throughout unperturbed cell cycles in single Xenopus embryos. Full mitotic phosphorylation occurs with near switch-like kinetics in vivo, and most phosphosites are CDK targets. Cross-species analysis shows that CDK-mediated phosphosites occur mainly on intrinsically disordered proteins (IDPs) that localise to membraneless organelles. Biophysical modelling and biochemical analysis show that CDK-mediated multisite phosphorylation can switch homotypic interactions of such IDPs between favourable and inhibitory modes for biomolecular condensate formation. These results provide insight into molecular mechanisms and kinetics of mitotic cellular reorganisation.

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

2B / 01

Projet Emergent

Blockade of CCK2R prevents the onset of vincristine-induced sensory neuropathy in mice

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Vincristine (VCR) belongs to the family of vinca alkaloids and is one of the most common and effective chemotherapeutic drugs for treating a broad range of cancers. VCR is the most neurotoxic agent of its drug class and is responsible for the onset of the VCR-induced painful peripheral neuropathy (VIPN). Recent reports have strongly linked the cholecystokinin type 2 receptor (CCK2R) to nociceptive process. CCK system acts as a neuromodulator, in sensitive and pain tract, where CCK2R exhibits pronociceptive properties. Moreover, our recent findings in a mouse model of VIPN showed an up-regulation of cck2r gene in DRG. Thus, we investigated the localization of CCK2R on sensory nervous system and the effect of CCK2R antagonists on the onset of VIPN, using two pharmacological tools already done in humans; proglumide and netazepide.

VIPN was induced by intraperitoneal (i.p.) injections of VCR at 100 µg/kg/d during 7 days (D0 to D7). Proglumide (30 mg/kg/d, i.p.) or NTZ (2 mg/kg/d or 5 mg/kg/d, per os) were administered one day before VCR treatment until D7. The onset of tactile allodynia induced by VCR was assessed with von Frey monofilaments. Immunohistochemistry and morphological analyses were performed on DRG, skin and sciatic nerve.

VCR induced tactile allodynia from D1 to D7 in mice, which was correlated to DRG neuron and intraepidermal nerve fiber (IENF) loss, and by enlargement and loss of myelinated axon in sciatic nerve. Treatment with proglumide or netazepide significantly reduced mechanical allodynia and prevented sensory nerve fiber damages induced by VCR. We found that CCK2R co-localized with S100, a cytoplasmic marker of schwann cells.
Targeting CCK2R could therefore be an effective strategy to prevent the onset of VCR-induced neuropathic pain and thus improve cancer survivor quality of life.

2B / 02

Synergistic effect of FOLFIRINOX with an ATR inhibitor on pancreatic tumor cells and its microenvironment

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Pancreatic Ductal Adenocarcinoma (PDAC) is an extremely aggressive disease. There is a clear need of new strategies and new researches to treat and diagnose these patients. Regarding treatments, surgery is possible in only 20% of cases, and the chemotherapeutic molecule Gemcitabine is unfortunately lacking a good response rate. Recently, a new polychemotherapy oxaliplatin-based (FOLFIRINOX), which is a combination of 4 drugs: oxaliplatin, irinotecan, 5-fluorouracil and leucovorin, has been approved. It has showed a significant increase of the overall survival in patients compared to gemcitabine, but associated with more toxicity and still limited efficiency. This resistance to chemotherapy can come from the stroma that represents up to 90% of the tumor mass, therefore the impact of the chemotherapies on the microenvironment can be a key to increase the efficiency of these treatments. Most chemotherapies induce their toxicity by provoking DNA damages and replicative stress, leading to the activation of DNA repair pathways. That is why our research project proposes to find a synergistic association of FOLFIRINOX with a specific inhibitor of DNA damage repair - Ataxia Telangiectasia and Rad3 related inhibitor (ATRi) - to increase the efficiency of the chemotherapy while reducing its toxicity. Moreover, in order to be as close as possible to the tumor models observed in the clinic, the impact of our combination is studied in vitro in 3D co-culture models of tumor cells associated with cells from the microenvironment and more particularly cancer-associated fibroblasts (CAFs). These co-culture models make it possible to study the effectiveness of the FOLFIRINOX association with ATRi on each population as well as the signaling pathways impacted in response to the treatment.

We demonstrated a synergistic effect of the association in vitro (2D and 3D) independently of the KRAS, ATM, TP53, BRCA1/2 mutation status in several pancreatic models (ATCC and derived from PDX) and in co-culture with CAFs. We observed chemoresistance from the CAF and a protection of the tumor cells in co-culture. Higher DNA damage were observed in tumor cells treated with FOLFIRINOX combined with ATRi compared to FOLFIRINOX alone. These results were associated with a decrease of DNA damage repair pathways leading to apoptosis. In vivo, the association FOLFIRINOX with ATRi significantly inhibits the tumor growth compared to each treatment alone and no toxicity was observed in both immunodeficient (PDX models) and immunocompetent orthotopic model. Furthermore, we were able to observe more immune infiltration in tumors treated with the association compared to the chemotherapy alone. The localization and the nature of infiltrating immune cells are now under investigation.

To conclude, our work shows that FOLFIRINOX associated with an ATRi is highly synergistic in vitro in our co-culture models and in vivo where it also induces more apoptosis, less DNA damage repair and more immune infiltrate. These results show that this combination could be a new therapeutic strategy in order to increase the survival of patients with PDAC for whom only a few solutions have been found until now and that is why this cancer represents a major challenge of public health today.

2B / 03

Cancer Stem Cell glycosylation markers: A promising biomarker for prognosis and disease progression

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Lung cancer is one of the leading causes of cancer deaths worldwide. Despite scientific advances, its diagnosis and the patient management are still difficult due to the lack of biomarkers to predict tumor aggressiveness and the risk of recurrence. This is even pronounced in early stages leading to a recurrence rate up to 15% at 2 years.
However, growing evidence supports that cancer stem cells (CSCs) are responsible for cancer initiation, progression, aggressiveness and also therapeutic resistance. Thus, CSCs might constitute useful prognosis biomarkers to lung cancer patient monitoring and to prevent recurrences.

In this context, this work focuses on specific and early detection of Cancer Stem Cells (CSCs) using a new diagnostic and prognostic approach based on the recognition of specific CSCs glycosylation patterns. An immunohistochemistry (IHC) kit, called LungSTEM and based on a mix of biotinylated plants was developed by Carcidiag Biotechnologies. This mix specifically detects CSCs through (over)expressed glycan patterns. This first study aims to demonstrate the potential of this new tool for efficiently detecting and sorting CSCs from a heterogeneous tumor cell population. In a second step, we analyzed the clinical significance of the lung STEM kit from a Non-Small cell Lung Cancer patient cohort by establishing the link between the detection of CSC with Immunohistochemistry in Tissue microarray and overall survival. So, we demonstrated through two different cell sortings (Magnetic or fluorescence-activated cell sorting - MACS and FACS) on A549 cell line that the fraction sorted using the Mix is significantly enriched in CSCs compared to CD133 sorted-fraction.

We also evaluated the capacity of Mix sorted-CSCs to induce tumorigenicity in collaboration with the Functional Genomics Institute (IGF-CNRS of Montpellier). Finally, the detection of CSCs using the mix could be correlated with the patient survival from a retrospective study based on 235 patients from a cohort from Lyon civil hospices.

Altogether these results confirm the clinical significance of this specific mix as a biomarker for detecting CSCs and predicting tumor aggressiveness at early stages. These promising results suggest that this new kit based on a specific mix able to detect CSCs biomarkers is of prime interest to improve patient management determining the prognosis value regarding therapeutic response in lung cancer patients.

2B / 04

Role of Furin in Colon Cancer Stem Cells Phenotype in KRAS and BRAF-Mutated Colon Tumors

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Found in respectively 50% and 10% of colorectal cancer (CRC) patients, KRAS and BRAF gene-inactivating mutations mediate colon cancer initiation through cancer stem cells (CSCs) activation. CSCs are involved in tumor progression, metastasis induction, chemotherapy resistance, and tumor relapse. **Proprotein convertases (PCs)** are known to regulate the malignant phenotype of colon cancer cells by different mechanisms, but their effects on cancer stem cells (CSCs) have been less widely investigated.

Here, we studied the P**Cs expression in colon CSCs, and the effect of their inhibition** by using general PC inhibitors α1-PDX or decanoyl-RVKR-chloromethylketone (CMK) on colon CSCs markers, growth, survival, and invasion in three-dimensional spheroid cultures.

Moreover, Furin convertase was reported to be a pro-oncogenic driver in KRAS and BRAF-driven colorectal cancer2. We evaluated the**specific repression of Furin in KRAS or BRAF mutant CRC cell lines** and wild-type KRAS and BRAF on the expression of the stemness markers and global PCs activity.

2B / 05

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

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Brain metastases (BM) from colorectal cancer (CRC) are associated with a poor prognosis. Cancer stem cells derived from patients with BM (BM-CSC) from breast and lung cancer have already been described, but those from CRC have not yet been identified. In this study, we identified and characterized BM-CSC from CRC patients (BM-CSC-CRC).

BM-CSC-CRCs were obtained by mechanical dissociation of patient's tumor and cancer stem cells selection by appropriate culture conditions. BM-CSC-CRCs were characterized in vitro and in vivo by performing clonogenic and limiting-dilution assays, as well as immunofluorescence and Western-blot analyses. A chicken chorioallantoic membrane (CAM) model and xenograft experiments using BALB/c-nude mice were performed to study BM-CSC-CRCs phenotype.

Four patient-derived CSC (BM-CSC-CRC1 to BM-CSC-CRC4) were obtained. These cells formed metaspheres and contained tumor- initiating cells with self-renewal properties. The BM-CSC-CRC lines expressed stem cell surface markers (CD44v6, CD44, ALDH1, CD133, Lgr5 and EpCAM) in serum-free media and CRC markers (CK19, CK20 and CDX-2) in fetal bovine serum-enriched media. The CAM model demonstrated invasive and migratory capabilities of these BM-CSC-CRCs. The phenotype of the tumor mice intracranial xenotransplantation of BM-CSC-CRCs tumorspheres adequately recapitulated the original patient BM.

For the first time we successfully characterized BM-CSC from CRC patients. These promising BM-CSC-CRC cell lines will be a useful model to understand dissemination of CSC in brain and identify future therapeutic targets.

2B / 06

Therapeutic efficiency of Fc-engineered human anti-cathepsin D antibody in mono and combotherapy in triple negative breast cancer

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Triple-negative breast cancer (TNBC) defined by the absence of estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 overexpression and/or amplification, accounts for 15-20% of all breast cancer (BC) cases. Resistance to systemic treatment is common in TNBC so new treatments are required. The aspartyl protease cathepsin D (cath-D), an independent marker of poor prognosis in BC, is overexpressed and hypersecreted into the tumor microenvironment. Recently our team has shown that cath-D is a tumor-specific extracellular target in TNBC suitable for antibody-based therapy (*Ashraf, Mansouri et al., JITC, 2019, 7:29*) and that the co-expression of cath-D and AR (androgen receptor) is an independent bad prognostic factor for overall survival in TNBC (*Mansouri, Alcaraz et al., Cancers, 2020,12(5):1244*).

The objective of this project is to develop a targeted therapy in TNBC using an optimized anti-cath-D antibody. The therapeutic efficacy of the anti-cath-D F1 antibody was studied in vivo in athymic nude mice (Foxn1nu) xenografted with TNBC (MDA-MB-231) cell line or PDX (Patient-derived xenografts). F1 antibody significantly reduces tumor growth of TNBC cells and PDX in nude mice and exhibits immunomodulatory activity with natural killer cell activation and tumor depletion of myeloid cells (*patent WO/2016/188911; Ashraf, Mansouri et al., JITC, 2019, 7:29*).

The mechanism of action of therapeutic antibodies is known to involve both binding to the target and immune cell recruitment by Fc part (crystallizable fragment). Here we mutated Fc-part F1 (F1Fc+ antibody) to augment its affinity for Fcγ receptors and C1q complement protein leading to increased ADCC (antibody-dependent cellular cytotoxicity), CDC (complement-dependent cytotoxicity) and ADCP (antibody-dependent cellular phagocytosis).

We showed in vitro that F1Fc+ antibody activates degranulation and cytotoxic activity of natural killer (NK) cells and lysis of TNBC cells via the ADCC mechanism. In addition, the therapeutic efficacy of the F1Fc+ antibody was improved over that of the non-mutated Fc F1 antibody in a TNBC model xenografted in nude mice. Recruitment, maturation and activation of NK cells have been analyzed by immunophenotyping in TNBC tumor xenografts. Besides, combination treatment strategies with this optimized antibody are being studied, particularly with chemotherapy, with promising results.

In this study, we showed the benefit of an Fc-improved antibody to target an extracellular protease, the cathepsin D, in TNBC. Based on these encouraging in vivo results, we hope that antibody-based targeting of cath-D may represent an attractive avenue for therapeutic applications in TNBC.

2B / 07

Platinum-based chemotherapy induce inflammation via a non-canonical STING pathway independent on cGAS and interferon production

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Upper Tract Urothelial Carcinomas (UTUC) are extremely aggressive tumors of ureter or renal pelvis. UTUC present less tumor mutational burden and low tumor immune infiltrate compared to bladder cancer. Despite this they are treated with the same protocol than bladder cancer with more than 50% of relapses justifying the need of new therapeutic options. To improve patient care, we suggest stimulating the immune system by platinum-based chemotherapies, Cisplatin-Gemcitabine (CisGem) or Carboplatin-Gemcitabine (CarboGem) in order to potentiate the effect of an anti-PD-L1, the Durvalumab. To that, we conduct an in vitro project that aims to determine if chemotherapies could transform cold tumor into a hot tumor, and if so by which mechanisms ?

Using 3 UTUC cell lines (UM-UC-14, UCC3 and UCC17) we have evaluated the cytotoxicity effects of the chemotherapies combinations in 2D and 3D cell cultures. We have assessed their potential (i) to induce DNA damage using image cytometry, (ii) to induce PD-L1 expression using flow cytometry, (iii) to induce immune cell death using ELISA kits, (iv) to activate the cGAS/STING pathway using qPCR and Western-Blot, and finally (v) to attract immune cells by using heterotypic spheroids model (tumoral cells+PBMCs).

Our results demonstrate that CisGem and CarboGem present synergistic effects in UTUC spheroid cultures. These treatments also induce DNA damage pathway demonstrated by an increase of γH2AX, P-ATM, P-CHK1 and P-CHK2 positive cells. We found an increase of PD-L1 membrane expression after treatment in UTUC cell lines. RNA Seq analyse indicates that the major pathways upregulated by these combinations are inflammatory pathways (TNF-α signaling via NFκB, interferon alpha response, inflammatory response, interferon gamma response) without induction of IFN I. We could observe an immune cell death induction demonstrated by an increase of ATP and HMGB1 release and calreticulin translocation. And finally, we showed STING pathway activation, independent of cGAS or interferon production but dependent on ATM and ATF3 . On the other hand, we have created heterotypic spheroids composed of UTUC cancer cell lines and immune cells, and we showed that CisGem and CarboGem increase the percentage of immune infiltration in the heterotypic tumor spheroids and enhance chemotherapy effect.

These results indicate that the combination of platinum salts + gemcitabine induces inflammatory pathways via a non-canonical STING pathway independent on cGAS and interferon production. These combinations induce an upregulation of PD-L1 expression and allow immune cell attraction at the tumor. All these data support that a combination CisGem or CarboGem with an anti-PD-L1 will be efficient for UTUC patients.

2B / 08

Repression of protein maturation inhibits PD-1 expression and enhances tumor clearance and tils: virtual ligand screening and drug repurposing approach

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Immune checkpoints, such as programmed death-1 (PD-1) are involved in the regulation of T cell effector function, are now exploited for the treatment of various solid and hematologic cancer. However, although therapies targeting PD-1 were clinically effective in various preclinical models and cancer patients, several patients with solid tumors are still refractory to these treatments. Indeed, solid tumors evade anti-cancer immune control by establishing immune privileged niches within the tumor microenvironment that reduce proliferation, viability, and/or activity of cytotoxic T lymphocytes (CTL). Interestingly, a wide range of proteins involved in the expression of PD-1 and CTL function require proteolytic activation by the proprotein convertases (known as PCs). Using general protein-based inhibitors of the PCs we previously reported the implication of the PCs in PD-1 expression and T cell exhaustion. In the current study we identified small molecule convertase inhibitors through virtual ligand screening and drug repurposing approach that inhibit the activity of the convertases. Using organoids culture, we found that some of these molecules were able to repress cancer cells viability, proliferation and invasion. These molecules were also able to mediate potent repression of PD-1 expression on T cells activated by CD3. In vivo, subcutaneous inoculation of mice with syngeneic cancer cells revealed their anti-tumoral efficacy that associated increased intratumoral T cell infiltration in the developed tumors. The treated mice showed improved overall survival while compared to controls. These and other findings highlight the potential use of PC inhibitors to increase the anti-tumoral immune response and could act as novel immunotherapeutic approach in cancer used alone or as adjunct therapy.

2B / 09
Programming lactic acid bacteria for cancer therapy

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

Our work aimed to engineer a colorectal and probiotic *Lactobacillus* strain as a new chassis for cold tumor therapy. As precision engineering of LAB (lactic acid bacteria) is currently limited by the lack of tools enabling reliable control of gene expression, a part of this project aims at building a collection of well-characterized regulatory elements to control transcription, translation and secretion levels. In parallel, We are optimizing the production of cytotoxic (Azurin, cytolysine A) and immunomodulatory proteins (VHHa-PDL1) in our chassis *Lactobacillus gasseri.* Ultimately, bacterial therapeutic activity will be controlled by biosensors responding to signals from the tumor microenvironment and external trigger combined together in biological logic gates. In order to test, improve and validate our recombinant strains, we are combining *in vitro* spheroid-based screening with animal models infection and therapeutic tests.

2B / 10
Unravelling the role of early dissemination in colorectal cancer

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Context: Most of the mortality attributed to cancer is due to metastasis; however, the mechanisms involved remain poorly understood. The literature mainly describes the late stages of tumorigenesis, ignoring early dissemination particularly in colorectal cancer. To overcome this lack, we have generated an inducible mouse model in which specifically in the intestinal epithelium the cells are fluorescently labeled with TdTomato, and simultaneously intestinal tumorigenesis is induced by a partial deletion of APC, known as the gatekeeper gene in CRC. This mouse model, thanks to the expression of tdTomato allowed us to detect early disseminated cells (eDTC) in the liver, the main distant organ for colorectal cancer metastases. The impact of eDTCs in the liver was assessed using CyTOF/hyperion and immunolabelling and we could detect a strong enrichment of macrophages and neutrophils suggesting a microenvironmental remodelling. Moreover, at the systemic level, a cytokine profile (M-SCF, SDF1, CXCL2, Timp1) was observed, which could be involved in this massive enrichment of myeloid cells (macrophages).

Objectives: Our aims are to decipher the mechanisms explaining the enrichment of macrophages in response to early dissemination and to validate some of our results, on blood samples from patients with intestinal polyps.

Materials and methods: Detection of eDTC has been possible by using imunolabelling. Identification of enriched macrophage subpopulations was first performed by RT-qPCR on livers mice and further characterization have then been performed by single cell RNA-sequencing. To identify genes and cytokines that are essential for macrophage subset enriched in the liver, co-culture assay of intestinal polyp cells purified from mice with HoxB8-derived macrophages were carried out; the macrophage phenotype was determined by RT-qPCR. The cytokine profile of patient plasmas was performed using an ELISA assay.

Results: The qPCR results obtained on the livers of APC mutated mice suggest an overall polarization towards an M2-like immunosuppressive phenotype. HoxB8-derived macrophages cultured for 2 days in the presence of intestinal polyps adopt a state of polarization mimicking macrophages in the liver of mice with mutated APC. Plasma TIMP-1 and M-CSF levels were elevated in patients with adenomas compared to healthy donors; however, SDF-1 and CXCL2 levels in plasma were patient dependent.

Conclusion: this project demonstrated for the very first time that not only tumor dissemination occurs much earlier than previously believed in intestinal tumorigenesis but also that at very early stages, concomitant with the presence of early disseminated cells in the liver, drastic remodeling is induced, including strong macrophage enrichment. In addition, macrophages tend to have an M2-like immunosuppressive, which strongly suggests that this early dissemination has a causal role in the establishment of a premetastatic niche to promote late colonization.

2B / 11

Sensitization of pancreatic cancer to radiotherapy and chemotherapy by proprotein convertase inhibition.

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The **pancreatic ductal adenocarcinoma (PDAC)** is a major health issue with a 5-year relative survival rate of only 6%. This aggressiveness is mainly due to a late diagnosis and a lack of curative option with a resistance to most conventional treatment (chemotherapy and radiotherapy).

Interestingly, the activation of the majority of the signaling pathways involved in the initiation and progression of pancreatic cancer is mediated by various protein precursors that require proteolytic activation by a family of nine enzymes called **proprotein convertases (PCs)**. Consequently, deregulation in the expression and activity of proprotein convertases (PCs) is associated with pathological conditions and they are known to behave as oncogenes in various types of cancer.

Indeed, we have found that four members of PC family are predominantly expressed in PDAC tissues while compared to noncancerous tissues. Therefore, due to their aberrant activity in PDAC, the inhibition of PCs, by siRNA or chemical inhibitors, in combination with conventional treatments have been tested in vitro on 2D adherent cancer cells as well as in 3D pancreatic tumorsphere. In both cases, PCs inhibition sensitizes pancreatic cancer cells to current chemotherapy and radiotherapy. The combination treatment shows a reduction of the different tumorigenic properties of pancreatic cancer, including cell growth, motility and survival.

These and other findings highlight that PCs inhibition, in combination with the current first line treatment, might be beneficial for treatment of PDAC and could improve the current therapeutic status of pancreatic cancer patients.

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The Effect of Benzodiazepine and Benzodiazepine-related Drugs on Survival after Surgery for Colorectal Cancer

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Introduction: Benzodiazepine, usually used for depression, was shown to increase the plasma brain-derived neurotrophic factor (BDNF) that may improve the survival of colorectal cancer (CRC) patients. The aim of this study was to evaluate retrospectively the associations between benzodiazepine and benzodiazepine-related drugs (BZRD) use and overall survival (OS) or disease-free survival (DFS) in patients operated for CRC.

Methods: It was a retrospective cohort study. It included patients who underwent surgery for CRC at Limoges' University Hospital between 2010 and 2019. Data on the characteristics of patients, CRC, comorbidities and drug exposure were collected from the electronic medical records. Patients were divided into two groups, benzodiazepine users and non-users. All patients were followed for five years after surgery for CRC. The outcomes were overall survival (OS) and disease-free survival (DFS). All cases of CRC recurrence were confirmed by computed tomography (CT) scan or magnetic resonance imaging (MRI) and verified by biopsy. Multivariate analysis using the Cox model was performed to adjust various confounding factors (age, sex, body mass index (BMI), tumor site, cancer stage, Charlson comorbidity index, benzodiazepine/BZRD use, propensity score, antipsychotic drugs, antidepressant drugs) and all statistical analyses were done with IBM SPSS Statistics 22.

Results: In total, 512 patients were included in this study. A third of patients were treated with benzodiazepine (33.4%). Univariate survival analysis using the Kaplan-Meier method and comparing benzodiazepine/BZRD users and non-users showed no statistically significant differences in 5-year OS (64.4±4.4% vs. 68.1±3.0% respectively, p = 0.69) and 5-year DFS (53.9±4.4% vs. 55.3±3.1% respectively, p=0.52) in patients operated for CRC. After adjustment to confounding factors, the use of benzodiazepine/BZRD was not associated with OS and DFS. Using further adjustment for propensity score, multivariate analysis provides similar results (aHR=1.10, 95%CI: 0.71-1.73 and aHR=1.01, 95%CI: 0.70-1.46 respectively).

Conclusion: the use of benzodiazepine doesn't seem to be associated with improved survival in patients operated for CRC.

Session 2C – Health technologies

2C / 1
How intravital microscopy helps us to study cancer events?

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Intravital microscopy is a technique used for direct visualization of fluorescent events in living animal. This technique consists in observing and recording, via an adapted microscope, fluorescent phenomena, kinetics in space and time, appearing at the scale of a tissue or an organ, in a sleeping intact animal. For cancer studies, the main applications concern microcirculation and angiogenesis, blood circulating cells recruitment and intravascular adhesion phenomena.

To observe these events, different models suitable for one or several acquisitions on the same animal can be used. For a single acquisition, animal has to be prepared for organ immobilization and exposition and for injection of cells or markers. For acquisition on several days, a chamber model is preferred requiring a surgical procedure for implantation of the chamber.
To study vessels different types of markers can be injected to mice: fluorescent plasmatic markers (dextran, liposomes or Qdots) to highlight vessels or fluorescent probes (lectins or antibodies) to label vessels wall. To observe cells behavior, either exogenous single cell suspensions or endogenous cells can be labeled with suitable markers but also transgenic mice with fluorescent cells can be used.
Depending on the temporal and spatial resolution needed, different instruments can be used, Macroscope, widefield video-microscope and multiphoton, all being adapted for small animal imaging. Therefore, we obtain 2D wide-field acquisitions of vascular network, 2D acquisitions at video rate of fast events occurring in vessels, or 3D acquisitions of this network and cells migration deeper into the tissue. For these visualizations, mice are anesthetized and a heating pad with temperature feedback is used to maintain the temperature of animals.
Several examples will be presented to illustrate the potentiality of intravital microscopy.
Observations of tumoral cells and vascularization using the dorsal skinfold chamber model will be presented with a special focus on endothelial cell-to-cell junctions modulation induced by electric fields. By wide field fluorescence microscopy, we thus demonstrated that permeability of normal vessels was transitory altered by electric fields. With multiphoton, we explained this permeabilization by alterations in cell-to-cell junctions induced by electric fields [1].
Observations of lymphocytes recruitment in tumor and lymph node in different cancer type allowed to demonstrate, by video-microscopy, that high endothelial venules (HEVs) were the main sites of lymphocyte capture, rolling and sticking in the tumor microcirculation during immunotherapy. Multiphoton imaging revealed that lymphocytes crawl and transmigrate through HEVs wall to enter treated tumors [2]. We also discovered that, within the mouse lymph node microcirculation, human chronic lymphocytic leukemia (CLL) cells bind to HEVs via a multistep adhesion cascade, which involves rolling, sticking and crawling of the leukemic cells on the endothelium. Functional analyses revealed that the lymphocyte homing receptor L-selectin was the key factor controlling the binding of CLL cells to HEVs wall in vivo [3].

All these examples show the power of intravital microscopy to better understand the events occurring in the tumor and its environment and thus consider improving efficacy of treatments or establish new strategies.

[1] Increased permeability of blood vessels after reversible electroporation is facilitated by alterations in endothelial cell-to-cell junctions. Markelc B, Bellard E et al. J Control Release. 2018; 276:30-41.
[2] Tumor-associated high endothelial venules mediate lymphocyte entry into tumors and predict response to PD-1 plus CTLA-4 combination immunotherapy. Asrir A et al. Cancer Cell. 2022; 40(3):318-334.
[3] L-selectin controls trafficking of chronic lymphocytic leukemia cells in lymph node high endothelial venules in vivo. Lafouresse F, Bellard E et al. Blood. 2015; 126(11):1336-45.

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Combination of MRI-guided HIFU, bioluminescence imaging and transgenic mouse model to assess efficiency of noninvasive thermal therapies for solid tumors and their microenvironments

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Purpose: To improve the efficiency of tumor treatments, one strategy is to combine therapies that alleviate tumor cells by inducing thermal coagulation necrosis while stimulating the tumor microenvironment (TME) for inducing anticancer effects. The aim of this work is to present a dual approach using MRI-guided high intensity focused ultrasound (MRgHIFU) for non-invasive thermo-therapy and bioluminescence imaging (BLI) to visualize concomitant thermal effects targeting both thermo-ablation of tumor cells and mild hyperthermia of the TME. This technology is combined with a sophisticated transgenic mice model having different BLI reporters for tumor cells and TME.

Material and methods: Transgenic mice were modified to express heat-induced luciferase with a heat shock protein promoter (Hsp-Fluc) to follow the TME response by in vivo BLI (n=10). These mice were implanted on the hind leg with cancer cells exhibiting constitutive-luciferase expression to follow tumor cell viability (RM1-CMV-Nluc). Prior and after MRgHIFU, BLI acquisition were performed.
MRgHIFU experiments were performed on a preclinical 9.4T MRI. The hind leg of the anesthetized mouse was positioned underneath a HIFU transducer (focal point = 5mm in length x 1mm in width). Scout MR-images were used to position the transducer relative to the tumor and to avoid sonicating in bones and/or viscera. For MR-thermometry, a FLASH sequence was used with 3 slices positioned orthogonal to the ultrasound beam axis and centered on the focal point (in-plane resolution= 667x667µm2/pixel, slice thickness= 1mm, 2.4s temporal resolution per stack of images).
Temperature images were processed on the fly and were displayed online in Thermoguide software which also regulated the tumor temperature in a single pixel by automatically adjusting the output power of the HIFU generator to follow a predefine temperature-time profile. Thermometry data were re-processed offline to compute the average temperature in each pixel during the plateau of heating (25 images for 1min) and over 9 adjacent pixels.

Results: Before heating, BLI revealed a strong signal from constitutive Nluc expression by tumor cells, but no signal from Fluc by the TME. Then, a thermo-coagulation heating protocol (temperature range from 54 to 62 °C for 1min) was applied to tumors by MRgHIFU. After heating, an overall decrease of BLI (NLuc) signal in the tumor is observed, indicative of area of tumor thermal necrosis. In tissues surrounding the tumor (TME), BLI signal (Hsp-Fluc) displays a "ring-shaped" photon distribution resulting from moderate, non-lethal, temperature increase surrounding the central necrotic area. This photon distribution is indicative of sufficient thermal stress to activate BLI signal (Hsp-dependent transcription of FLuc), while remaining nondestructive for the tissue.

Conclusion: This proof-of-concept study highlighted the advantage of modulating heat deposition by MRgHIFU in order to exploit different thermal effects in the tumor (ablative therapy by coagulation necrosis) and its microenvironment (moderate temperature increase) (P. Jeanjean and D. El Hamrani et al. Adv. Mater. Technol. 2022). Combination with this transgenic mouse model allows direct in vivo evaluation of innovative thermo-therapies strategies (ablation associated to drug delivery and/or immunotherapy) by assessing the physiological response of the tumor and TME non-invasively.

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Fluorescent Peptide Biosensors Reporters of Kinase Activities: profiling signatures in human tumour biopsies through a multiplex approach for cancer diagnostics

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Protein kinases (PK) are frequently hyperactivated in many human cancers thereby constituting relevant biomarkers and attractive pharmacological targets for anticancer therapy (Fleuren et al. Nat Rev Cancer 2016; Roskoski R. Jr. pharmacol. Res. 2021; Cohen et al. Nat Rev Drug Discovery 2021). Although these biomarkers may be detected through antigenic, proteomic, transcriptomic or genetic approaches, there are currently no approaches that report on their functional activity for diagnostic purposes. In order to monitor the kinase activities of cyclin-dependent kinases (CDKs), we have developped a toolbox of fluorescent peptide biosensors through conjugation of environmentally-sensitive probes to synthetic peptides derived from CDK substrates (Morris M.C. Life 2022a, European J Organic Chem 2022b). Specifically, we have engineered a CDK4-specific biosensor which enables quantification CDK4 hyperactivity in skin cancer cell lines, biopsies and melanoma xenografts (Prével et al. Biosens. Bioelectron. 2016; Gonzalez-Vera et al. Chem. Commun. 2017; Henri et al. Br. J. Dermatol. 2019), a CDK6 biosensor which was implemented to compare CDK6 and CDK4 activities in lung cancer (Soamalala et al. ChemBioChem 2020), a CDK5-selective biosensor for neuronal disorders such as glioblastoma (Peyressatre et al. Frontiers Chemistry 2020), and a CDK1 biosensor conjugated to carbon nanotubes for in vivo imaging in tumour xenografts in mice (Tilmaciu et al. Small 2021). These synthetic biosensors offer straightforward means of quantifying differences in kinase activities between healthy and cancer cells, and of sensing alterations in response to therapeutics.

With the aim of implementing CDKACT technology to profile CDK activity signatures in biopsies in human tumours, we developed a multiplex approach combining four different biosensors and established a protocol for standardized and calibrated quantification of CDK activities. In collaboration with the CRB-CHU Montpellier, we characterized the CDK activity profiles from 40 lung adenocarcinoma and 40 lymphoma samples and performed Western blots to determine CDK expression levels in parallel. We further correlated our results with age and sex of patients, as well as with the genetic and immunohistochemical characterization of the biopsies performed by the CHU (Royet et al. in preparation). This study shows that CDKACT biosensing technology provides new and complementary information relative to current genetic and immunohistochemical characterization of tumour biopsies, enabling further stratification of patients and potential to develop a diagnostics approach based on kinase activity profiling using synthetic fluorescent biosensors.

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TranSipedia: a novel framework for large scale RNAseq data analysis with applications in cancer from research to diagnosis

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Driven by myriads of projects, public RNA-seq databases are exploding. To date, over 850,000 RNA-seq are deposited on SRA for human. This huge body of publicly available RNA-seq libraries is a precious resource to identify specific transcriptional events. The challenges lie in the complexity of RNA biological content and the exponential increase in data volume. We want to make RNA-seq data easily accessible providing a better capture of the whole transcriptome complexity, in the context of human health applications. New computational methods that perform indexing of k-mers across huge datasets constitute interesting solutions to interrogate "Omics data" at a large scale from dataset collections. Here, we developed TranSipedia, a new framework based on k-mer approach, constructed with several modules: 1/ **The RNA-seq indexing step** constructed with Reindeer (REad Index for abuNDancE quERy; Marchet et al., 2020), a novel computational method that serves as an efficient platform to request all transcribed information, 2/ **a module to generate k-mers as signature** of transcripts (Kmerator; Riquier et al, 2021), 3/ **a supporting web site** to facilitate the queries easily shared by biologists (TranSipedia, https://transipedia.montp.inserm.fr).

Reindeer performs indexing of k-mers and records their counts across a large collection of datasets. Interestingly it associates k-mers to their counts instead of only recording the presence/absence of k-mers as frequently done in previous works. Moreover, Reindeer provides an ultra-fast performance in the query process while indexing several thousands of RNA-seq. One of the great advantages of indexing raw data is also that it integrates reference-free and annotation-free approaches. For applications where gene expression level is required, the k-mer count must be sufficiently sensitive and representative to be applicable. The quantitative accuracy with k-mers counts from Reindeer indexed datasets was compared to classical quantitative methods like Kallisto. Secondly, the design k-mer module uses Kmerator, a tool developed to construct specific k-mers, already avalailable on github. Thirdly, the website is also available to facilitate index queries by the biologists with sequences on fasta file format. The Transipedia platform now includes several thousands of datasets from public and private collections mainly from acute myeloid leukemia (AML) for cancer applications. We indexed the whole CCLE cohort representing 1019 RNA-seq samples for a total of 10 To and indexes from Leucegene, BEAT-AML, SRA and TCGA collections represented with more than 1000 RNA-seq samples for AML application. Concerning biological applications, we already requested in selected public datasets biomarker tissue specificity as well as tumor specific signatures comparing normal/tumor, for simple and useful medical usage. In perspectives, based on data structures such as k-mer features, diagnosis applications are in developpement. Moreover, Machine learning models could be used to search for signatures and explore better diagnostic and prognosis models.

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Estimating spatial distribution of oxygen and hypoxia in tumor microenvironment: a mechanistic approach

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Being a hallmark of several solid tumors, hypoxia - a state of reduced level of tissue oxygen tension and a result of aberrant vasculature - leads to several alterations in the tumor microenvironment. Hypoxic regions of neoplasm are prone to be more resistant towards radiation therapy than compared to well oxygenated ones (A. L. Harris 2002). Furthermore, hypoxia and its mediators influence multiple signaling pathways and gene regulation to promote neovascularization, invasion, migration, adhesion, metastasis, and phenotypic switches (D. S. Widmer et al. 2013, A. Tameemi et al., 2019). Hence hypoxia is one of the leading factors which contributes towards intratumor heterogeneity and resistance against treatments, these two features being particularly important and common in many invasive tumors including melanoma (B. Bedogni et al. 2009, D'Aguanno et al. 2021). Estimation of accurate hypoxia profile would be key for better prognosis and design of more efficient treatment approaches. Mathematical modeling has been proven a useful tool to understand and predict such complex dynamics. Several computational and mathematical models have been proposed to describe tissue oxygenation, however the majority of them are restricted to synthetic data and qualitative results, lacking application to and connection with real tumor tissues and experimental results.

We propose mechanistic modeling frameworks, which are driven by experimental data, to explain and mimic oxygen-hypoxia dynamics. The data is in the form of tissue scans of Patient Derived Xenograft (PDX) of breast, ovarian and pancreatic as well as human melanoma tumors. These scans of tumor tissue slices are immunohistochemical stained with CD31 -cluster of differentiation 31, marking the presence of endothelial cells- and CAIX- carbonic anhydrase IX, regulated by the hypoxia-inducible factor (HIF) 1, is an intrinsic marker of tumor hypoxia - markers. Keeping the data availability in mind, the distribution of oxygen is described by a reaction-diffusion partial differential equation with the source term incorporating the contribution from blood vessel density (obtained from CD31 staining) for the 2D model and from the vasculature architecture and the geometry of each blood vessel (reconstructed from several 2D tissue slices) for the 3D model. Next, hypoxia is modeled from the obtained oxygen distribution using an algebraic equation. The further steps include estimation of parameters and validation. The obtained parameters demonstrate biological relevance. 3D reconstruction, which is underway, is required for obtaining 3D profiles of oxygen and hypoxia. This requirement leads to another aspect of this work consisting in quantification of the error made when 2D models are used instead of more realistic 3D models. This is important since the 3D reconstruction is not always feasible, especially for patient tissue samples. A framework to quantify this approximation error would be essential for evaluating the hypoxia profile for clinical applications. Future work involves development of a general framework, applicable to most of the solid tumors, to estimate oxygen and hypoxia distribution based on the 3D reconstruction of blood vessels as well as for the 2D case with an error bound due to the approximation.

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Involving the biologists in the design of in silico models

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In recent years, mathematical and computational tools have allowed a new abstraction: in silico modelling. In the continuity of in vivo and in vitro models, in silico models allow the exploration of working hypotheses with relatively low costs and precise control of experimental conditions. Among in silico models, agent-based models are used in biology to study population-scale phenomenon by reproducing the individual behavior of the cells. In comparison to other modeling paradigms, agent-based models have the benefits to be easier to understand by non-modeler because they are based on the description of simple rules described at the agent level (often the cells in our context). Additionally, they allow an individual cell scale analysis of mechanisms emerging at the population scale. Designing these models thought requires a good intercomprehension between the biologists and the modelers to capture the essence of the cell behaviors and interactions. All in all, building a model of a given biological system often takes weeks or months.

To overcome the complexity of building these in silico models, we propose a participatory methodology based on schemas to interactively build models. With these schemas, the modeler can draw:
- a state-transition diagram that represents the different phenotypic states of a cell and their conditions of evolution in time,
- an activity diagram that describes the sequence of actions (e.g. divide, migrate, produce/consume molecule, etc.) a cell follows in a given state.
Additionally, our methodology allows to discuss efficiently about the environment and experimental conditions to simulate in the model.

To support this methodology, we also propose a web-based modeling platform, named ISiCell, that allows the automatic translation of the drawn diagrams into an executable simulation. The aim of ISiCell is both to reduce the development duration of the models and consequently to improve the interaction with biologists. With this platform, the modelers and the biologists can together draw the above-mentioned diagrams in a dedicated interface. Once designed, the platform generates the corresponding code of the model and compiles it on-the-fly to build an executable program. The simulation can be run in the cloud and be interactively parametrized, visualized and explored within the platform. Moreover, modelers and biologists can together explore the impact of different parameters with the dedicated tool. This exploration tool allows to (1) select one or multiple parameters of the model to be explore, (2) start a batch of simulations to screen the selected parameters and (3) display the kinetics of the model with different sets of parameters. It also enables to build an exploration tree to manually drive the calibration of the model.

First results with our methodology and platform shows the capacity to reproduce different models from the literature (intestinal crypt and muscle inflammatory process). Additionally, we were able to build new models with biologists in one-day workshops repeated in time to incrementally build and refine specific models. Namely, together with biologist partners, we have developed models representing the growth of multicellular tumor spheroids, oncolytic virus interaction with cancer cells, cytotoxic T lymphocytes interacting with target cell and generic inflammatory process. Initial feedbacks from the biologists show the capacity of the methodology and the platform to proactively involve the experts in the modeling process, additionally improving their understanding the internal functioning of the final model.

Session 3 – Heterogeneity & single cell

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Epigenomic evolution of breast cancers in response to treatment

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The dynamic nature of chromatin and transcriptional features are expected to participate to tumor evolution. Our group focuses on the study of the dynamics of histone modifications in cancer cells upon cancer treatment as well as during the initial steps of tumorigenesis. We develop experimental and computational approaches to map histone marks at single-cell resolution, enabling the investigation of the dynamics of chromatin marks in tumor samples (Grosselin et al. Nat Genet 2019; Prompsy et al. Nat Comm 2020).

We have recently combined single-cell epigenomic and transcriptomic approaches to lineage tracing strategies to reveal the initial epigenomic events driving tolerance to chemotherapy in triple-negative breast cancer (Marsolier & Prompsy et al., Nat Genet 2022). We show that the repressive histone mark H3K27me3 is a lock to the activation of a drug-persistent expression program in breast cancers. Under chemotherapy, very few cells can survive the treatment, and these cells have a remodeled repressive epigenome, with targeted loss at key promoters. Using demethylase inhibitor in combination to chemotherapy, we improve the response rate and delay recurrence both in vitro and in vivo.

We also study mechanisms of cell plasticity in early breast tumorigenesis in vivo. We have recently mapped state transitions during Brca1-tumorigenesis in the mouse. We discovered that luminal progenitor cells undergo a partial epithelial to mesenchymal transition at the onset of tumorigenesis (Landragin &Saichi, biorxiv 2022).

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Drug persisters arise from transcriptionally and mitochondrially distinct stem cell subpopulations in acute myeloid leukemia

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Acute myeloid leukemia (AML) is a heterogeneous hematological malignancy with a poor prognosis due to frequent relapse caused by tumor regrowth initiated by therapy-persisting leukemic stem cells (LSCs). We and others have shown that LSCs are rare and phenotypically heterogeneous when assayed in NSG-deficient mice. Strikingly, chemotherapy cytarabine (AraC)-residual disease is enriched in neither immature, quiescent cells nor LSCs but require oxidative metabolism in vivo. We further demonstrated that the ectonucleotidase CD39 maintains this high mitochondrial activity by inducing an adaptive mitochondrial stress response in a cAMP-PKA and ATF4 dependent manner. A recent study also showed that the ATF4-mediated stress response is restricted to LSCs as compared to more differentiated AML cells, suggesting an underappreciated functional, transcriptional, metabolic and mitochondrial heterogeneity of LSCs after treatments.

To address this hypothesis, we assessed LSCs heterogeneity using single-cell RNA sequencing (scRNA-seq; 10X Genomics) coupled to CITE- and CYToF-based approaches in PDX treated with AraC or targeted therapies (selective BCL2 inhibitor venetoclax). First, study of more than 30,000 single AML cells followed by Seurat and unsupervised hierarchical clustering analysis highlighted eight distinct transcriptional states. Cell clusters persisting after AraC displayed gene signatures enriched in oxidative stress response, lipid metabolic process, CD39 and CD36, consistent with our previous work at the bulk level. Unexpectedly, three new cell clusters appeared in VEN+AraC combination therapy-residual disease and their trajectories analysis revealed three different developmental stages: HSC-like, myelo-erythroid progenitor-like or monocyte-like, respectively. Further, we observed an upregulation of OxPHOS activity, ribosome, inflammatory response and CD39 pathway and a downregulation of LSC gene signature in these three clusters. Next, we used SCENIC approach, a clustering method to identify stable cell states and gene regulatory networks governing those states. Using this method, we confirmed the clear cell state distinction of VEN+AraC-treated cells and of CD39 cell subpopulations compared to other AML cell subpopulations. Moreover, SCENIC predicted a complex regulatory network involving regulons leaded by MITF and TP53. Similar workflow is pending to assess CD39 role and transcriptional heterogeneity following additional therapies such as VEN+azacitidine or FLT3 inhibitors gilteritinib in vivo. In addition, we showed that drug-persisting CD39high cell subpopulation was enriched in CD34+ cells with an increased cell granulosity and senescence gene signature at the bulk level. However, this cell fraction with high oxidative metabolism displayed characteristics distinct from therapy-naïve LSCs at diagnosis. Similarly, limiting dilution assay of FACS-sorted and purified LinnegCD39high cell fractions uncovered that AraC increased LSC frequency in LinnegCD39high subpopulation at a greater extent than in LinnegCD39low subpopulation. LSC gene signatures and the naïve LSC frequency were enriched and higher in LinnegCD39low subpopulation compared to LinnegCD39high subpopulation before treatment, respectively. Finally, metabolic prediction using our novel machine learning-based algorithm uncovered that AML patients overexpressing CD39 with a particular metabolic reaction network at diagnosis have a poor overall survival in public AML databases.

Collectively, these results indicate that the cell surface CD39 discriminates heterogenous subpopulations of therapy-naïve and -resistant LSCs with distinct functional, transcriptional, phenotypical, metabolic and oxidative states. Accordingly, we are developing a novel therapeutic solution combining standard chemotherapies, mitochondrial inhibitors and anti-CD39 immunotherapies targeting both therapy-naïve LSC and therapy-adaptive LSC subpopulations to overcome relapse in AML.

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TREM1+ CD163+ myeloid cells are potent immunosuppressive cells and associate with poor survival in human liver cancer

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Background. Hepatocellular carcinoma (HCC) is an inflammation-associated cancer and is among the deadliest cancers worldwide. Despite well-known risk factors, i.e. chronic viral infection with hepatitis B virus (HBV) primarily in Asia and HCV in western countries, excessive alcohol consumption and the metabolic syndrome-associated non-alcoholic steatohepatitis (NASH), HCC is diagnosed late in most patients (Llovet et al., 2021). The landscape of clinical trials for the treatment of advanced HCC has recently shifted to the field of immunotherapy and therapeutic options now includes the immune checkpoint inhibitors (ICI) nivolumab and pembrolizumab, and since 2020, the combination therapies (atezolizumab/bevacizumab and nivolumab/ipilimumab) (Finn et al., 2020). However, despite significant therapeutic advance with ICI, ~75% of patients do not respond to these immunotherapies for unclear reasons (Giraud et al., 2021). Recently, a meta-analysis of three randomized phase III clinical trials administering ICI to patients with advanced HCC showed a superior efficacy of immunotherapies in virally-infected patients compared to NASH-affected patients with HCC (Pfister et al., 2021). This suggests that the tumor microenvironment (TME) of HCC is an important determinant of therapeutic success and highlight the urgent need to further explore human liver-specific immunity towards the identification of theranostic immune biomarkers for patients' stratification and novel immunotherapies.

Expansion of suppressive myeloid cells is a hallmark of chronic inflammation and cancer. Their heterogeneity in HCC is not fully resolved and might underlie immunotherapy resistance. Several studies have employed single cell analyses, including single cell RNA sequencing (scRNA-seq) and mass cytometry, to characterize the cellular landscapes of HCC. However, the bulk of these studies included all liver cells, limiting the granularity of the analysis.

Objective. In this study, we setup to discriminate and localize human liver-specific innate immunity cells to improve the stratification and the treatment of patients with HCC.

Methods. Here we implemented scRNA-seq on purified CD45+panTCRαβ-CD19- cells freshly isolated from tumoral and juxta-tumoral tissues from 10 patients with HCC of different etiologies, and performed spatial transcriptomics (10x Genomics) to map their localization. We validated our results by multiplex immunofluorescence, by functional analyses performed on*ex-vivo* FACS-sorted cells co-cultures, on a mouse model of HCC, and by computational analyses of published HCC data sets.

Results. We report a high-resolution atlas of innate immunity cells (around 100,000 transcriptomes) in HCC and unravel a strong myeloid bias in NK cell differentiation and a remarkable myeloid cell heterogeneity. In particular, we identify three phenotypically distinct myeloid-derived suppressor cells (MDSC) populations, including polymorphonuclear MDSC, monocytic MDSC and a distinct population expressing a variety of myeloid lineage-affiliated genes and selectively marked by elevated expression of triggering receptor expressed by myeloid cells-1 (TREM1) in conjunction with CD163. We show that TREM1+CD163+ MDSC are the most potent immunosuppressive subset *ex vivo* and expand in models of liver inflammation and fibrosis *in vivo*. A specific gene signature defining TREM1+CD163+ MDSC correlate with poor patient survival in HCC and response to immune checkpoint blockade in different cancers. Accordingly, TREM1+CD163+ MDSC correlate with signatures of other myeloid cells with pro-tumoral activities, including TREM2+SPP1+ tumor-associated macrophages and VSIG4+ monocytic DCs. We further show that TREM1+CD163+ MDSC are high producers of TGFβ and spatially localize at liver fibrotic lesions in close association with scar-associated profibrogenic fibroblasts.

Conclusion. Collectively, our data support for a myeloid subset-targeted immunotherapies to treat HCC.

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Regulatory γδ T cells in solid cancer: characterisation, role and ecosystem

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γδ T cells contribute to the anti-tumor immunity within the tumor microenvironment (TME) in various cancers. Despite their well-described effector functions, recent studies correlated their presence in the TME with solid tumor progression suggesting that γδ T cells may display pro-tumor activities. My project aims to characterize those pro-tumoral or regulatory γδ T cells and decipher their role in cancer.

We demonstrated *in vitro* that inflammatory signals promote the development of a regulatory γδ T cell sub-population characterized by the expression of CD73 and displaying immunosuppressive functions through the production of immunosuppressive molecules such as IL-10, adenosine and the angiogenic and chemotactic factor IL-8. The challenge associated with the characterization of CD73+ γδ T cell resides in assessing their existence in vivo as well as their relevance in human cancers. We showed in human breast cancer that ~20% of γδ tumor infiltrating lymphocytes (TILs) expressed CD73 and displayed the same immunosuppressive functions as described *in vitro*, suggesting that they could promote tumor development via these mechanisms. In line with these observations, we showed that the presence of γδ TILs is associated with late tumor grades in breast cancer. We extended such observations to ovarian cancer and showed that the density of CD73+ γδ TILs negatively correlates with patient survival, suggesting that CD73+ γδ TILs density could be used as a prognosis factor. Using Imaging by Mass Cytometry, we are now investigating the cellular networks of regulatory γδ TILs (CD73+) and their effector counterpart (CD73-) in breast and ovarian tumors to better understand their role in cancer. Our data show different immediate ecosystems for CD73+ compared to CD73- γδ TILs, with more cancer-associated fibroblasts in contact with CD73+ γδ TILs, while CD73- γδ TILs interact more with activated αβ TILs reinforcing the idea that CD73+ and CD73- γδ T cells are functionally different.

Altogether, these data improve our knowledge on human γδ T cell immunobiology during cancer development, with the in-depth characterization of the new regulatory γδ T cell subset, their localization and their functions within the TME.

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Single-cell transcriptomics for a better understanding of tumor infiltrating heterogeneity

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The single-cell (sc) technology increased the amount of information that we could obtain from a sample, allowing us to be more precise in our understanding of tumor complexity and microenvironment. However, the most challenging on single-cell analysis are the methods we need to develop or apply to unveil this cellular complexity or heterogeneity. We thus used single-cell technology and developed a series of tools to investigate the tissue complexity of Splenic marginal zone lymphoma (sMZL). SMZL is a very rare small B cell malignancy which is still poorly described. Based on our former expertise in single cell RNA sequencing (scRNASeq) analyses, including the development of newer and powerful scRNAseq analytic softwares (Perchey et al., 2019; Pont et al., 2019; Pont et al., 2020), our study aims to characterize this disease through multiple single-cell technologies [RNA sequencing (scRNAseq), CITE-seq 3' chemistry, scRNAseq 5'-VDJ chemistry] on paired samples (blood/spleen) from 3 patients. Characterization of gene expression profile of tumor and microenvironment cells, as well as immunophenotype at single cell level was first performed together with CITE-seq 3' chemistry. ScRNAseq 5'-VDJ chemistry was also added to this analysis in order to obtain the B and T lymphocytes clonal information. To integrate and analyze all these data together from different perspective, many tools and pipelines were developed by using different programming languages, such as R, Julia, Perl, and GO. Altogether, these single-cell data showed intra and interpatient tumor cell heterogeneity with a loss of synchrony of tumoral cells during differentiation, but a homogeneous and conserved microenvironment between blood and spleen compartments. Finally, to better understand the spatial context of the studied cells in a tissue, we increase the complexity of the analysis by adding spatial transcriptomics data (Visium Spatial Gene Expression - 10X). This data adds a topographical information which was associated to our previous single-cell data. The spatial exploration of this data required a software development, single-cell Spatial Explorer (Pont F., & al, BioRxiv 2022), which allowed us to better understand the B and T cell organization and structure in sMZL tissues, and how these tissue organization is characterized in terms of molecular functions.

Session 4 – Innovations in intracellular targetting

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Targeted protein degradation as a cancer therapeutic modality

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Degrader molecules (also known as PROTACs) recruit proteins to E3 ligases for targeted protein degradation. Formation of a ternary complex between the PROTAC, the E3 and the target leads to the tagging of the target protein by ubiquitination, and subsequent proteasomal degradation.

Over the last decade, PROTACs have witnessed significant development and exponential rise in interest and adoption across academia and the biopharma industry, enabled by the discoveries of high-quality, drug-like small-molecule ligands for the E3 ligases von Hippel-Lindau (VHL) and cereblon (CRBN). They are today firmly established both as chemical tools to study biology and as next-generation medicine, with a first wave of >20 PROTAC drugs advancing in clinical trials against cancer and other diseases. Degrading rather than inhibiting a target protein offers a novel modality of chemical intervention, and advantages such as more efficacious drug response at lower doses, and enhanced target selectivity, with potentially reduced side effects and disease resistance.

This lecture will offer an introduction and a perspective on this rapidly evolving field. It will outline some of the key discoveries from our laboratory that helped to elucidate their mechanism of action and to provide a rational basis for their design and optimisation. I will also highlight current trends and future direction we and others in the field are taking in the design and development of the next generation of targeted protein degradation systems, including recent expansion beyond bivalency, tackling of challenging targets, and the development of novel PROTAC-inducible technologies to degrade any target protein e.g. BromoTag.

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The use of the HTRF® and AlphaLISA® technologies to support compounds identification and characterization in Targeted-Protein Degradation

Eric TRINQUET

Perkin Elmer, Cisbio Bioassays, Parc Marcel Boiteux, Codolet

Targeted protein degradation is a new therapeutic modality which appears as a promising alternative to the inhibition of protein activity. Compounds like Proteolysis-targeting chimeras (PROTACs) or molecular glues selectively adress targeted proteins to the ubiquitin-proteasome system and therefore induce their degradation.

To support the identification and the characterization of such compounds, Perkin Elmer has developed a unique set of biochemical and cell-based assays based on its proprietary no-wash assay platforms called HTRF® and AlphaLISA®.

Due to their « no-wash » design and their miniaturization capabilities, both technologies are compatible with High Throughput Screening (HTS) approaches, allowing a fast and accurate identification of new chemical entities.

The available biochemical assays allow researchers to determine the binding properties of the compounds towards E3 ligases. They are also able to monitor the formation of ternary complexes between the targeted protein, the compounds and an E3 ligase.

The HTRF or AlphaSureFire cell-based assays can monitor the selective degradation of a protein of interest expressed at endogenous levels in a large variety of cell systems. They are therefore highly suitable to determine the efficacy of selected compounds in physiologically relevant cellular models.

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Targeting microtubule detyrosination activity of VASOHIBINS as a new therapeutic approach for cancer and neurodegeneration

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Microtubules are essential cytoskeletal elements composed of α- and β-tubulin heterodimers. They are involved in many different functions including intracellular transport, cell motility, cell division and cell morphogenesis. The functional adaptation of microtubules is often achieved through posttranslational modification, which regulate their interactions with Microtubule Associated Proteins and molecular motors. The first tubulin modification to be discovered almost half a century ago is called detyrosination, which consists of the removal of the very C-terminal tyrosine encoded by most α-tubulin isotypes. Abnormally high levels of detyrosination have been found to be associated with various diseases including cancer and neurodegeneration.

Recently, we have discovered the two members of the Vasohibin family, VASH1 and VASH2, as the first class of enzymes involved in tubulin detyrosination. The identification of VASHs has been made possible by the development of custom-designed covalent inhibitors compatible with click chemistry. Further optimization of the original inhibitors through the use of medicinal chemistry and molecular docking led to the development of highly potent and cell penetrant compounds with IC50 in a lower nanomolar range. This will allow for the first time to evaluate the therapeutic potential of Vasohibin inhibitors against cancer and neurodegeneration using cell-based and animal models.

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Novel strategies to study epigenetic mechanisms in cancer

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Epigenetic mechanisms, including histone methylation and acetylation, and DNA methylation, control gene expression and play a fundamental role in cell homeostasis. Aberrant epigenetic pathways showed to favour cancer progression. For instance, overexpression of histone methyltransferases (HMTs), such as EZH2 and SETD8, was identified in several cancers [1-2], while DNA hypermethylation of specific tumour suppressor gene promotors leads to their transcriptional repression particularly in haematological cancers [3]. A variety of chemical agents modulating epigenetic modifications has been developed and reached the clinics, which validates these targets for cancer treatment [4-5]. However, currently available drugs are highly toxic and at low doses show poor efficacy.

Our group focuses on finding new pharmacological strategies to overcome these issues: 1) we first developed novel non-nucleoside DNA methyltransferase (DNMT) inhibitors to go beyond the high toxicity and poor stability of FDA- and EMA-approved nucleoside azacytidine and decitabine [6]. Our compounds showed good potency in the purified DNMT3A-c enzyme and in our reporter gene model in KG-1 leukaemia cell line; 2) we employed the multivalent strategy to enhance the potency and selectivity of currently used epigenetic inhibitors and identify new compounds with a significant increase in potency and a positive multivalent effect; 3) we finally applied the proteolysis targeting chimera (PROTAC) strategy to target an HMT and induce its degradation through E2/3 ligase-mediated ubiquitination and following proteasomal degradation in cancer cells. We designed and synthesised bifunctional compounds featuring an HMT inhibitor and different E3 ligase binders, linked together by several spacers of different lengths and chemical natures. We then evaluate their potency in cancer cell lines assessing their cytotoxicity and their ability to lower histone methylation levels and induce target degradation.

We hope that our diversified epigenetic toolbox will boost the potential of epigenetic drug discovery for cancer research and treatment, benefiting both the scientific community and future patients.

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Targeting hard-to-drug oncoproteins with intracellular antibodies

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Many disease causing proteins such as mutated oncoproteins are localised inside the cells. These deregulated proteins often lack a drugabble pocket as they can work by protein-protein interactions and therefore require specific binding molecules to interfere with their function. Intracellular antibodies are binding molecules that generally only comprise the antigen-binding regions of antibodies. They are expressed in the cells and can be used to exploit the natural features of antibodies' specificity and high affinity to interfere with the function of target proteins. In addition, intracellular antibodies can be easily modified by molecular engineering to add a warhead that will affect target antigens by activating cell-intrinsic functions such as the proteasomal degradation with antibody-based degraders. In this presentation, we will discuss how intracellular antibodies can specifically target major oncoproteins such as KRAS and highlight novel inhibitory mechanisms but also how they can be used to defeat the resistance to treatment in cancer.

Afternoon opening session

FOXC2 promotes vascular mimicry and resistance to anti-angiogenic therapy

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Vascular mimicry (VM) describes the existance of pseudo-blood vessels formed of tumour cells that have acquired endothelial-like properties. VM channels endow the tumour with an alternative vascular system that directly connects to host blood vessels, and VM has been postulated as a mechanism of resistance to anti-angiogenic therapies (AAT). However, evidence for VM-driven resistance to AAT and a molecular understanding of how tumour cells acquire endothelial-like characteristics have been lacking. Using a mouse model of breast cancer heterogeneity, we recently identified lineages for which VM drives metastasis. We find that these VM-proficient lineages coopt an endothelial transcription factor, Foxc2, to promote ectopic expression of vascular/endothelial genes in tumour cells and survival under hypoxia. Moreover, VM-proficient tumours are largely resistant to AAT and suppression of Foxc2 enhances the response to AAT thus motivating the search for VM-inhibitory agents that could form the basis of combination therapies with anti-angiogenics.

Session 5A – Genome Dynamics & Cancer

5A / 1

Cytidine deaminase controls replicative stress and protects cancer cells from DNA-targeting drugs

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DNA replication stress and genome instability are general hallmarks of cancer that fuel oncogenesis and tumor diversity. Therapeutic approaches to leverage replication stress to intolerable levels in tumors is gaining momentum, especially for pancreatic cancer. However, the molecular mechanisms involved in replication stress response in pancreatic tumors are unclear. We show that cytidine deaminase (CDA), which functions in the pyrimidine salvage pathway for DNA and RNA synthesis, is overexpressed in human pancreatic tumors and is essential to cell proliferation and tumor growth in mice. We unveil that CDA locates at the replication fork and promotes DNA replication by increasing replication fork speed, limits replication stress, DNA breaks and chromosomal instability. CDA expression in pancreatic cancer cells decreases the number of genetic alterations in long-term culture, and is positively associated with genetically unstable PDAC tumors. In functional studies, CDA expression protects pancreatic cancer cells from DNA-damaging agents, while CDA targeting in vitro and in vivo in PDOX increases replication stress as revelaed by scRNAseq and senstizes human primary cells to oxaliplatin. Our findings reveal new evidence on how pancreatic cancer controls replication stress and genome stablity, and suggest avenues into new therapeutic opportunities to defeat tumor resistance to treatment.

5A / 2
Targeting RUVBL1/2 chaperones in colorectal cancer

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Colorectal cancer (CRC) is a heterogeneous disease, both at the clinical and molecular levels, which is a major challenge for prognosis and treatment. However, recent efforts aimed at classifying CRC into distinct consensus molecular subtypes (CMS) brought new prospects for biomarker-guided targeted therapies. Colorectal tumors can be stratified into four subgroups, with different transcriptomic profiles, activated cellular pathways, histology and immune status. Consequently, CMS also differ in response to treatments, and prognosis. Importantly, only tumors from the CMS2 respond well to standard-of-care therapies, while CMS4 is associated with the worst prognosis. A recent study opened new therapeutic perspectives, finding that pre-clinical models of CMS 1 and 4 are sensitive to HSP90 inhibition (Sveen et al. , 2018).

HSP90 is a cellular chaperone responsible for the folding, stabilization, and activation of thousands of cellular proteins, with numerous substrates involved in cell signaling, growth and proliferation. Yet, past clinical trials based on HSP90 inhibition in cancer treatments have been unsuccessful, with severe side-effects. This could result from the pleiotropic functions of HSP90 and the lack of patient stratification for HSP90 inhibition. We propose to tackle these issues in two ways. First, we characterize inhibitors of HSP90 co-chaperones, in order to selectively target a fraction of HSP90 substrates. Second, we test the cytostatic effect of these inhibitors in pre-clinical models representative of the different colorectal CMS.

Specifically, we studied the HSP90 co-chaperones R2TP and TTT, which share the RUVBL1/2 AAA+ ATPases as a catalytic core. R2TP and TTT fold, stabilize and assemble into functional complexes proteins with crucial roles in transcription, DNA damage response, translation, cell growth and proliferation. Both co-chaperones are over-expressed in colorectal tumors and we found that elevated levels of R2TP is a bad prognosis factor in colorectal cancers. Accordingly, we showed that R2TP sustains cell growth in the murine intestinal epithelium (Maurizy et al., 2021). Finally, we leveraged a recently developed inhibitor of RUVBL1/2, CB-6644, to inhibit R2TP and TTT activities in CRC cells (Assimon et al. 2019). We found that CB-6644 efficiently and selectively inhibits the proliferation of colorectal cancer cell lines of CMS 1, 3 and 4. We will discuss the underlying mechanisms that may explain this differential sensitivity.

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Mechanical regulation of bivalent gene expression via the nuclear lamina

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Extracellular matrix (ECM) stiffness is of critical physio-pathological importance regulating mesenchymal and epidermal stem cell fates and promoting metastatic programs of cancer cells. At cellular levels, mechanical cues induce cytoskeleton and nuclear envelope rearrangements. However, how and to what extent such changes influence chromatin organisation, chromatin landscape and gene expression remain largely elusive. Using hydrogel-based culture to mimic physiological stiffness combined with super-resolution microscopy and western-blot approaches, we first characterised the modifications in the organisation of the nuclear lamina. From soft to stiff conditions, thickness of the lamina is changing as well as lamina components expression (3 to 30 folds). Since the lamina influences chromatin organisation through direct contacts, we next analysed how mechanical cues impact 3D genome organisation, chromatin accessibility, active and repressive histone marks and gene expression programs using Hi-C, ChIP-seq, Cut&Run and RNA-seq in primary fibroblasts. We found ~5000 genes that are differentially expressed between cells platted on soft or stiff matrix. These modifications in gene expression programs do not alter TADs or chromatin loops but large loci are switching from the A to B compartments. While ECM stiffness remodels the lamina organisation, its association to the chromatin do not vary indicating robustness of these structures. However, under soft conditions hundreds of apparent bivalent genes marked by both H3K4me3 and H3K27me3 histone marks escape efficient repression and are up-regulated. Using siRNA targeting lamina components, the LINC complex which bridges the lamina to the cytoskeleton as well as toxin targeting the cytoskeleton organisation, we show that these elements are required for an efficient repression of bivalently marked genes under stiff conditions. Our work uncovers how the nuclear lamina acts in cell adaptation and in gene expression control through its composition plasticity in response to mechano-sensation.

5A / 4

The E3 ubiquitin ligase TRIP12 induces the formation of heterochromatin altering gene expression and DNA damage repair independently of its catalytic activity.

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TRIP12 is a nuclear HECT-type E3 ubiquitin ligase that is overexpressed in numerous cancers. TRIP12 is involved in several nuclear functions via its catalytic domain. Indeed, TRIP12 is involved in pancreatic carcinogenesis by controlling the stability of PTF1a, an essential transcription factor for pancreatic homeostasis. TRIP12 regulates the chromatin remodelling complexes SWI/SNF and PRC1 by inducing the degradation of BAF57 and ASXL1, respectively. It is also involved in DNA damage repair by targeting RNF168 and parPARP1. Recently, we identified in the N-terminal extremity of TRIP12 an intrinsically disordered region (IDR) which is responsible for TRIP12 interaction with the chromatin. However, the consequences of TRIP12 overexpression observed in cancers on chromatin homeostasis and the involvement of its IDR in this process remain largely unknown.

First, we performed BioID experiments to establish an exhaustive mapping of TRIP12 protein partners. We identified 328 statistically enriched proteins, among them, already known substrates such ASXL1 or BAF57. In silico functional network analysis revealed that TRIP12 partners are massively involved in chromatin organization and histone modifications which are in favor of a global function of TRIP12 on chromatin organization. Second, we evaluated the effects of a TRIP12 overexpression on global chromatin organization by high-resolution microscopy. Interestingly, we observed that TRIP12 expression modifies the organization of the genome by forming chromatin condensates in a dose-dependent manner and independently of its catalytic activity. Using a series of TRIP12 deletion constructs, we identified the IDR as the domain responsible of the chromatin condensates formation. Moreover, we proved that the chromatin condensates are enriched in heterochromatin marks such as HP1a, EZH2, H2AK119Ub and H3K27me3. By using a nanobody coupled-degrader and half-bleach FRAP experiments, we demonstrated that the formation of these chromatin condensates is reversible and governed by polymer-polymer phase separation.

In parallel, we measured the functional consequences of TRIP12 induced-chromatin condensates on biological processes such as cell cycle progression, transcription, DNA replication, genome accessibility and DNA damage response. By live cell microscopy and immunofluorescence, we demonstrated that these chromatin condensates impair cell cycle progression and global transcription but do not affect DNA replication. By ATAC-seq approaches, we demonstrated that the global genome accessibility is drastically modified in response to IDR-expression. Finally, it is well known that chromatin compaction regulates DNA damage repair efficacy. Interestingly, in TRIP12-transfected cells, we observed a drastic inhibition of NHEJ pathway effectors (53BP1 and MDC1) after irradiation in a dose dependent manner and independently of its catalytic activity.

Altogether, our results reveal a new dynamic role for TRIP12 on heterochromatin homeostasis independently of its catalytic activity and through its IDR which alters major biological processes such as transcription and DNA damage pathways. Therefore, TRIP12 overexpression could dramatically modify the genome organisation/expression of cancer cells and sensitize them to DNA damage-inducing chemotherapies.

5A / 5

A small RNA-based innate immune system guards the integrity of germ cell genomes

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PIWI-family proteins and their associated small RNAs (piRNAs) act in an evolutionarily conserved innate immune mechanism that provides an essential protection for germ cell genomes against the activity of mobile genetic elements. piRNA populations comprise a molecular definition of transposons that permits them to be distinguished from host genes and selectively silenced. piRNAs can be generated in two distinct ways. Primary piRNAs emanate from discrete genomic loci, termed piRNA clusters, and appear to be derived from long, single-stranded precursors. The biogenesis of primary piRNAs involves at least two nucleolytic steps. Zucchini cleaves piRNA cluster transcripts to generate monophosphorylated piRNA 5' ends. piRNA 3' ends are likely formed by exonucleolytic trimming, after a piRNA precursor is loaded into its PIWI partner. Secondary piRNAs arise during the adaptive ping-pong cycle, with their 5' termini being formed by the activity of PIWIs themselves. At least in Drosophila, piRNAs are maternally deposited and transmit an epigenetic signal essential for the effective control of at least some transposable elements. Our continuing efforts combine genetics, biochemistry, structural biology, and evolutionary and computational approaches to understand how the piRNA pathway effectively discriminates self from non-self at the genomic level.

Session 5B – NanoCancer, new devices for therapy

5B / 1

From workbench to Phase 2 clinical trials: development of the nanodrug candidate AGuIX - history and perspectives

Géraldine LE DUC

NH TherAguiX, Meylan

Widely used for decades, radiotherapy is one of the reference treatments for cancer and it concerns 60% of patients. NH TherAguix (NHT) has developed an innovative theranostic approach in nanomedicine: designed to increase the dose and efficacy of radiotherapy within the tumor itself without increasing damage to healthy tissue, the nanoparticle AGuIX® also allows for very precise guidance by imaging. AGuIX® is based on the use of nanoparticles composed of gadolinium, already known for its magnetic properties and used as a positive contrast agent for MRI. In addition, gadolinium has a high atomic number (Z = 64) allowing for a very strong interaction with X-rays. Once inside the tumor after an intravenous injection (EPR effect), it has the potential to increase the effectiveness of radiotherapy even though the dose of X-rays passing through normal tissue remains unchanged. NHT aims to make AGuIX® a new standard of care in oncology in the field of precision radiotherapy.

Backed up by >70 scientific publications and 14 patent families, AGuIX® is a chemistry innovation born in the lab of Prof. O. Tillement (Institut Lumière Matière, Université de Lyon) and developed worldwide thanks to academic collaboration, at the in vivo scale. NHT was created in Dec. 2015 by S. Dufort, F. Lux, O. Tillement and G. Le Duc who is the CEO since inception. NHT has raised dilutive funds of €3.5M in 2015-18, before to perform a A Series in 2019 (€13M) with BPI, Omnes, Arbevel, Supernova as Venture Capitalists funds. At present the team consists of 16 people and its board of directors is chaired by H. Brailly (Co-founder and chairman of Innate Pharma).

The first human study was conducted during the NANORAD 1 Phase 1b trial, that evaluated AGuIX® in terms of (i) tolerance after intravenous injection, (ii) MRI contrast uptake and (iii) initial evidence of radiosensitization. Beyond safety and pharmacokinetic aspects, NANORAD 1 has demonstrated a dose effect between the concentration of AGuIX injected and the tumor volume reduction and a clinical benefit for the majority of patients in this study [1,2]. At present, the company is running 5 clinical trials: NANORAD (Ph. 2, brain metastases treated by pan-encephalic irradiation, 100 patients, randomized, multicentric, Dr. C. Verry, CHUGA), NANOCOL (Ph. 1b trial, cervix cancer treated by radiotherapy and brachytherapy, 12 patients, Prof. C. Chargari, IGR, Paris), NANOBRAINMETS (Ph. 2, brainmets using stereoradiosurgery, 134 patients, randomized double-blind, Dr. A. Aizer, DFCI, Harvard), NANOSMART (Ph. 1b/2, pancreas/lung treated by MRI-Linac, 99 patients, randomized, Dr. J. Leemann, DFCI, Harvard) and NANOGBM (Ph. 1b/2, glioblastoma, 66 patients, multicentric and randomized, Dr. Julian Biau, Centre Jean Perrin).

The presentation will be a testimony of an entrepreneurship venture based on an academic innovation, including science/medicine key points for the project

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Where, when et how many: MRI for theranostic AGuIX nanoparticles

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Theranostic nanoparticles, defined as agents with coexisting therapeutic and imaging functions, offer the possibility of bringing diagnosis and therapy closer together. They are expected to play an important role in personalized and precision medicine, with individualized therapies and optimized treatment approaches. Among these theranostic agents, MRI-traceable nanoparticles are mostly based on paramagnetic (Gd^{3+} and Mn^{2+} ions) and superparamagnetic (iron oxide) particles bound to the therapeutic part of the agent that can interact chemically or physically with the biological target (e.g., by heat or radiation). In some cases, the MRI contrast agent is the therapeutic agent itself, as it is the case for Gd-based radiosensitizing agents.

Regardless of the mechanism of action of these MRI-traceable theranostic agents, the measurement of their tissue concentration is essential. As a matter of fact, the measurement and the mapping of their concentration allow to (i) correlate their concentration and the magnitude of the therapeutic effect observed locally, (ii) to estimate the expected therapeutic effect knowing the local concentration and (iii) to spatially adjust and intensity modulate any controllable external therapeutic modality to the local concentration of the theranostic agent. In the case of theranostic radiosensitizers, the spatial and amplitude modulation of the ionising radiation can be carried out using standard radiotherapy equipment or MR-Linac systems.

In this presentation, we will show how MRI can help answer these three related key questions: **where** (the theranostic agents are), **when** (these agents are distributed in tumors and tissues) and **how many** (agents are present in tumors and tissues)? To illustrate our point, we will focus on the use of the Gd-based radiosensitizing agents AGuIX[1] with applications in animal models of tumor and in patients with multiple brain metastases[2-4].

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5B / 3

Pharmaceutical development to enhance small Extracellular Vesicles (sEV) therapeutic potential

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Despite the proof of concept of their efficiency as drug delivery systems (DDS) compared to synthetic nanoparticles, the rationale of using extracellular vesicles (EVs) in therapy for their intrinsic properties or as DDS still requires improvements due to technical limitations (handling, drug loading reproducibility and rates, blood instability of allogenic EVs).

Working in a chemistry institute and coming from the field of drug delivery vector, we decided to use the tool we use with synthetic vectors to manipulate mMSC EV and try to find solution to alleviate these limitations. One of our research lines focuses on transient functionalization of EVs surface to increase their plasma stability while maintaining their cell internalization capacity. Our strategy relies on the post insertion of fine-tuned bio-inspired polymers: the poly(2-oxazoline)s (POx). Known for their excellent biocompatible properties, POx also constituted an excellent alternative to poly (ethylene glycol) (PEG) as clinical awareness has risen around its overuse (e.g. anti-PEG Abs).

We will present here a quick overview of what we have been doing with EV field in term of pharmaceutical development, especially on the evaluation of surface modification to develop allogenic EV that can be more rational as therapeutics.

5B / 4

Targeted thermal or mechanical nanotherapy of pancreatic adenocarcinoma

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Magnetic nanoparticles (MNPs) are already widely used in nanomedicine, particularly as MRI contrast agents or in magnetic hyperthermia therapy. The first clinical trial using nanotherapy was conduct in 2011 to treat high-grade brain tumors. Currently, the efficacy of nanotherapy combined with radiotherapy is investigated as a new treatment against prostate cancer. However, the benefit on life expectancy remains negligible and neither radiotherapy nor magnetic hyperthermia can distinguish between normal and cancerous tissues, responsible of adverse effects. Our strategy is based on the vectorization of iron oxide magnetic nanoparticles called **NanoFlowers** (NFs) capable of recognizing targeted cells and therefore specifically treating cancerous tissue thought the application of an external magnetic field, minimizing damage to healthy tissue. Under a **high frequency magnetic field** (AMF) exposure, the heat of NFs will specifically eradicate these cells, without macroscopic temperature elevation. Therefore, the rotation of the NFs under a **low frequency rotating magnetic field** (RMF) application generates mechanical forces leading to cell destruction. As a proof of concept, we have chosen a model of **pancreatic adenocarcinoma** (PDAC), a cancer with a very poor prognosis. The therapeutic failure is especially due to the development of multidrug resistance resulting from many mechanisms such as the lysosomal sequestration of chemotherapies. Moreover, tumor microenvironment plays a critical role in the development of PDAC resistance. By secreting extracellular matrix proteins, Cancer-Associated Fibroblasts (CAFs) create a physical barrier that limits the penetration and the efficacy of treatments (chemotherapy and radiotherapy). PDAC cancer cells and CAFs can overexpress the type 2 cholecystokinin (CCK2) receptor that is internalized after its activation. The graft of a specific agonist of the CCK2 receptor, the Gastrin, at the Nanoflower surface (NF@Gastrin) allows their accumulation into the lysosomes of pancreatic cancer cells and CAFs overexpressing the CCK2 receptor. The RMF (1Hz, 40 mT) or AMF (275 kHz, 30 mT) application kills up to 45% of cancer cells and CAFs that have internalized NF@Gastrin, slows down their proliferation without affecting cells lacking the nanoparticles. We showed that these two strategies also inhibit cell migration and stimulate the expression of Damage-Associated Molecular Pattern (DAMP) proteins such as Calreticulin and HSP70, well known to induce an immunogenic anti-tumoral response. Current studies are performed in order to determine the impact of these two strategies, consisting in thermal or mechanical energy delivery through magnetic nanoparticles excited by an external magnetic field, on spheroids & preclinical in vivo models.

Session 5C – Nouveaux dispositifs numériques de prévention

5C / 1

Epidaure Market, évaluation de l'efficacité et de la transférabilité d'une intervention en milieu scolaire visant à améliorer les choix alimentaires équilibrés et durables chez des collégiens

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Au moins 40 % des cancers sont liés à des comportements ou à des facteurs environnementaux sur lesquels on peut agir. La prévention est donc une priorité pour lutter contre les cancers, et en particulier la prévention nutritionnelle. Il n'est plus à démontrer que les comportements de consommation sont largement influencés par le marketing, la publicité, ou encore les pressions extérieures (pairs, famille, milieu social…), comme le signale entre autre une expertise collective INSERM de 2017. Le comportement alimentaire est aussi très fortement influencé par les motivations de choix alimentaire (e.g. l'importance de choisir des produits bons pour ma santé, favorable à l'environnement…). Cette motivation de choix dépend de facteurs environnementaux comme le marketing et les médias, l'accessibilité et la disponibilité alimentaire ; de facteurs sociaux tels que la culture, l'influence des parents et des pairs ; mais aussi individuels (e.g. auto-efficacité, normes sociales perçues, connaissances…). Les facteurs influençant les motivations alimentaires n'ont de surcroit pas le même sens et le même poids selon les âges de la vie. Comme le montre une revue de la littérature sur les interventions visant à modifier l'alimentation des jeunes, l'école est un lieu idéal pour promouvoir l'alimentation saine.

L'intervention Epidaure Market a précisément pour objectif d'améliorer les choix alimentaires (équilibrés et durables) des jeunes grâce à des séances pédagogiques basées sur l'utilisation d'une application simulant un supermarché virtuel. Cette intervention se déroule en milieu scolaire et a été co-construite avec des chercheurs, des enseignants de l'Education Nationale et des acteurs de prévention, afin de permettre une bonne acceptabilité de l'intervention par les enseignants et de la rendre accessible à tous les élèves.

L'objectif principal est d'évaluer l'efficacité d'Epidaure Market auprès de collégiens de 5ème et 4ème (âgés de 12 à 14 ans) sur la motivation de choix alimentaires plus durables. Les objectifs secondaires sont 1) d'évaluer l'efficacité de l'intervention sur le sentiment d'auto-efficacité, les normes sociales perçues, les connaissances sur l'alimentation durable, des techniques de marketing et de l'influence de ces dernières sur leurs choix ; 2) d'évaluer l'acceptabilité, l'applicabilité et les conditions de transférabilité de l'intervention. Pour construire l'intervention et impacter au mieux les variables ciblées, nous avons utilisé les travaux de Carey et al, (2019) basés sur les techniques de changement de comportement (BCT) et le modèle du COM-B de Michie et al, 2011.

Le design de l'étude d'efficacité est un essai randomisé contrôlé en cluster, comparant un groupe de 36 classes recevant l'intervention et un groupe contrôle de 36 classes de 5ème et 4ème (sans intervention) (40 à Montpellier et 32 à Dijon, voir figure 1 ci-dessous). L'unité de randomisation est la classe, stratifiée selon le niveau social et la zone rurale ou urbaine du collège. La population est composée de 1800 élèves âgés de 12 à 14 ans. L'applicabilité et les conditions de transférabilité seront évaluées par une étude mixte qualitative-quantitative auprès des différentes parties prenantes (élèves, enseignants, personnels d'encadrement, parents). Les données des questionnaires auprès des élèves éclaireront les informations issues des focus groups, des entretiens individuels semi-dirigés et le recueil des éléments de la grille ASTAIRE (Cambon et al. 2013). Celle-ci prévoit la description de la population, les facteurs de l'environnement susceptibles d'influencer les effets de l'intervention, les éléments d'implantation, et les moyens d'accompagnement au transfert qui contribue à l'adaptation au nouveau contexte.

5C / 2

Le soin par la musique : Music Care© un dispositif médical innovant dans le domaine de la prévention

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Music Care est la première thérapie numérique validée par plus de 150 études scientifiques réduisant significativement la douleur et l'anxiété de plus de 50 % grâce à ses algorithmes de composition musicale conçus cliniquement.
-Nous avons créé un algorithme modulant les paramètres musicaux pour diminuer significativement la douleur, l'anxiété et les troubles du sommeil *.
-Music Care utilise la recherche scientifique pour composer une musique synchronisée avec les paramètres vitaux **
-Notre induction musicale est fondée sur les résultats de la littérature scientifique.
-Notre algorithme est basé sur notre méthode innovante de la Séquence "U" utilisant le principe de la méthode d'hypnoanalgésie*.
-Nous mettons en œuvre notre thérapie numérique sur des appareils destinés aux établissements de santé et aux patients.
Music Care utilise la musique comme médicament pour soulager la douleur et réduire l'anxiété, sans effets secondaires pour les patients.
Music Care a été mis en œuvre dans certains des hôpitaux et cliniques les plus réputés du monde. Les résultats montrent :
- Une diminution du niveau de douleur de 50% (Clin J Pain. 2012).
- Une diminution du niveau d'anxiété de 70% (JAMA. 2021)
- Une diminution de la durée des soins de la douleur de 30 % (Ann Phys Rehabil Med. 2018).

*Evaluation de l'application standardisée MUSIC CARE© dans le traitement de la douleur : la technique de composition en U. Musique et Médecine. 2014
**Les effets d'une intervention musicale dans la gestion de la douleur chronique : un essai contrôlé, randomisé, en simple aveugle (n=87).
Clin J Pain. 2012

5C / 3

Etudes pilotes multi-méthodes évaluant l'intérêt d'une intervention musicale associée à un substitut nicotinique versus substitut nicotinique seul sur le craving lié au sevrage tabagique

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La réduction du tabagisme demeure une priorité absolue du nouveau plan de lutte contre le cancer 2021-2030, dont l'un des objectifs est de supprimer l'exposition de la population au tabac, premier facteur de risque de cancer. Notre équipe a montré les effets préventifs et curatifs de l'environnement enrichi ie des conditions environnementales positives et stimulantes, dans plusieurs modèles animaux d'addiction, pour différentes drogues dont la nicotine (Chauvet et al 2009 ; Solinas et al 2008 ; Sikora et al 2018). Nous avons suggéré que les effets in vivo de l'environnement enrichi sur le craving, ce besoin compulsif de consommer, considéré comme une composante centrale de l'addiction, seraient liés à sa capacité d'agir sur le système de stress en diminuant sa réactivité (Solinas et al 2010 ; Solinas et al 2020).

La musique qui constitue une forme d'environnement enrichi, pourrait permettre d'améliorer l'accompagnement des patients en sevrage tabagique. Effectivement son rôle a été étudié depuis plusieurs années avec des effets identifiés dans différentes pathologies (Robb et al 2018). Des recherches ont envisagé d'utiliser la musicothérapie pour intervenir sur le craving (Perkins et al 2013 ; Silverman 2011). A notre connaissance, aucune étude randomisée n'a évalué l'intérêt d'une intervention musicale dans l'arrêt du tabac alors qu'une telle intervention pourrait aider à la gestion du craving des personnes qui souhaitent arrêter de fumer.

Nous proposons donc de mener 2 essais pilotes randomisés et contrôlés comparant l'efficacité et la faisabilité d'une intervention musicale associée au traitement substitutif nicotinique (TSN) versus TSN seul dans l'amélioration de la gestion du craving à 1 mois, sachant qu'1 mois sans fumer est la période de sevrage qui augmente de 5 fois les chances d'arrêter de fumer à long terme. Le rythme des séances sera de 2 par semaine le premier mois, 1 par semaine le deuxième et 1 tous les 15 jours le troisième. Les participants seront vus en consultations à 1, 2 et 3 mois après la date d'arrêt du tabac, et entre les visites, seront contactés par téléphone. Ces études pilotes combineront la méthodologie des essais cliniques avec des techniques qualitatives (entretiens, étude des possibles mécanismes cognitifs et affectifs sous-jacents l'efficacité de l'intervention musicale). Elles sont financées dans le cadre de deux AAP nationaux.

La 1ère étude ciblera des étudiants fumeurs (120), chez lesquels la prévalence du tabagisme quotidien observée en 2018 restait encore trop élevée quel que soit le sexe, avec peu de programmes spécifiques d'arrêt du tabac disponibles dans cette population (Karekla et al 2020). Or des études montrent que le stress et le craving influencent l'arrêt du tabac chez les jeunes adultes (Villanti et al 2016).

La 2ème étude s'intéressera au sevrage tabagique chez les professionnels de santé (50) qui jouent un rôle essentiel dans cette lutte anti-tabac. Or ces professionnels sont également concernés par le tabagisme avec une prévalence de 16% à 43% selon les professions (SPF 2017) et leur statut tabagique a une incidence sur la manière dont ils dispensent les traitements de sevrage tabagique (Duaso et al 2014 ; Duaso et al 2017).

Le recrutement et le suivi des participants se fera au sein du service de santé universitaire de l'Université de Poitiers et du Centre d'Investigation Clinique CIC P1402 du CHU de Poitiers. Les séances de musicothérapie seront réalisées via l'application Music Care©. Les résultats de ces études pilotes permettront d'ajuster le schéma d'études ultérieures de puissance adéquate et pourraient ouvrir de nouvelles perspectives dans les stratégies de sevrage tabagique. Les programmes de santé publique pourraient inclure cette technologie innovante d'une intervention musicale via un logiciel sur tablette, simple à mettre en œuvre, dont l'utilisation pourrait par ailleurs être élargie à d'autres populations de fumeurs.

Session 6A – Aging & stem cells

6A / 1
Cancerstemflammaging - state/fate impact of cancer cell senescence

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Activated oncogenes and cancer therapies evoke cellular stress responses with similarities to tissue regeneration upon injury, but typically derailed outcome in the malignant setting ("wounds that never heal" [Harold F. Dvorak, 1986]). Reminiscent of senescence as the state switch at the end of a cell's replicative capacity during aging, and accompanied by the senescence-associated secretory phenotype (SASP) composed of largely pro-inflammatory cytokines underlying "inflammaging", cancer cell senescence hijacks features of wound healing, especially the de novo conversion into cancer stem cells. Besides senescence-associated stemness and adding to its prime phenotype as a terminal cell-cycle arrest, therapy-induced senescence (TIS) exhibits aberrant plasticity and transdifferentiation states affecting biological presentation, immunogenicity, host immune exhaustion, and, ultimately, organismic fate and treatment failure. Simultaneously, stemness and plasticity imply hitherto underrecognized opportunities for conceptually novel therapeutic strategies, in particular signaling- or immune-based senolytics. Moreover, since senescence occurs under various triggers as replicative senescence in aging, oncogene-induced senescence or TIS in cancer, and virus-induced senescence in COVID-19 and other highly inflammatory viral infections, senescence-associated plasticity might have critical implications beyond cancer.

6A / 2

A single short reprogramming early in life improves fitness and increases lifespan in old age

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Although, the iPSCs reprogramming process, using the 4 Yamanaka factors, OCT4, SOX2, KLF4, C-MYC (OSKM)[1], has been described to favor senescence, establishing senescence as a barrier to reprogramming, we were able to derive iPSCs efficiently from senescent cells and from centenarian donor cells, using an optimized reprogramming strategy based on 6 factors (OCT4, SOX2, KLF4, C-MYC, NANOG, LIN28)[2]. This strategy allowed to overcome the senescent state and both gene expression patterns, telomere length and metabolism were rejuvenated after reprogramming into iPSCs and re-differentiation into fibroblasts.

We and others further investigated for a different reprogramming regimen using OSKM factors to avoid senescence promotion and promote rejuvenation[3]. Consistent with previous results, we showed that inducing transiently OSKM factors in fibroblasts decreases DNA damage and senescence and activate autophagy, in vitro[4].

To reproduce this activity in vivo, we derived a mouse transgenic mice model, allowing both a controlled expression of OSKM by doxycycline and recapitulating the human phenotype of the accelerated aging syndrome of Hutchinson-Gilford Progeria (HGPS). We firstly established a specific induction protocol that significantly extend the lifespan of this accelerating aging mice confirming our hypothesis and previous results[5]. Then, we investigated for tissue integrity improvement. Strikingly, we observed that a single transient reprogramming induction for a short period of time, in the early life was able to improve body composition and functional capacities of mice over the entire lifespan. In addition, treated mice have improved tissue structures such as bone, cartilage, lung, spleen, kidney and skin, leading to an increased lifespan of 15%, in old age. Moreover, this single short reprogramming, applied early in life initiates and propagates an epigenetically related rejuvenated cell physiology, to promote a healthy lifespan[6].

This new transient reprogramming strategy is a pertinent approach to explore and deconstruct potential rejuvenation mechanisms and its propagation to prevent age-related pathologies, promoting healthy aging.

1. Takahashi et a., Cell 2006
2. Lapasset et al., Genes and developement 2011
3. Sarkar et al., Nature Communications 2020
4. Alle et al., Biorvx 2021
5. Ocampo et al., Cell 2016
6. Alle et al., Aging Cell 2022

6A / 3
Stem cells, aging & cancer across species

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Both aging and cancer involve accumulation of mutations and alteration of stem cells, but for many reasons, it is unclear what the difference is exactly between the two processes. A consistent view has emerged of cancer being a certain trajectory of aging driven mainly by the accumulation of random somatic mutations with a constant probability of occurrence. However, certain facts, mainly from evolution and phylogenetics, do not fit well into the picture and suggest an alternative view of the role of stem cells in aging and cancer should be proposed.

Among the recent facts that seem to support this traditional view, one may think of Tomasetti & Vogelstein (2015)'s work on the existence of a strong correlation between the number of replications of stem cells and the lifetime risk of cancer, in different tissues in humans. Cagan et al. (2022) have similarly established an inverse correlation between somatic mutations rates and lifespan across species, while Whittemore et al (2019) have established that lifespan is predicted by the rate of telomere shortening, and Vincze et al (2022), that there is no correlation between adult lifespan and cancer risk.

Yet this picture does not fit well with the fact that, if cancer does not develop in postmitotic tissues, these tissues may both age exceptionally slowly (e.g., human neurons) or very quickly (e.g., C. elegans). Second, some exceptionally quickly replicative tissues (e.g., in Cnidaria) neither age nor have cancer. All of this suggests that the replicative rate alone does not explain both aging and cancer.

Based on my recent work on aging across the tree of life (Lemoine, 2021), I propose the alternative hypothesis that cancer risk is proportional to replicative rate only in aging tissues. In this view, tissues do not age because they accumulate mutations (Vijg 2021), but accumulate mutations because they age. 'Aging,' in this sense, involves the degradation of the organization of the tissue, both structural and functional, as a consequence of imperfect repair capacity, which would in turn allow cells to accumulate mutations and other forms of damage that would, in a non-senescent tissue, normally lead to their elimination. This hypothesis relies on the distinction between unicellular and metacellular aging I have proposed, and partially turns on its head the cell-centric view of cancer and aging usually accepted. In short, unicellular aging is the aging of cells as they contribute to the aging of the tissue, while metacellular aging is the aging of the tissue as it forces cells into dysfunctional states. I will try to show that this theoretical distinction can help clarify the confused facts of aging, of cancer, and the entanglement of the two processes.

In the last part of the talk, I will sketch some of the consequences regarding innovative treatments for cancer, in particular, senolytics.

Session 6B – Nouveaux dispositifs numériques de dépistage

6B / 1
Depist&vous

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Contexte : En France trois programmes de dépistages organisés (DO) de cancers sont proposé à des populations cibles : le dépistage du cancer du sein, du côlon-rectum et du col de l'utérus. L'adhésion de la population aux propositions de DO est un enjeu majeur qui conditionne l'efficience d'une politique de santé publique, tant du point de vue sanitaire qu'économique. Afin d'encourager la participation, de nombreuses actions promotionnelles ont été mises en place, cependant les taux de participation à ces programmes de DO restent faibles et en deçà des recommandations européennes et de celles du dernier plan cancer. C'est pourquoi la 1ère plateforme digitale e-santé, Dépist&vous, a été développée. Il s'agit d'un outil numérique qui permet d'accompagner chaque personne de manière simple et personnalisée vers une prévention efficace et un dépistage précoce des cancers. Cet outil se présente sous la forme d'un site applicatif qui ne nécessite pas de téléchargement préalable et qui permet d'accéder à un compte personnel sur un navigateur internet.

Objectifs : Un projet de recherche a ainsi été mis en place afin 1) d'étudier la faisabilité et l'acceptabilité d'un outil d'accompagnement personnalisé pour les DO proposés et 2) d'augmenter la participation au DO du cancer colorectal (CCR).

Méthode : Ce projet a pour population cible les femmes et les hommes de 50 à 55 ans éligibles au DO du CCR, vivant dans les départements de Charente, Charente-Maritime, les Deux-Sèvres, Gironde et la Vienne. Tous sont invités par courrier à réaliser le test de DO par le CRCDC de Nouvelle Aquitaine : un groupe reçoit l'invitation usuelle par courrier papier (Groupe Témoin) ; un second groupe reçoit l'invitation usuelle accompagnée d'un flyer explicatif de l'outil Dépist&vous présentant un numéro d'identification unique permettant aux personnes de se connecter à la plateforme. Sur son compte, l'utilisateur a la possibilité de créer son profil ainsi que son parcours personnalisé de prévention et de dépistage des cancers. Un calendrier de rappels de rendez-vous de dépistage permet l'envoi de rappel par l'intermédiaire de notifications, emails, sms, selon le choix de l'utilisateur. Un questionnaire permet de définir le profil de la personne et ses facteurs de risque pour les différents types de cancer.
Nous évaluerons le taux de participation au DO du CCR dans les 2 groupes, le nombre de personnes qui se sont connectées, la perception de cet outil par les utilisateurs à partir d'un questionnaire proposé sur la plateforme.
Premiers résultats : 105 personnes se sont inscrites dans le mois suivant le lancement du projet, et toutes ont finalisé leur inscription (renseignements personnels et historique de dépistage), 95% des inscrits ont complété le questionnaire médical détaillé. Parmi les 82 personnes réellement éligibles au DO seules 4 personnes ont récupéré leur kit (5%) et 2 ont réalisé le test.

Conclusion : Cet outil simple d'aide aux DO apporte à la fois des informations sur les trois propositions de dépistage des cancers (sein, colorectal et du col de l'utérus) et sur l'état de la réalisation ou non des différentes démarches de dépistage. Cette plateforme numérique peut être accessible à tous, quel que soit l'âge et sur tout support. De plus, des vidéos explicatives et des quizz informent et sensibilisent à l'importance des dépistages précoces des cancers. Ainsi, Dépist&vous pourrait être un levier pour augmenter la participation aux DO.

Perspectives :Dépist&vous est testé sur la population éligible au dépistage du CCR, mais la finalité est de mettre à disposition des personnes concernées, un outil personnalisé et simple pour la réalisation et le suivi de tous les DO.
L'outil est développé par la société Liber présidée par Charlotte Berthault. Ce projet est porté par l'équipe Epicene (U1219, Université de Bordeaux) en partenariat avec le Centre Régional de Coordination des Dépistages des Cancers de Nouvelle Aquitaine et le SIRIC BRIO.

6B / 2

Predict-O: Projet d'Evaluation personnalisée du risque et Dépistage Individualisé du Cancer - TOulouse

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Le projet PREDICT-O (Projet d'Evaluation personnalisée du risque et Dépistage Individualisé du Cancer - TOulouse) a pour objectif général d'améliorer le dépistage des populations à haut risque (risque élevé à très élevé) de cancer en se centrant initialement sur le cancer du sein, de cancer du col de l'utérus, de cancer colorectal et de cancer du poumon.
La majorité de la population est à risque moyen de cancers fréquents dans notre pays (sein, colorectal, poumon, etc) et malgré un dépistage de masse organisé (pour les cancers du sein, du col de l'utérus et colorectal) la participation est largement insuffisante. Une partie minoritaire, mais numériquement non négligeable de la population (5 à 10%), est à risque élevé. Le dépistage de ces individus est donc d'autant plus justifié, mais il est souvent défaillant. L'une des premières raisons est l'absence d'identification des sujets à risque élevé, viennent ensuite les obstacles déjà identifiés pour tout dépistage de cancer (réticence, accès aux soins, etc.).
Comme pour tout dépistage les personnes défavorisées sont particulièrement concernées par ces obstacles. Toute démarche d'amélioration du dépistage devra donc veiller ne pas les laisser de côté.
Le projet PREDICT-O comprend 3 phases principales :
1) Le développement d'un outil d'auto-évaluation du niveau de risque de ces cancers sous forme d'une application web sécurisée (via tablette, smartphone…)
2) L'application de l'outil sur une population définie et la mise en place d'une organisation (en lien avec les médecins traitants) permettant d'inciter, de guider, voire d'accompagner, les personnes vers le dépistage adapté à leur niveau de risque. Ainsi une étude multicentrique, dans une population plutôt défavorisée (en cabinets de médecine générale et hôpitaux publics), sera menée afin d'évaluer l'impact du dispositif sur l'amélioration de l'identification des populations à haut risque et de leur accès au dépistage adapté
3) La mise en place d'une coordination régionale (médecins généralistes, communauté professionnelle territoriale de santé, réseau régional de cancérologie, caisse d'Assurance Maladie…) sur l'organisation du déploiement en population générale.
L'objectif final est l'évaluation de la pertinence du dispositif en termes d'amélioration du pourcentage de personnes à haut risque accédant à un dépistage adapté, d'impact à long terme sur le pronostic des cancers et d'impact médico-économique.
Portée par le CHU Toulouse, elle sera réalisée en association avec les départements universitaires de Médecine Générale de Toulouse, de Santé publique du CHU de Toulouse et le Centre d'Epidémiologie et de Recherche en santé des POPulations (CERPOP).

Prestige conference

Evolution & Cancer

Frédéric THOMAS

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Although the medical and evolutionary sciences have traditionally developed in relative isolation, it is increasingly recognized that cancer is a process shaped by Darwinian evolution. In my talk, I will first briefly present when cancer appears in the history of life and why it is primarily an evolutionary problem. Then, in order to stimulate discussion, I will present different research directions that are currently being explored by evolutionary biologists. These include: Why are some animal species resistant to cancer? Why are some cancers transmissible, as in the Tasmanian devil ? Can ecological and evolutionary science contribute to improved cancer therapies ? I will conclude my presentation by arguing that the traditional separation between medicine and evolutionary ecology remains a fundamental limitation that must be overcome if we are to fully understand complex processes such as oncogenesis.

Session 7A – Cell signaling & Therapeutic targets

7A / 1

EIF2A represents the central node of UPR-mediated cell death in hig-risk medulloblastoma

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Cancer cells often display basal activation of Unfolded Protein Response (UPR) as an adaptive mechanism to cope with ER stress due to their high demand in protein synthesis. UPR activates 3 distinct arms, ATF6, IRE1a and PERK which control different transcriptomic program as a cytoprotective mechanism. The PERK kinase also inhibits global protein synthesis by phosphorylating the eIF2a translation factor but promotes the specific translation of two transcription factors, ATF4 and CHOP. Rather than being cytoprotective, a strong and sustained UPR induces cell death, opening a therapeutic window. Through a targeted RNAi screen, we identified HSPA5 (Heat Shock Protein 5), the master regulator of UPR, as an interesting hit in group 3 (G3) medulloblastoma. Medulloblastoma (MB) is the most frequent malignant brain tumor in childhood. Four different groups have been identified, G3 having the worse prognosis and being of high risk. Transcriptomic analyses of human patient datasets showed that G3 tumors display a basal UPR transcriptomic signature. This basal activation was confirmed in G3 cell lines. We further showed that, thanks to this basal activation, G3 cell lines are highly sensitive to HA15, a HSPA5 chemical inhibitor, in comparison to normal cells. shRNA-mediated knockdown (KD) of HSPA5 induces a strong UPR coupled to cell death. Importantly, we also showed that the HSPA5 KD delays tumor growth in vivo. We investigated the molecular mechanisms involved. We showed that the PERK arm is the main mediator of cell death in HSPA5 KD cells. Indeed, its pharmacological inhibition partially rescues cell viability of HSPA5-KD cells and also prevents the induction of its two downstream mediators, ATF4 and CHOP. However, KD of ATF4 or CHOP does not rescue cell death by them-self. In contrast, re-establishment of protein synthesis by ISRIB, a compound that bypasses inhibition of eIF2a by phosphorylation, partially rescues cell death in HSPA5 KD cells. According to a key role of eIF2a phosphorylation in this process, we showed that shRNA-mediated inhibition of eIF2α phosphatase induces a strong cell death in G3 and inhibits tumor growth in vivo. Our work showed that G3 tumors displays a high basal of UPR activity sensitizing them to UPR-mediated cell death opening an interesting therapeutic window. Control of protein synthesis by eIF2α represents a crucial node in UPR-mediated cell death and could represent the Achille's heel of G3 MB.

7A / 2

Farnesyltransferase inhibition overcomes the adaptive resistance to targeted therapy in lung cancer

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Since the discovery two decades ago that single-agent targeted therapies could induce drastic responses in oncogene-addicted tumors, the systematic emergence of resistances remains to date a major public health issue for all types of cancer, that has not been solved despite the improvements of successive generations of inhibitors. In this context, Drug-tolerant "dormant" cells (DTC) have emerged as one of the major non-genetic mechanisms driving resistance to targeted therapy in lung cancer, although the sequence of events leading to entry and exit from dormancy remain poorly described.

Here, we report an in-depth phenotypic and molecular characterization of the early events leading to drug resistance using EGFR-mutant non-small cell lung cancer (NSCLC) as a reference model, and we extended our findings to other oncogenic contexts. We established an innovative approach by combining real time monitoring of the cell cycle dynamics and single cell RNA sequencing in a broad panel of cell lines and Patients Derived Xenograft. We identified a rare population of S/G2 cycling cells (referred to as early escapers) that emerged in the first hours of treatment amongst stably arrested and progressively dying G1 cells. We determined that early escapers evolved from a non-proliferative differentiated alveolar type 1 (AT1) phenotype which was invariably associated with cytoskeletal remodeling through Rho/ROCK pathway activation. Using a panel of Rho-pathway inhibitors, we found that the farnesyltransferase inhibitor tipifarnib induced a complete clearance of drug-tolerant cells *in vitro* and fully prevented relapse to targeted therapy, not only in EGFR-mutant models but also in ALK and KRAS-driven lung adenocarcinoma and BRAF-mutant melanoma. *In vivo*, co-treatment with tipifarnib prevented relapse to osimertinib for up to 6 months in a xenograft and a PDX model of EGFR-mutant lung adenocarcinoma, with no evidence of toxicity. Osimertinib and tipifarnib co-treatment completely suppressed the emergence of the AT1 phenotype, prevented mitosis of S/G2-treated cells and increased the apoptotic response through activation of ATF4-CHOP-dependent Integrated Stress Response (ISR) pathway. Our data strongly support the use of tipifarnib in combination with osimertinib in patients to effectively and durably prevent relapse.

7A / 3

Sensitizing the tumor microenvironment to immune checkpoint therapy trough monoclonal antibody-based therapeutic combinations in pancreatic cancer

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Pancreatic duct adenocarcinoma (PDAC) is one of the most lethal solid tumors, with an extremely unfavorable prognosis. The complex tumor microenvironment (TME) is responsible for the failure of many clinical trials including combination chemotherapies, targeted therapies or immunotherapies and there is a real need to develop new effective clinical strategies against this disease. This TME is characterized by an extremely low ratio of neoplastic to stromal tissue (< 20%). Cancer-associated fibroblasts (CAFs) make up the vast majority of this stroma and constitute an heterogeneous population with essentially pro-tumor characteristics. Moreover, PDAC tumors are poorly infiltrated by T cells, and the majority of immune cells present at the tumor site, such as macrophages (type 2), MDSCs and regulatory T cells (Tregs), are immunosuppressive.

This cellular context leads to the failure of clinical trials using immune checkpoint inhibitors in PDAC. The objective of this project is to develop a combinatorial approach using a monoclonal antibody that targets the microenvironment combined with interleukin-15 (IL15) or with conventional chemotherapies (Gemcitabine or Folfirinox) in order to reactivate the tumor microenvironment and obtain higher response rates to the immune checkpoint inhibitor programmed cell death 1 (anti-PD-1). We thus develop three-dimensional in vitro spheroid models 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. Different 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, also described in patients, are one of the trademarks of pancreatic cancer.

In parallel we have shown that, upon 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 that IL15 modify 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 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 steps will be to find the best synergistic mechanisms in our combination therapies based on monoclonal antibodies or IL15 combined with different chemotherapies (FOLFIRINOX, Gemcitabine) to promote and increase the infiltration of immune effector cells and sensitize the PDAC microenvironment to anti-PD1 treatments.

7A / 4

De novo generation of the NPM-ALK fusion recapitulates the pleiotropic phenotypes of ALK+ ALCL pathogenesis and reveals the ROR2 receptor as target for tumor cells

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Background: Anaplastic large cell lymphoma positive for ALK (ALK+ ALCL) is a rare type of non-Hodgkin lymphoma. This lymphoma is caused by chromosomal translocations involving the anaplastic lymphoma kinase gene (ALK). In this study, we aimed to identify mechanisms of transformation and therapeutic targets by generating a model of ALK+ ALCL lymphomagenesis ab initio with the specific NPM-ALK fusion.

Methods: We performed CRISPR/Cas9-mediated genome editing of the NPM-ALK chromosomal translocation in primary human activated T lymphocytes.

Results: Both CD4+ and CD8+ NPM-ALK-edited T lymphocytes showed rapid and reproducible competitive advantage in culture and led to in vivo disease development with nodal and extra-nodal features. Murine tumors displayed the phenotypic diversity observed in ALK+ ALCL patients, including CD4+ and CD8+ lymphomas. Assessment of transcriptome data from models and patients revealed global activation of the WNT signaling pathway, including both canonical and non-canonical pathways, during ALK+ ALCL lymphomagenesis. Specifically, we found that the WNT signaling cell surface receptor ROR2 represented a robust and genuine marker of all ALK+ ALCL patient tumor samples.

Conclusions: In this study, ab initio modeling of the ALK+ ALCL chromosomal translocation in mature T lymphocytes enabled the identification of new therapeutic targets. As ROR2 targeting approaches for other cancers are under development (including lung and ovarian tumors), our findings suggest that ALK+ ALCL cases with resistance to current therapies may also benefit from ROR2 targeting strategies.

Ref: *De novo generation of the NPM-ALK fusion recapitulates the pleiotropic phenotypes of ALK+ ALCL pathogenesis and reveals the ROR2 receptor as target for tumor cells* Mol Cancer. 2022 Mar 4;21(1):65. doi:10.1186/s12943-022-01520-0.

7A / 5

Towards new approaches to target integrins in immune cells: the case of tumor-associated macrophage

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Integrins are a widely expressed family of cell adhesion receptors that participate in cellular interactions in many tissues. Their role in immune cells is critical for leukocyte trafficking, activation, and function to shape successful immunity. Therefore, integrins are attractive targets for recalibrating immune responses in local microenvironments. They are proven therapeutic targets in many diseases, but direct targeting of their extracellular domain to interfere with their function may prove ineffective and often lead to adverse effects limiting the use of integrin antagonists. Instead, we intend to target intracellular signaling events that orchestrate integrin activities in immune cells. We have identified several pathways of integrin activation in lymphocytes, neutrophils, and platelets; these pathways coexist but their relative contribution depends on the type of cell and integrin. We are now extending our study to macrophages, particularly in the context of cancer. Macrophages are massively recruited in most solid tumors. Their abundance is correlated with a poor prognosis. They promote tumor progression and interfere with the effectiveness of various cancer therapies. Not surprisingly, therapies that aim to deplete or reprogram macrophages deserve considerable attention. However, care must be taken when modulating immune responses so as not to interfere with the essential functions of macrophages and with their antitumor properties. Our results reveal that macrophages use integrin regulatory pathways uniquely and differently from other immune cells and suggest that interference with these pathways might specifically target protumor macrophage subsets. Thus, our study contributes to designing new, unforeseen pathways to block macrophage migration and tumor infiltration to create synergy with cancer cell-targeting therapies.

Session 7B – Radiotherapy & resistance

7B / 1
Flash Radiotherapy

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7B / 2

Radiosensitization of digestive tumors by bioactive food components

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7B / 3

Role of extracellular vesicles during bystander cytotoxicity and bystander immunity of Targeted radionuclide therapy

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Background: Over the past 30 years, extracellular vesicles (EVs) emerged as a major mechanism for cell-cell interactions and prominent regulators of the immune response. Radiation therapy is known for its ability to kill cancer cells in an immunogenic manner, however the role of EVs in its efficacy is not yet understood. Here, we investigate the role of EVs in triggering anti-tumor immunity, specifically in the context of targeted radionuclide therapy (TRT) also known as radioimmunotherapy, consisting in the administration of radiopharmaceuticals.

Methods: B16F10 melanoma cells were subcutaneously injected in C57BL/6J and athymic mice. Mice received intraperitoneal injections of TA99 mAb targeting TYRP-1/gp75 tumor antigen radiolabeled with 5 MBq 177Lu-TA99 (beta TRT), or intratumoral injection of EVs purified from B16F10 cells exposed to 2 MBq/ml 177Lu-TA99 (TRT-EVs) or from non-treated cells (NT-EVs). EVs were also purified from DU145 prostate cancer cells exposed to 9.25 kBq/ml Xofigo (223RaCl2, alpha TRT).

Results: In vivo, beta TRT efficacy was shown to require T-cells for adaptive immunity, with a significant tumor growth delay (**p = 0.001) compared to non-treated (NT), and no difference was observed between TRT and NT in athymic nude mice.

In addition, quantitative nanoparticle tracking analysis showed that cells upon TRT released vesicles between 30 nm and 300 nm, a size range of major EVs subtype including exosomes and microvesicles (MVs). For instance, in B16F10 cell line exposed to TRT, the average size of released EVs shifted from (132.8 ± 4.4) nm to (153.9 ± 8.2) nm (***p = 0.0001). This result suggests potential difference mechanisms involved in the biogenesis and release the EVs NT versus TRT, and therefore of their cargo contents. Next, EVs purified from beta-TRT were administered intratumorally in the same animal model, and demonstrated a strong tumor growth delay and survival (***p = 0.0007) in immunocompetent mice, suggesting an immunostimulatory effect during TRT. The role of EVs in TRT efficacy was confirmed by using imipramine to prevent the translocation of acid sphingomyelinase (aSMase), inhibiting MVs release [1].

Conclusion: EVs contribute to TRT efficacy by mediating an antitumor immune response in vivo. Cells under physiological conditions (NT) are prone to release exosome-like EVs, while upon TRT and a massive accumulation of cytosolic DNA, MVs will be generated at the plasma membrane from its outward budding.

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7B / 4
Glioblastoma heterogeneity: a model for resistance

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Session 7C – Nouveaux dispositifs numériques de prise en charge

7C / 1

Thess : Dispositif de sécurisation et délivrance des traitements oraux

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De plus en plus de patient suivent hors des murs de l'hôpital des traitements oraux qui nécessitent une connexion continue avec l'équipe soignante. Les hôpitaux ou les structures HAD, s'organisent pour valoriser et développer cette prise en charge. Les outils numériques facilitent l'organisation et le développement de ces services santé. Le dispositif médical Thess sécurise les traitements oraux à domicile en permettant plus d'autonomie pour le patient. Il élimine les erreurs de prises, favorise l'observance et facilite le monitoring du patient. L'efficacité du traitement est optimisée. Le patient est connecté et sécurisé. Le réseau ville hôpital des professionnels est interconnecté.
L'innovation en matière de dispositifs médicaux a apporté d'énormes avantages aux patients. Le développement et la diffusion de dispositifs médicaux innovants ont été plus lents que pour certains produits de consommation en raison des obstacles à l'innovation. Bien que certains obstacles soient nécessaires et doivent rester en place en raison de la sécurité des patients et de la définition de l'efficacité du dispositif, d'autres obstacles pourraient être supprimés ou abaissés par des politiques améliorées et une coopération plus étroite entre les différentes parties prenantes.

7C / 2

Un robot d'assistance sociale auprès d'enfants hospitalisés en isolement protecteur

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Durant les périodes d'isolement en hospitalisation, le lien entre l'enfant et sa famille est altéré. L'éloignement de son domicile ainsi que de son cercle social et familial peut être délétère pour l'enfant hospitalisé. Un projet conjoint a ainsi été mené à l'Hôpital des Enfants du CHU de Toulouse avec l'association The Hope of Princesse Manon pour permettre aux enfants hospitalisés et en isolement d'utiliser le robot BUDDY de la société Blue Frog Robotics. L'objectif de l'utilisation de ce robot est de favoriser un maintien du lien entre l'enfant et sa famille. Malgré la présence grandissante de ce type de robots, la littérature reste pauvre en la matière. Un projet de recherche a ainsi été construit pour mesurer l'impact de l'utilisation d'un tel dispositif tant sur l'enfant lui-même que sur sa famille et les professionnels infirmiers intervenant auprès de l'enfant.

7C / 3

La réalité virtuelle : un outil de soutien pertinent dans la relation de soins en chimiothérapie ?

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Dans le système de santé actuel, et peut-être d'autant plus dans le cadre de la crise sanitaire que nous traversons, la relation avec les professionnels de santé est considérée comme essentielle pour les patients lors de la chimiothérapie, jouant un rôle primordial pour faire face au cancer. De nombreuses études ont attesté que l'aspect relationnel est le facteur le plus important pour garantir la satisfaction du patient durant le soin. Or, les conditions de travail parfois pénibles, tant sur le plan physique qu'émotionnel, auraient tendance à mettre à mal la relation infirmière-patient. Les infirmières n'auraient pas la capacité de développer une réelle relation de soin susceptible d'optimiser la gestion émotionnelle des patients et manqueraient de temps au risque de négliger leurs besoins. Face à ce paradoxe, il semblerait que la modernité et les outils technologiques, notamment lorsqu'ils sont proposés par le personnel soignant, permettent de pallier une relative disponibilité. Cette question mérite d'être appréhendée dans une phase critique du traitement du cancer du sein où l'anxiété des patients atteint généralement son paroxysme. Si des effets prometteurs de la Réalité Virtuelle (RV) ont été observés, notamment dans le parcours de kinésithérapie, le ressenti émotionnel n'est probablement pas aussi intense que celui qui se manifeste durant la phase critique. Notre étude s'inscrit dans la continuité de cette première recherche en kinésithérapie et a pour objectif de tester l'efficacité de la RV en tant qu'outil de soutien permettant de facilité le lien entre patients et personnels soignants en phase aiguë de traitement du cancer du sein (i.e. chimiothérapie).

L'efficacité de la RV tiendrait en partie dans son caractère multimodal et interactif permettant d'engager les individus dans le monde virtuel. La possibilité de façonner son propre environnement augmenterait la prégnance du sentiment de présence en maintenant l'attention de l'individu sur l'expérience virtuelle. À ce titre, l'immersion participative devrait soutenir davantage la régulation émotionnelle qu'une immersion contemplative. Par ailleurs, la RV devrait être un outil plus efficace que la musique tant cette dernière ne nécessite qu'un engagement attentionnel passif de la part des patientes.

Pour cela, nous avons mis en place un protocole quasi-expérimental dans un service d'oncologie auprès de 120 femmes atteintes d'un cancer du sein. Lors d'une séance de chimiothérapie, durant les 10 premières minutes de soin : un groupe a été placé sous une immersion participative où les patientes avaient la possibilité d'agir dans un environnement naturel relaxant, comme contrôler la météo, planter des arbres ou donner à manger aux animaux ; un groupe a été placé sous une immersion contemplative où elles avaient pour seule consigne de naviguer en observant la nature ; un groupe écoutait une musique classique via un casque audio ; tandis qu'un groupe ne disposait d'aucun dispositif de distraction.

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

Les résultats indiquent que la présence de la RV durant la chimiothérapie favorise la diminution de l'anxiété et apaise l'état de tension émotionnelle. Le caractère multisensoriel de cet outil de soutien à la régulation émotionnelle s'avère plus efficace que la musique pour induire une émotion positive, et ce d'autant plus lorsque l'immersion est interactive. Proposée par le personnel soignant, la VR fournit un soutien efficace aux patientes en leur offrant la possibilité d'être actrices de leur propre bien-être, et pourrait les préserver d'une perception dégradée du lien soignant/patient.

Session 8 – Urological cancers

8A / 1

Novel drivers of castration-resistant prostate cancer

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8A / 2

Prostate cancers and immunotherapies : hot or cold tumors?

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8A / 3

The role of PXR (Pregnane X receptor) and drug metabolism in the resistance of castration-resistant prostate cancers to kinase inhibitors

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While prostate cancer patients with localized tumors may benefit from surgery and/or radiotherapy, patients with metastatic disease are treated with androgen deprivation therapy but invariably become resistant to castration. Despite the approval of taxane-based chemotherapies and new-generation hormone therapies targeting the androgen receptor (AR) axis, the 5-year survival rate of metastatic castration resistant patients is hardly exceeding 30%, urging the need for new alternative strategies. Resistance to castration is mainly due to an alteration of the androgen signaling. Nevertheless, AR-independent mechanisms have also been described. As it is the case for many cancer types, these mechanisms are associated with alterations of signaling pathways that directly or indirectly stimulate tumor cell proliferation and/or survival. Most, if not all of these pathways involve a cascade of phosphorylation signals that are mediated by one or more of the ~600 tyrosine or serine/threonine kinases encoded by the human genome. Several studies using large cohorts of patients with CRPC reported rather frequent gene alterations in a limited number of these kinases such as ATM or PI3KCA, but a more in depth analysis of those sequencing data show the presence of alterations in most kinases. Targeting these kinases has therefore emerged as a promising strategy with a series of clinical trials that were conducted during the past 20 years. Unfortunately, none of these trials led to the approval of a kinase inhibitor for the treatment of castration-resistant prostate cancer (CRPC) patients despite some interesting responses. This is in striking contradiction with the 38 kinase inhibitors that are currently approved in oncology for the treatment of other kind of malignancies. The reasons for these disappointing results are still not clearly understood and may be due to various mechanisms including drug metabolism at the tumor level as kinase inhibitors are known to be good substrates for drug metabolizing enzymes and transporters (DMET), many of which are under the control of the nuclear receptor PXR (Pregnane X Receptor). Because PXR is expressed at a significant level in prostate tumors and is more frequently detected in advanced forms of the disease, we evaluated the effects of PXR expression on the sensitivity of PCa cells to a small panel of kinase inhibitors. We found that stable expression of PXR sensitized prostate cancer cells to erlotinib, dabrafenib, and afatinib, while it rendered cells resistant to dasatinib and had no effect for other inhibitors tested. In the case of afatinib, sensitization to the drug was due to an alteration in drug transport that involved the SLC16A1 monocarboxylate transporter. These results indicate that PXR might serve as a clinically relevant biomarker of response to kinase inhibitors in castration-resistant prostate cancers to select patients that could benefit the most from these targeted therapies. This is of particular importance in light of the increasing number of kinase inhibitors that are currently in development and that will enter in clinical trial soon for the treatment of CRPC.

8A / 4

Collagen remodeling leads to inflammation-free expansion of periprostatic adipose tissue and promotes prostate cancer progression

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Prostate cancer (PCa) is the second most common form of solid tumor in men worldwide and the fifth leading cause of death from cancer. It is a highly heterogeneous disease, ranging from slow-growing indolent tumors to fatal metastatic carcinomas. Risk stratification is, therefore, the cornerstone for selecting the most appropriate treatment for these men. Progression from indolent to aggressive PCa depends on endocrine signaling (mainly through the androgen receptor) and genetic alterations leading to cancer cell-autonomous progression; however, paracrine signaling from the tumor microenvironment also plays a role. The adipose tissue (AT) surrounding the prostate, called periprostatic adipose tissue (PPAT), has emerged recently as an important factor in the progression of the disease. Periprostatic adipose tissue (PPAT) accumulates independently of body mass index and its abundance correlates with PCa progression, but the mechanism remains unexplained. Here, we used a statistical approach to define abundant PPAT by normalizing PPAT volume to prostate volume in a cohort of 351 patients with a linear regression model. Applying this definition, we find that tumors specifically from patients with the highest amount of PPAT (called abundant PPAT) exhibit several hallmarks of aggressiveness such as undifferentiated tumors, and changes in the tumor microenvironment including increased fibrosis (Masson's trichrome staining), angiogenesis (CD31 staining), and macrophage infiltration (CD68 staining). Multivariate analysis using a logistic regression model indicate that PPAT abundance is an independent risk factor for occurrence of aggressive PCa as defined by the ISUP (International Society of Urology Pathology) score. We show that abundant PPAT expands by adipocyte hypertrophy but this does not result in inflammation as defined by the infiltration of pro-inflammatory macrophages or the secretion of pro-inflammatory cytokines. A proteomic study using isolated adipocytes reveals that abundant PPAT display under-representation of proteins involved in mechano-sensing and cytoskeletal contractile force generation as compared to non-abundant PPAT. Using 3D confocal microscopy, we find that abundant PPAT has a looser collagen network than less abundant PPAT, supporting the hypothesis that mature adipocytes in abundant PPAT are subject to fewer mechanical constraints than those in less abundant PPAT. Abundant PPAT exhibits increased collagen degradation likely by MMP9, which expression and activity is increased in these samples. Moreover, collagen VI degradation in abundant PPAT is associated with production of endotrophin, a matrikine (i.e. biological active peptides liberated by the proteolysis of ECM macromolecules) already known to promotes breast cancer progression. We find high levels of endotrophin specifically in the urine (obtained after prostatic massage) of patients with abundant PPAT, indicating the clinical relevance of our findings. Our new and robust definition of PPAT abundance can be applied to future translational studies and, potentially, to clinical practice to improve risk stratification. Endotrophin is a good candidate biomarker and a potential pharmacological target to dampen PCa progression. Finally, knowledge of the mechanism(s) that initiate PPAT expansion might allow clinicians to identify preventive approaches to decrease PPAT abundance in order to improve the outcome of PCa patients.

8A / 5

Molecular characterization of the combined chemotherapy of SUV4-20h epigenetic enzymes inhibitor with Topoisomerase II poisons in metastatic prostate cancer

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Despite the long-term survival in localized prostate cancer (PCa), this disease is still a major cause of death notably because its advanced forms remain largely incurable even after intensive multimodal therapy. The search for new therapeutic targets and strategy is therefore essential. In last few years, pathologists revealed that the progression of prostate cancer and its resistance to current therapy often coincide with chromatin and epigenetic alterations, a facet that could be exploited for identification and development of new therapeutic strategy. In this regard, we discovered that a subset of prostate cancer patients display an up-regulation of the epigenetic enzymes SUV4-20H1 and SUV4-20H2, two methyltransferases responsible for the di- and tri-methylation of histone H4 at lysine 20 (H4K20me2/3). We found that the upregulation of SUV4-20H2, but not of SUV4-20H1, is associated with a poor prognosis and the appearance of metastases in patients, suggesting an important role of this enzyme in cancer progression. We hypothesized that SUV4-20h enzymes and H4K20 methylation marks could constitute predictive markers of advanced forms of PCa and might be exploited as new targets to improve therapeutic strategy. Consistent with this last hypothesis, we demonstrated that the pharmacological inhibition of SUV4-20H1/2 enzymes and the complete loss of H4K20me2/3 states impair replication fork progression and chromatin compaction but, surprisingly, prostate cancer cell survival and proliferation were not affected. Nevertheless, the inhibition of SUV4-2H1 and SUV4-20H2 also affects DNA repair mechanisms, which creates a specific synthetic lethality with innocuous concentrations of topoisomerase II (TOPO2) poisons used for cancer treatment. Yet, this new drug synergy between inhibitors targeting SUV4-20h and TOPO2 enzymes is not observed with other DNA damaging agents, suggesting a specific interplay between these chromatin-associated enzymes in genome stability and repair. We will present here our last data and discuss the mechanisms by which the inhibition of SUV4-20h enzymes could specifically impair DNA repair pathways upon the poisoning of TOPO2 in metastatic prostate cancer cells. Altogether, our results show that the targeting SUV4-20h enzymes and H4K20me2/3 methylation dramatically improves the treatment responses with TOPO2 poisons and might constitute a novel promising drug combination for cancer treatment.

Posters – Axis 1 “Cell signaling and Therapeutic Targets”

P101

SUMOylation Controls AML Cells Migration Through the Regulation of CD36 Gene Expression

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Acute Myeloid Leukemias (AML), is a group of deadly hemopathies, resulting from a deregulation of hematopoiesis in the bone marrow. Despite recent advances in the characterization and prognosis of AML and the hope raised from therapies, the relapse rate is considerable and the overall prognosis remains poor, therefore, therapy improvement is still needed. Our team has previously shown that SUMOylation plays a critical role in the AML response to chemotherapies and differentiation therapies. Thus, targeting SUMOylation constitutes a promising approach in the AML treatment. This hypothesis is being tested by our team, thanks to a collaboration with 'Takeda Pharmaceutical' which developed the TAK-981, a first in class inhibitor of SUMOylation. Our team demonstrated that TAK-981 has a promising anti-leukemic effect both in vitro and in vivo AML models.

Our aim is to understand on the transcriptional level, the TAK-981 effect on AML cells, and to determine the function of the TAK981- induced genes in AML.

We performed an RNA-seq analysis on a TAK981-treated AML cell line, U937, and we observed limited effect on gene expression. Among the few overexpressed genes, we identified the CD36 gene, expressed in different cell-types, in particular, hematological cells. These results were confirmed by flow cytometry and RT-PCR. We also showed that the transcription factor PPARδ is involved in the TAK981-induced CD36 overexpression. Moreover, we demonstrated that the TAK981 treatment didn't affect lipid uptake & accumulation, however, it increased AML cells migration. We finally found that the CD36 was highly expressed in migrating cells and that its inhibition by a specific antibody decreases AML cells migration.

In conclusion, TAK-981 upregulates CD36 expression, via PPARδ, which increases AML cells migration. Therefore, CD36 could be considered as a target in order to improve AML response to TAK-981 which could pave the way to developing clinical-grade antibodies specific to CD36.

P102

Chronic pancreatitis a risk factor for pancreatic cancer: PI3Kα a target to limit the risk

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Despite remarkable improvements in cancer treatment, pancreatic ductal adenocarcinoma (PDAC) still presents an extremely dismal prognosis. What has emerged clearly from the most recent studies on this cancer is that PDAC is a very difficult disease to treat even when diagnosed at stage 1 or 2. Efforts should be made to prevent the development of this cancer at the earliest stage. As of now, the molecular events underlying risk factors for PDAC development are insufficiently understood. Those risk factors include amongst others, chronic pancreatitis (CP) and diet rich in red meat. We have already demonstrated the key importance of PI3Kα in PDAC initiation, progression and dissemination [1, 2]. The role of PI3Kα activation by risk factors is unknown.

We developed a Pdx1-Cre p110αlox/lox mouse model (αinC mouse model), in which PI3Kα activity is inhibited selectively in pancreatic lineage. To mimic CP, those mice were subjected to caerulein intraperitoneal injections. We next analyzed tissue damage by histological analysis.
We developed an in vitro chronic pancreatitis model using AR42J cell line, an acinar pancreatic cell line. We treated this cell line with dexamethasone and EGF to induce cell transdifferentiation towards a pseudo-ductal cell state. These parameters were assessed by measuring the genic expression of acinar and ductal markers by RT-qPCR. We performed proliferation assay and western blot analysis on acinar and pseudo-ductal cells in context of PI3Kα inhibition and / or PHGDH inhibition.
Thanks to transcriptomics data from patients, we compared expression levels of PI3Kα activation gene signature in normal, alcoholic CP, autoimmune CP, PDAC and stroma of CP patients. Finally, we analyzed activation of identified pathways by nutrition (including high content of red meat).

PI3Kα inactivation reduced both the frequency of early cancer pancreatic lesion (such as ADM acino-ductal metaplasia) and fibrosis inside the pancreatic parenchyma. Immunohistochemistry analysis of these samples showed a reduction of phospho-Akt substrate staining in context of CP when PI3Kα is inactive. PI3Kα inactivation however led to an increase of acinar proliferation markers (increase of Ki67 marker and decrease of p27 nuclear expression).
When analyzing the expression of a panel of genes shown to be transcriptionally regulated by PI3Kα in patient samples, we found a higher expression of PHGDH (phosphoglycerate deshydrogenase) in CP patients compared to normal and PDAC patients. PHGDH is an enzyme involved in in de novo serine biosynthesis. The role of PHGDH is known in the development of pulmonary fibrosis [3].
We observed an increase of PHGDH expression in αinC acinar cells in vivo. The pharmacological inhibition of PHGDH alone or in combination with PI3Kα genetic or pharmacological inhibition reduced acinar cell proliferation but increased pseudo-ductal cell proliferation rate.

Conclusion
The combined inhibition of PHGDH and PI3Kα blocks acinar-to-ductal transdifferentiation and induces the proliferation of stressed acini to maintain pancreatic parenchyma integrity. PHGDH and PI3Kα appear as good targets for preventing tissue damage and early cancer lesion development in CP patients and might limit the risk for CP patients to develop PDAC.

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P103

Eukaryotic translation initiation factor 3 complex promotes invadosome formation and matrix remodeling through PI3K/AKT/mTOR pathway upon Src induction

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Introduction

Metastasis formation is the first cause of death in patients with cancer. Remodeling of interstitial extracellular matrix is a key step for cancer cell migration and tumor progression. ECM degradation by tumoral cells is partly due to cellular structures called invadosomes. With an innovative method that combines laser microdissection and mass spectrometry analysis our lab showed that invadosomes were enriched with specific components of the translational machinery but also contained mRNAs and were an active translation site. Among enriched proteins involved in translation process, we identified several eukaryotic translation initiation factors (eIFs) including 8 eIF3 subunits among the 13 subunits that compose this complex.

Objective

We hypothesized that translation through eIF3 complex has a determinant role in invadosome formation and matrix degradation activity leading to tumoral progression.

Results

Using the classical model for invadosome study (3T3-Src), we demonstrated that the endoplasmic reticulum recruitment into invadosome associated with an active translation are determinant for invadosome formation and for their maintenance along the time. Affecting the translation using siRNA strategy against eIF3 subunits found enriched in invadosomes (3H and 3E) revealed that some subunits are mandatory for invadosomes stability while the depletion of other (3K) has no effect on invadosome development and on the invasiveness of 3T3-Src cells. Furthermore, we showed that Src signaling has a determinant role on translation by modulating the protein expression of several eIF3 subunits including eIF3H and eIF3E. To decipher the relationship between Src and translation through eIF3 complex, we combined two proteome analyses from 3T3-Src +/- Src inhibitor and proteome from isolated invadosomes revealing an Src-dependent enrichment of components involved in mTOR/S6K signaling into invadosomes. Our preliminary data demonstrated inhibition of PI3K/AKT/mTOR pathway reduced invadosomes formation and their associated degradation.

Conclusion

These results revealed the determinant role of Src-induced translation through regulation of eIF3 subunits and the activation of PI3K/AKT/mTOR pathway, in invadosome formation and matrix degradation activity leading to tumoral progression.

P104

Involvement of Reptin in invadosome formation and tumor invasion

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

In a previous study, we used an approach combining laser microdissection and mass spectrometry analysis to define the invadosome proteome in the NiH3T3-SrcY527F cell model. These cells overexpress a constitutively active form of Src protein promoting rosette invadosome formation. This approach revealed that Reptin is 6 times enriched in invadosomes in comparison with the total cell lysate. Reptin is a AAA+-ATPase involved in different cellular functions including DNA repair, replication and molecular co-chaperoning complexes. Reptin is a member of the R2TP complex, which is required for the assembly and conformation of many protein complexes.
We demonstrated that Reptin, as well as the other members of the R2TP complex (Pontin, RPAP3 and PiH1D1), co-localize with rosette invadosomes. By a siRNA approach we have shown that Reptin depletion significantly decrease the NiH3T3-SrcY527F ability to form invadosomes and to degrade the ECM. Moreover, in Reptin depleted cells, we noticed a recover of the wildtype phenotype characterized by the presence of stress fibers and the absence of invadosomes. That point reflects a loss of SrcY527F activity suggesting a molecular link between Src and Reptin. We confirmed this hypothesis by showing a Reptin and Src co-localization and by highlighting a decrease of Src-Tyr419 phosphorylation state in Reptin depleted cells without affecting its total expression level.
To identify the molecular mechanism involved in the modulation of Reptin-dependent Src activity, we use bibliographic and exploratory proteomic approaches to determine Reptin partners and their major functions in invadosomes. Therefore, we revealed the involvement of autophagy in the regulation of proteins, such as Fak, in a Reptin-dependent manner. We confirmed this result showing an increase of autophagy in cells treated with siRNA against Reptin and a rescue of Fak protein level when we inhibit autophagy in these cells.

Taken together these results will allow a better understanding in the mechanism of invadosome formation mediated by Reptin and the R2TP complex. This work will lead to a better comprehension of mechanisms involved in the process of tumor invasion.

P105

Effect of proprotein convertases inhibition on cancer stem cells tumorigenic and invasive properties in gastric adenocarcinoma

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Proprotein convertases (PCs), also called proprotein convertases subtilisin/kexin (PCSKs), are enzymes involved in the maturation of a large panel of precursor proteins implicated in fundamental cellular processes: proliferation, survival, adhesion, invasion, and immunity. PCs have a plethora of substrates such as growth factors (TGF-β, VEGF-C, …), integrins α-chains, pro-hormones, receptors (IGF-1R, Notch, …) and matrix metalloproteases. The role of PCs in tumorigenesis has been extensively studied: it contributes to tumor progression in many malignancies such as rhabdomyosarcoma, colon carcinoma, and others. They have been shown to activate proteins implicated in proliferation, angiogenesis, cell survival, invasion, and metastasis. However, the involvement of PCs in gastric adenocarcinoma (GC) tumorigenesis has been poorly studied until now and need to be investigated.

GC is the fourth leading cause of cancer-related death worldwide, it is very often detected at an advanced stage and is associated with poor prognosis and high risk of relapse. This can be explained by the presence of cancer stem cells (CSCs), a subpopulation of cancer cells able to self-renew, differentiate in other cancer cell types, initiate tumor growth and metastasis. CSCs hold their properties and survival through hijacked signalling pathways such as the Hippo pathway as it has been demonstrated in GC. They possess an epithelial to mesenchymal transition (EMT) signature reflecting cancer aggressiveness and metastasis. Those cells are also resistant to conventional therapies and are therefore held responsible for cancer relapse. For these reasons, targeting CSCs may be a very promising therapeutic strategy in cancers with a high recurrence rate.

Using decanoyl-RVKR chloromethyl ketone (CMK), a general chemical PCs inhibitor, we have tested to inhibit PCs as a strategy to target CSCs tumorigenic and invasive properties in four different GC cell lines. Various in vitro experiments were performed such as live immunofluorescence, tumorsphere assay and flow cytometry to evaluate the effect of PCs inhibition on CSCs properties and phenotype. It was also assessed whether the observed effects could involve the Hippo signalling pathway and the EMT process by performing Boyden's chamber assay, immunofluorescence, western blot, dual luciferase reporter assay.

This study allowed to highlight that PCs inhibition by CMK has a significant effect on GC CSCs ability to initiate tumorspheres, and to sustain tumorspheres growth. Indeed, it substantially inhibited CSC's tumorigenic and drug efflux properties, as indicated by decreased tumorsphere formation and growth as well as reduced drug efflux capacities. However, there seemed to be no impact on the expression nor enzymatic activity of two specific GC CSCs markers: CD44 and ALDH. It was also demonstrated that PCs inhibition decreased the transcriptional activity of downstream YAP/TAZ/TEAD oncogenic effectors of the Hippo pathway, suggesting that CMK could inhibit CSCs properties via YAP/TAZ/TEAD activity. Moreover, the invasiveness of GC cell lines was highly reduced by PCs inhibition. This effect was associated to a decrease of expression of some invasive and mesenchymal markers and of the nuclear expression of ZEB1 and Snail, that are the main transcription factors of the EMT. So, PCs inhibition impaired EMT process and invasive properties of CSCs in GC.

To conclude, PCs inhibition seems to be a potential strategy to target CSCs in GC notably by reducing their tumorigenic, drug efflux and invasive properties. It also decreases the activity of the YAP/TAZ/TEAD Hippo signalling pathway oncogenic effectors which contribute to maintenance of CSC properties and EMT. Further investigations are required to refine PCs inhibition strategy and better understand the molecular mechanisms implicated in anti-CSC effects in GC.

P106

Deciphering the role of circRNAs in drug resistance of anaplastic large cell lymphoma expressing the oncogenic tyrosine kinase ALK

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Circular RNAs (circRNA) are new non-coding transcripts belonging to the long non-coding RNAs family. They are characterized by a covalently closed structure due to back-splicing and lack for the 5' and 3' ends providing them a high level of stability and resistance to degradation. Several classes of circRNAs with different functions, mainly as miRNA and RBPs sponges, can be generated through back-splicing event during the processing of pre-mRNAs. circRNA can be localized either in the nucleus or in the cytoplasm and also in all human body fluids making them potential biomarkers for human diseases. Their abberant expression is shown to promote tumorigenesis. We are focusing on the study of circRNAs in Anaplastic Large Cell Lymphoma (ALCL) expressing NPM-ALK fusion protein in which inspite of the responsivness to chemotherapy, 30% of the patients relapse with very bad prognosis. Thus, the discovery of new theragnostic biomarkers is crucial for treating relapsing patients.

Whole transcriptome deep sequencing was performed on 9 reactive lymph nodes as healthy tissue and 39 primary biopsies of NPM-ALK (+) ALCL including 18 patients with early relapse after diagnosis and 21 patients without relapse. Using CIRI2, circRNA prediction algorithm, and differential analysis of NPM-ALK (+) ALCL patient samples, we identified overexpression of 6 circRNAs in the relapsed group compared to the non-relapsed group and reactive lymph nodes. Divergent primers spanning the back-splice junction were designed to allow specific circRNA detection and quantification by qRT-PCR in 5 ALCL NPM-ALK (+) cell lines. The resistance to degradation was verified by treating the RNAs with RNaseR, a 3'-5' exonuclease. Thus, all 6 circRNAs were detected in the 5 NPM-ALK (+) ALCL cell lines and were enriched after RNaseR treatment proving their circularity status. Subsequently, using Sanger sequencing, both the back-splice junction (BSJ) and the complete sequence were validated. Moreover, by performing subcellular localization followed by qRT-PCR, we detected all circRNAs in the cytosolic fraction, suggesting their potential role as miRNA and RBP sponges. Currently, we are generating models overexpressing these circRNAs in order to study their impact on cell survival, proliferation and disease progression, in vitro and in vivo.

All together, our preliminary data reveal a signature of circRNAs upregulated in resistant NPM-ALK (+) lymphoma cells. This circRNA signature could be used as a potential biomarker for NPM-ALK (+) ALCL resistant patients.

P107

Cancer cells transfer invasive properties through tracks, a novel collagen associated extracellular vesicle entity

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Metastasis is the leading cause of cancer-related deaths. During this process, tumor cells acquire invasive and migratory capacities in order to invade the surrounding tissues. To achieve this, the tumor microenvironment including the extracellular matrix (ECM) is altered to facilitate cancer cell proliferation and dissemination. Extracellular vesicles (EVs), such as exosomes or migrasomes, are already known to induce pro-tumor features such as migration, promoting tumor development and metastasis formation. Here we highlight a new type of EVs, referred as tracks, released by cancer cells during migration and specifically attached along collagen fibers. These tracks, identified by discoidin receptor 1 (DDR1) enrichment, are promoted when cell-ECM interactions are increased, such as in tumors. We characterized these tracks, their ultrastructure as well as their molecular composition in terms of proteins and nucleic acids, showing that they are different from classical EVs known so far. Moreover, these tracks are very stable structures and can be internalized by surrounding cells. After internalization, they modify the differentiation status and the phenotype of recipient cells, promoting epithelial to mesenchymal transition, matrix degradation and invasion.

Thus, we identified a new class of collagen-associated EVs, tracks, that play a role in cell-cell communication by transferring invasive properties. Consequently, these cancer-related tracks could be a new player in the tumor invasion process.

P108

Fascin-1 as a new potential target of aggressive hepatoblastoma

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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. However, β-catenin is an essential protein and is not a druggable target.

Here, we investigate one of ß-catenin 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.

Our results show that the inhibition of Fascin-1 using siRNA strategy and a commercialized Fascin-1 inhibitor reduces cell migration, cell survival, and increases cell death in two HB cell lines (HepG2 and Huh6 cells). Moreover, we confirmed these results in HB patient-derived xenograft cells. To further understand the way Fascin-1 influences gene regulation, we analyzed the Hippo/YAP pathway, that plays a key role in HB development. We found that YAP activity is reduced after Fascin-1 depletion. Indeed, YAP is found translocated from the nucleus to the cytoplasm, upon Fascin-1 inhibition.

In conclusion, our results show that Fascin could be an interesting druggable target in HB.

P109

Targeting tryptophan metabolism as a new therapeutic approach in hepatoblastoma.

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Hepatoblastoma (HB) is the most frequent pediatric liver cancer, affecting children between 1 and 5 years old. Every year, 1 to 9 children per million develop this pathology which represent 1% of all malignant pediatric liver tumor with highly increased incidence during the last years. In term of therapy, standard therapy is not efficient in 20% of cases and has many long lasting severe side effects underlining the need to find new and less toxic treatments.

In many types of cancer, inhibiting the tryptophan (Trp) metabolism could be beneficial for the treatment as the kynurenine (Kyn) one of the metabolites of this pathway seem to have immunosuppressive and oncogenic functions. Surprisingly, this pathway is not active in HB, as key enzymes metabolizing Trp into Kyn such as tryptophan dioxygenase 2 (TDO2) are weakly expressed.

In this project, we focused on TDO2 which we overexpressed in 2 different HB cell lines Huh6 and HepG2 and validated its metabolic activity. In this context, TDO2 overexpression led to a decrease in cell proliferation in both cell lines as assessed by 2D and spheroid cell culture 3D systems. We show that TDO2 was capable of inducing senescence as indicated by the increase of specific markers such as p16, p21 and β-galactosidase staining. Using electron microcopy, we noticed some morphological changes of mitochondria reflecting additional features typical of senescence.

Our findings underscore a metabolic signature of Trp in hepatoblastoma which could be targeted as a new approach to treat this pediatric liver cancer.

P110

Analysis of the involvement of Nurse-like cells exosomes in the survival of Chronic Lymphocytic Leukemia B cells

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Chronic Lymphoid Leukemia (CLL) is a malignant lymphoproliferative disease characterized by an accumulation of mature B lymphocytes (LB-CLL) in the blood, bone marrow and lymphoid organs (ganglia, spleen), linked to their resistance to programmed cell death or apoptosis. This resistance is linked to the overexpression of anti-apoptotic proteins of the Bcl-2 family (Kitada et al., 1998), including Bcl-2, Bcl-xL and Mcl-1, while the pro-apoptotic proteins of the same family, Bax and Bak are underexpressed (Dighiero and Hamblin, 2008). The activation of these survival pathways is also linked to the aberrant activation of protein kinases located downstream of the B cell receptor (BCR), such as the kinases BTK, Lyn, Syk, ZAP-70 and PI3K (Stevenson et al., 2011).

Current therapies that target the BCR pathway or the inhibition of anti-apoptotic proteins do not prevent the progressive forms of the disease, showing that other mechanisms exist in the pathophysiology of CLL. The tumor microenvironment plays a major role in the survival of LB-LLC, and in particular type 2 macrophages (or macrophages associated with CLL tumors) represented by Nurse-Like Cells (NLC). These cells secrete BAFF/APRIL chemokines and B lymphocyte survival factors (Nishio et al., 2005) and ensure the maintenance, survival and a proliferative component of B cells within tumor niches in peripheral lymphoid organs (Burger et al., 2000).

Among the modes of communication between the tumor cell and the microenvironment, exosomes perform an important function, and the study of this mode of communication is one of the focus of the UMR 1308 team. In the present study, we investigated the role of exosomes in the communication between LB-LLC and Nurse-Like cells (NLC). In a first approach, we evidence that NLC efficiently secreted exosomes that were significantly incorporated into LB-LLC and that these exosomes increased the survival of LB-LLC in an NLC deficient environment.

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P111

Interferon-independent STING activity in Non-Small Cell Lung Cancer translates into cancer 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.

In an effort to dig deeper into STING function in lung cancer cells, we took advantage of a Kras driven mouse model of lung cancer, from which we have generated cancer cells lines showing different STING expression levels.

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. Furthermore, monitoring the immune infiltrates in tumors generated in vivo from STING deficient cancer cells, revealed only a minor impact on the tumor immune microenvironment. Consistently with these findings, in vitro evaluations revealed an alteration in the canonical STING signaling in cancer cells that translates into an absence of IFNβ-induction, while these cells were able to produce high amounts of this cytokine upon TLR3 triggering. On the contrary, STING pathway in lung cancer cells seems to be rewired toward the Epithelial to Mesenchymal Transition (EMT) program throughout a not fully understood mechanism. Indeed, bioinformatics analysis on human lung cancer cells transcription profiles and investigation of EMT and stemness markers in autochthonous KP tumors derived cancer clones revealed that STING expressing cells have enhanced migration and EMT-like features. Furthermore, STING expression and its IFN-independent pathway is induced after EMT promoting stimuli, such as TGFβ or the ectopic expression of SNAIL, one of the major EMT transcription factors. We are currently investigating the signaling mechanism implying the connection between STING, cell plasticity and EMT reprogramming. We are also pushing forward our exploration of this signaling pathway to better understand how type-I interferon induction is repressed in lung cancer cells. As a key message from our results, we have accumulated evidences suggesting that along with tumor progression, cancer cells may switch toward a mesenchymal state that requires the alternative STING signaling that does not lead to type-I interferon response.

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

P112

PCSK9 expression on Liver Sinusoidal Endothelial Cells during Liver Metastasis

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The protein convertase PCSK9 has been extensively studied in hypercholesterolaemia, but has recently been shown to be involved in cancer. This protein of the convertase family is overexpressed in several types of cancer, especially during the process of metastasis. One of the most common is colon liver metastasis, a process in which tumour cells from the colon migrate to the liver to create a secondary tumour. In this process of tumour dissemination, the tumour microenvironment plays a key role. Our research focuses on the function of PCSK9 in hepatic sinusoidal endothelial cells (LSECs) involved in blood vessel formation. We have shown in a study *in vitro* by qPCR that PCSK9 protein is expressed in LSECs under basal conditions. Furthermore, we activated the cells with media from SW620 tumour cells as well as from SW620-derived cancer stem cells (CSC) during 24 hours. Thus, we have shown that activation of LSECs with cancer stem cell (CSC)-conditioned media significantly increases PCSK9 expression at the mRNA level. On the other hand, PCSK9 expression has been confirmed by Western Blot (WB), appearing to be expressed in the three conditions studied (LSEC Control, LSEC SW620-conditioned media and LSEC SW620-CSC-conditioned media). This is the first time this convertase in detected in LSECs. In addition, immunofluorescence staining of PCSK9 was performed to determine the cellular localisation of the protein in this cell type. PCSK9 was found to be localised in the nucleus of LSECs in cell culture. Furthermore, we have confirmed the nuclear location by a WB analysis of the cytoplasmatic and nuclear fraction of the cells. As far as we know, this localisation of PCSK9 has never been observed which open the possibility of new function for PCSK9 in LSECs.

In order to test whether this phenomenon occurred in other models than in vitro, we performed immunofluorescence staining in human tissues, both healthy and tumour tissues. Thus, LSECs were labelled with the specific marker CD31 and PCSK9. The colocalisation of both markers were observed, demonstrating that PSCK9 is also expressing in in vivo LSECs. Expression was seen in both healthy and tumour tissue, although the signal was higher in tumour tissue. Regarding cellular localisation, the protein appears to be localised in the nucleus according to the observations made in vitro.

Once both the expression and cellular localisation of PCSK9 in LSECs were determined, the function of this protein in the nucleus was assessed. For this purpose, PCSK9 was inhibited chemically by the inhibitor PF-06446846 and proliferation studies were performed as well as massive RNA sequencing (RNA seq) of the different conditions studied. Regarding proliferation, PCSK9 inhibition reduce the proliferation in the three conditions studied. This point to a possible involvement in the proliferation capacity of LSECs. On the other hand, RNAseq of control conditions, SW620 conditioned media, CSC conditioned media and PCSK9 inhibition and subsequent addition of CSC conditioned media was performed. We observe differences in mRNA expression of different proteins in the absence of PCSK9. In this way, the involvement of PCSK9 in the expression of certain proteins could be determined. The results showed that when PCSK9 was inhibited, certain molecules such as ERN1 (involved in apoptosis) or FAP (involved in extracellular matrix invasion) significantly decreased their expression. This would show that PCSK9 is necessary for the expression of these molecules, demonstrating its involvement in tumour development at the level of LSECs.

This study shows that PCSK9 appears to play a key role in the formation of blood vessels during colon liver metastasis by interacting with different proteins like ERN1 or FAP. These interactions need to be studied in depth to determine the role of PCSK9 in this process.

P113

HIF-1α implication in the gastric carcinogenesis mediated by Helicobacter pylori

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Context and objectives. Hypoxia is a common feature of several solid cancers, and the cellular response involves hypoxia-induced factors (HIF). HIF-1α is an oxygen-dependent subunit belonging to a transcriptional complex, along with HIF-1β, a constitutively expressed subunit, irrespective of the level of oxygen. This complex promotes the transcription of several genes involved, for instance, in cellular metabolism and angiogenesis.

Helicobacter pylori infection is the major risk factor for gastric adenocarcinoma, an important cause of death worldwide. *H. pylori* is a gram-negative bacterium that infects half of the world's population. *H. pylori* infections are mainly asymptomatic, underlined by chronic gastritis. Some may evolve towards gastro-duodenal ulcers, and preneoplastic lesions leading in ~10% of cases to gastric cancer.

In vitro, infection with *H. pylori* leads to cells' elongation, called the "hummingbird" phenotype. These cells undergo epithelial-mesenchymal transition (EMT) and express high levels of the CD44 stem cell marker, presenting tumorigenic potential, both in vitro and in vivo. Thus, *H. pylori* infection induces the emergence of cells with cancer stem cell (CSCs) properties. CSCs targeting remains a challenge in gastric cancer. Consequently, improving the understanding of its emergence is crucial to enable further therapeutic options.

Previous reports indicate that Infection with *H. pylori* induces HIF-1α protein expression. Association of HIF-1α, EMT and stemness has been studied and demonstrated in different types of cancer. However, the involvement of HIF-1α protein in the gastric carcinogenesis mediated by *H. pylori* has not yet been studied. Therefore, this project aims at determining the implication of HIF-1α protein in the EMT process and the generation of CSCs in gastric carcinogenesis.

Methods. Gastric epithelial cells were transduced with short hairpin RNAs against the HIF-1α protein or with a control short hairpin, and co-cultured or not with different *H. pylori* strains for 24 h.

Results. *H. pylori* infection increased the percentage of cells harbouring the hummingbird phenotype, but the knockdown of HIF-1α protein did not significantly affect these values. The study of the expression of EMT markers at the gene and protein level showed that *H. pylori* infection increased the expression of certain mesenchymal markers such as ITGB1 and Snail. The knockdown of HIF-1α impacted the protein expression of both markers, depending on the bacterial strain. The nuclear translocation of Snail, revealing its transcriptional activity, increased upon infection and was also decreased by the knockdown of HIF-1α protein. *H. pylori* infection increased the tumorigenic ability of cells to form tumorspheres, which decreased with HIF-1α knockdown. Immunofluorescence analysis for CSC markers showed an increase of the percentage of CD44+ cells upon infection, and a decrease in the HIF-1α knockdown cells, depending on the bacterial strain. Similar results were obtained with flow cytometry for CD44+ cells.

Altogether, the results obtained indicate a role of HIF-1α in the EMT process induced by *H. pylori* infection and in the emergence of CSCs. These data suggest that HIF-1α is implicated in the gastric carcinogenesis mediated by *H. pylori*, but further experiments are required to decipher the role of this pathway.

P114

Mechanistic models and machine learning for survival analysis helps predicting resistance acquisition in metastatic melanoma patients

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Metastatic melanoma is highly resistant to conventional therapies, but particularly sensitive to treatments targeting protein kinases involved in the canonical MAPK signaling pathway. In fact, the MAPK/ERK pathway is an essential intracellular signaling pathway in metastatic melanoma, and the discovery of activating mutations of the BRAF oncogene has opened new therapeutic avenues with BRAF protein kinase inhibitors. These inhibitors have shown either insufficient efficacy due to primary resistance of tumor cells or transient efficacy due to the deve- lopment of acquired resistance especially linked to the plasticity of the MAPK and PI3K/AKT signaling networks. Our hypothesis is that immediate sensitivity and acquired resistance to inhibitors can be predetermined by biomarkers related to the initial state of intracellular molecular networks.

By using phosphoproteomic data of melanoma cell lines, we built a predictive mechanistic model that mimics the response of MAPK and PI3K/AKT signaling pathways to kinases inhibitors. To generate the experimental data, we use a panel of cell lines recapitulating the genetic heterogeneity of melanoma. After disrupting intracellular signaling networks with inhibitors, we use validated quantitative phosphoproteomics technology based on multiplexed ELIS. We then developed an original version of the Modular Response Analysis method to describe how the observed metabolic network responds to small perturbations. In contrast to previous version of the same method, our method copes with missing data and performs model correction.
An other issue in oncology is the personalization of the treatment to the patient, the response to treatment being generally variable from one patient to another. Thus, a general descriptive model does not allow to fully understand the variation of treatment. From the perspective of developing a strategy for adapting treatment to each patient, we believe that it is essential to understand the individual clinical or biological characteristics influencing the treatment outcome.

There is a large amount of redundant data in the literature on patients with metastatic melanoma treated with kinase inhibitors. Thus, we built a curated SQL database from pre existing studies from the literature.

All data are for now from clinical studies gathering patients with metastatic melanoma treated with kinase inhibitors between 2014 and 2020 and the database was designed to be easily extended by adding information from other studies. Data were cleaned and homogenized between studies to be integrated in the curated database in which are stored both classical clinical data but also biological information such as Partial/Whole exon sequencing mutation information or copy-number alterations.
We use several statistical and machine learning (ML) models on retrieved data, such as Cox model, penalized Cox model, survival trees, Random (survival) Forests, or SVMs to predict the progression free disease time of patients from individual characteristics.

Multimodal data integration gives us the opportunity to understand the phenomen globally through the integration of multiple pieces of information, however the main difficulty in this approach lies in choosing how to properly integrate these different data types. Several strategies for integrating multimodal data have been addressed in this project, targeting genomic data processing.

We believe that the developed mechanistic model which takes into account the complex interactions present in intracellular molecular networks can be used in combination with ML models to predict the genotype/phenotype relation better. These hybrid approaches could exploit the complementary of mechanistic models and ML methods and represents a current trend in data processing and learning as they cope better with accountability and explainability, and are therefore more easily adopted and employed by biologists.

P115

Deciphering SRC-Like Adaptor Protein (SLAP) functions in intestinal homeostasis and tumorigenesis

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The tyrosine kinase and signaling protein, SRC, is an important player of tumorigenesis and metastasis, found deregulated in 80% of colorectal cancer (CRC). SRC deregulation induces intestinal cancer stem cell proliferation and dissemination, it is also a marker of poor prognosis in patients and a potent driver of metastasis in CRC. While the tumor-promoting role of SRC in CRC is well-defined, the underlying associated deregulation mechanisms are not fully understood. Interestingly, we identified one important mechanism controlling the SRC tumor activity in CRC which implicates the SRC-like Adaptor Protein SLAP. In normal conditions, SLAP is gradiently expressed in the crypts of the intestine, with the highest expression level in the differentiated part, and it is inversely correlated to the SRC and Wnt activities. Interestingly, we found that SLAP is frequently down regulated in CRC and displays important tumor suppressive functions via controlling the oncogenic activity of SRC. In line with this, SLAP overexpression suppresses cell tumorigenicity, tumor-initiating abilities and invasiveness, whereas SLAP silencing leads to an increased tumor growth and liver metastasis as well as well as cancer stem cells properties. To further investigate the pathophysiogical functions of SLAP in a more integrated system, we developed total constitutive and intestine specific conditional SLAP knockout (KO) mouse models. I observed, that the total KO of SLAP increases the colonic epithelium thickness, the number of proliferative cells and the intestinal stem cell marker LGR5. Our objective is now to take advantage of these mouse models to better understand the SLAP functions in tumorigenesis and stemness.

P116

Targeting cytidine deaminase with intracellular antibodies to defeat resistance to treatment in pancreatic cancer

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Cytidine deaminase, or CDA, is an enzyme that deaminates cytidine into uridine to maintain the pyrimidine pool of cells. In several tumors including pancreatic cancer (PDAC), CDA participates to therapeutic resistance by deaminating cytidine analogues, such as gemcitabine. In addition, we recently showed in the group that CDA controls replicative stress of PDAC cells and induces resistance to DNA targeting drugs. Catalytic inhibitors exist but they are not specific and failed in clinical trial so far. Therefore, developing alternative strategies to inhibit CDA and defeat resistance to treatment in PDAC is necessary. We describe here the development and the use of intracellular antibodies able to degrade CDA through the proteasome (i.e. antibody-based degraders). We identified CDA degraders via a cell-based screening, characterized them and showed their potency to deplete endogenous CDA protein in several PDAC cell lines. CDA degraders induce the chemosensitization to gemcitabine of all PDAC cell lines tested in vitro. This strategy will be soon addressed in vivo, so as other combinations of CDA degraders with antitumoral compounds using high content screening. Last, we are currently addressing the delivery of CDA degraders in experimental PDAC models, both in vitro and in vivo. Collectively, we show here promising evidences for the selective targeting of CDA to relieve therapeutic resistance of PDAC tumors.

P117

Targeting the SUMO pathway activates Natural Killer cells and enhances their anti-tumoral activity in Acute myeloid leukemia

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Acute myeloid leukemias are a group of hematological malignancies with poor prognosis. NK cells play a key role in the antitumoral immune response against AML. However, disease associated mechanism leads to alteration in circulating NK cells number and phenotype with decreased cytotoxicity and ability to produce cytokines. Several studies reported the role of SUMOylation, a posttranslational modification of the proteins, in the regulation of the innate immune response. Our team has shown that TAK981, a first in class inhibitor of SUMOylation used in various phase I/II clinical trials for solid tumors and lymphomas, upregulate the expression of the NK activating ligands on the surface of AML cells and favors their killing by NK cells. Moreover, several recent studies highlight the role of TAK981 in activating a global antitumoral immune response mediated by IFN type I induction. Here, we wondered whether TAK-981 could directly affect NK cells anti-leukemic activity.

In this study, we show that the inhibition of SUMOylation enhances the activation of primary NK cells and increases their cytotoxicity against AML cell lines and patient cells. In addition, RNAseq analysis showed a strong enrichment of a type I Interferon signature in primary NK cells treated with TAK981. The inhibition of the type I IFN receptor suppresses this direct effect of TAK981 on NK cells activation and cytotoxicity. We also describe a cross talk between NK cells and other immune cells, mainly monocytes, leading to an indirect activation of NK cells upon TAK981 treatment mediated by IFN type I and immunomodulatory cytokines production.

In conclusion, we show that targeting SUMOylation induces an IFN-I dependent activation of primary NK cells, which increase their cytotoxicity against AML cells. This could pave the way to the development of new AML treatment through either the restoration of patients' NK cells activity, or by the activation of primary NK cells for autologous infusion in AML patients.

P118

Analysis of the expression of ABC transporters linked to drug resistance in adult T cell leukemia (ATL)

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Adult T-cell Leukemia (ATL) is a lymphoproliferative disease of CD4+ T-cells infected with Human T-cell Leukemia Virus type I (HTLV-1). ATL leads to the death of thousands of infected people in worldwide and there are no effective treatments to date. On top of that, ATL cells often acquire resistance to conventional chemotherapeutic agents. This study aims to unravel the ATP-binding cassette (ABC) transporters 1 (ABCB1)- dependant chemoresistance mechanisms. This transporter is involved in multidrug resistance in many cancers via an ATP-dependent promotion of drug efflux. Gaining a better understanding of ABCB1 expression molecular mechanism could allow us to propose an alternative therapeutic strategy for ATL.

To do so, we started by evaluating ABCB1 expression in fresh ATL patients' cells. We observed an upregulation of ABCB1 and its regulatory kinase PIM-1. After that, using luciferase assay, we identified a list of factors (DHX9, Sp1, Fra2-JunD, Ras, NFkB, p53, Tax) which are able to either activate or to inhibit the proximal promotor of ABCB1. Next, we overexpressed Tax in HEK293T cells and we observed though Western Blotting analysis, a decreased of endogenous expression of ABCB1 in this model. Then, we used Valproate, a HDAC inhibitor to treat PBMCs from ATL patient and found a reactivation of Tax in 1 to 2 samples. We hypothesize that decreasing the expression level of ABCB1 by Tax may contribute to re-sensitize ATL cells to chemotherapeutic agents used in clinic to treat ATL.

In conclusion, our aim is to propose therapeutics strategies which consist to target regulators factors involved in the expression of ABCB1 during drug administration, in order to improve treatment of ATL.

P119

New function(s) of chk1 kinase in the autophagy process

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DNA damage response (DDR) is a complex signalling network activated upon different kinds of genotoxic events. Activation of this pathway, which implicates the phosphorylation and activation of the CHK1 kinase by the ATR kinase, leads to cell cycle arrest and DNA repair to preserve genomic integrity. Autophagy is a biological process activated in response to different types of metabolic stress, including DNA damage. It is a highly conserved self-eating process during which cells digest some of their structural components to maintain cell homeostasis and metabolic equilibrium.

Both autophagy and DDR are therefore essential for survival and maintenance of cellular homeostasis in response to different categories of stress. Several studies report that autophagy can be activated by different types of DNA damage, and that it is involved in cellular responses such as DNA repair, senescence or cell death. Although, functional links exist between DDR and autophagy, few studies have reported the implication of CHK1 in this cross-talk.

The hypothesis of this project is that CHK1 is by itself an actor of autophagy. The aim of my project is to demonstrate the existence of this new CHK1 function, and to decipher the molecular elements of this pathway. Our first results suggest that CHK1 is involved into autophagy induction in response to different types of metabolic stress independently of DNA damage. Also, we observe a modification of the CHK1 phosphorylation status (Serine 280) in response to different types of stress. In addition, our preliminary data suggest that the role of CHK1 in the survival of leukemic cells during metabolic stress is dependent on an "autophagic" function of the kinase.

Altogether, we propose a new function of the CHK1 kinase as an autophagy actor. In the future, CHK1 inhibition in combination with autophagy inhibition, or autophagy inhibition as an alternative to CHK1 inhibition, could be proposed as new therapeutic strategies.

P120

Inter-organ exchanges and cachexia: focus on the gut

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Cachexia, is an acute involuntary weigh loss (>5% of body mass), associated with different chronic illnesses, and several cancers (pancreas, colon...). It is a global metabolic syndrome triggering adipose tissue and skeletal muscles atrophy, that cannot be reversed by increased calorie intake. Cachexia leads to a general weakness and represents the primary cause or contributes to circa 30% of cancer patients' deaths. Although a major public health problem, there are currently no standard guidelines for treating cachexia, and a better understanding of the cellular and molecular mechanisms involved is urgently needed.

Cachexia is a complex syndrome in which inducing tumours promote the futile and inefficient usage (wasting) of target reserve tissues (lipids in adipose tissue, proteins in muscles) through organ metabolic rewiring. The involvement of relay and amplifying tissues, such as the liver or the brain, indicates that cachexia represents a perverted pathological state of the integrated physiology and exchanges between organs.

The gastrointestinal system is responsible for food digestion and absorption. The intestine is not only the place of nutrient intake, it also participates in the fine regulation of energy metabolism. Indeed, entero-endocrine cells, in response to several local stimuli, secrete different hormones and signals which control energy balance of the whole organism. The gut appears therefore, as an obvious candidate to study in the establishment of wasting syndromes.

Using unique non-mammalian cachexia models in Drosophila larvae triggered by localised tumorous growth in the wing imaginal disc, we showed the gut is atrophied and profoundly affected during peripheral organ wasting. Our first results have shown that the cachectic gut undergoes a phenotype remodelling. At early stage of cachexia, lipid accumulation can be observed in larvae. In the following days, the phenotype is defined by a shrinking of the midgut associated with changes in cell population dynamics: the AMP (adult midgut precursor) pool is depleted and the size of enterocytes nuclei increases. This phenotype will provide a steady read-out to test potential mediator identified from RNAseq analysis and progress towards a better understanding of cachexia.

P121

Characterization of the interplay of SG33 oncolytic virus and tumor metabolism in pancreatic cancer

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Oncolytic viruses (OV) infect, replicate, kill and spread into tumors and metastasis. We recently demonstrated that SG33 virus, that derives from Myxoma virus, has therapeutic potential in pancreatic adenocarcinoma (PDAC), a disease with no cure. OV are professional parasites that rely on infected cells for replicating, but still host factors and pathways that support SG33 infection of PDAC cells are still unknown. During this work, we highlighted a metabolic relationship between SG33 virus and PDAC patient primary cells. We found that following infection, SG33 nests within extranuclear replisomes in the core of the cellular mitochondrial network. In cells that support viral replication, SG33 infection strongly inhibits mitochondrial respiration capacity without any detectable effect on mitogenesis, while glycolysis is increased. Experiments are currently ongoing to map the metabolic rewiring of PDAC cells following infection. Importantly, unsupervised high-throughput screening further revealed that targeting mitochondria respiratory chain promotes SG33 replication and killing efficacy. In permissive cells, SG33 infection increases the production of reactive oxygen species and oxidized mitochondria, that correlate with increased DNA damage. Mechanistically, we found using RNAseq that SG33 infection results in a time-dependent induction of the mTORC-1 pathway and in the inhibition of the expression of major proteins from the TCA cycle. In summary, our results shine a new light on the interplay between oncolytic virus and cancer cell metabolism, that may translate into new opportunities for patient selection and therapeutic combinations to alleviate the dismal prognosis of PDAC.

P122

Sex steroids production in periprostatic adipose tissue and its role in prostate cancer progression and resistance to anti-androgen therapy

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Prostate cancer (PCa) remains the most commonly diagnosed malignancy in men in western countries and the fifth common cause of cancer death in men worldwide. As one of the important components of prostate cancer microenvironment, periprostatic adipose tissue (PPAT) plays a very large role in prostate cancer progression, by secreting chemokines, pro-inflammatory cytokines and lipids. Androgens promote the growth of both normal and cancerous prostate cells by binding to and activating the androgen receptor, but the role of androgens secreted by PPAT in PCa progression has never been investigated. Using human PPAT and a control tissue, abdominopelvic adipose tissue (APAT) from patients with PCa who underwent a radical prostatectomy, the objectives of our study were to investigate whether PPAT has a specific sex steroids metabolism and whether the sex steroids secreted from PPAT can promote the growth of PCa cells and resistance to the anti-androgen therapies used for PCa care. Our results showed that PPAT has a unique androgen metabolic profile compared with APAT. The dosage of sex steroids content by mass spectrometry indicated that 5α androstanedione and epiandrosterone, two precursors of the active androgens (17β testosterone and 5α dihydrotestosterone), were more abundant in PPAT than in APAT. These 2 compounds have been described to promote the intratumoral androgen synthesis which is one of the mechanisms of castration-resistant PCa. One inactive metabolite of 17β testosterone, etiocholanolone, was also more abundant in PPAT compared to APAT. We confirmed by RT-qPCR an overexpression of the enzymes involved in the accumulation of these intermediates. Anti-proliferative effects of etiocholanolone and epiandrosterone have been shown in colorectal cancer cell models while epiandrosterone can induce PCa cell proliferation but the molecular mechanisms involved have not been elucidated. Altogether, these results suggest that PPAT could represent a neglected source of androgens that may contribute to tumor progression and resistance to anti-androgen treatment. Protein expression of the enzymes involved in sex steroids synthesis is in progress to reinforce and confirm these results. The effects of PPAT-secreted androgens on survival, proliferation and response to anti-androgens (coculture, conditioned medium) are under investigation in in vitro PCa models. Understanding the role of PPAT in tumor progression and the development of resistance mechanisms to anti-androgen therapies may allow us to propose new risk stratification strategies and/or identify new therapeutic targets.

P123

Role of endothelin signaling pathway in diffuse IDH1 mutant low-grade and high-grade gliomas cell proliferation and fate

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Introduction and Objectives

Adult gliomas represent 40% of all brain tumors and are classified in 3 grades of malignancy with glioblastomas, the deadliest. Among them, Diffuse IDH-mutant Low-Grade (LGG) are slowly-growing tumors which often progress into secondary glioblastomas (GBM). These glial tumors are characterized by specif ic mutations such as a recurrent missense mutation in IDH1/2 gene coding for the isocitrate deshydrogenase, recurrent mutations in ATRX gene or a 1p/19q codeletion. Recently, our group has unveiled the presence of 2 non-overlapping tumoral cell population, Sox9+ astro-like and Olig1+ oligo-like cells in IDH1-mutant tumors. We also highlighted the role of Notch signaling in their generation (1). This tumoral heterogeneity observed in patients may defeat currently used therapies. Further exploration of the molecular mechanisms and pathways underlying the generation of this cellular diversity are thus needed. The endothelin signaling system encompassing cytokines (ET1-3) secreted by vessels and two receptors (EDNRA and EDNRB) is highly expressed in the CNS (2). In the normal brain, this signaling is known to regulate stem cell properties such as maintenance and proliferation (3) but its role in gliomas and LGG is still ill-defined (4). We thus interrogated whether IDH-mutant gliomas and high grade express endothelin receptors and explored whether this pathway influences tumoral proliferation and fate.

Material and Methods

Here, we used cultures of low and high-grade gliomas to explore the expression and role of EDNRA and EDNRB by RNA-seq, RT-qPCR, Western blot and immunofluorescence. We used immunohistochemistry to study the expression of these receptors on IDH1-mutant patient tumor cryosections. We investigated whether the expression of these receptors is regulated during differentiation of glioma cancer stem cells. Moreover, as these receptors are known to be coupled with G-protein and generate calcium f lux, we studied them with HTRF technology (Homogeneous Time Resolved Fluorescence) based on FRET (Fluorescence Resonance Energy Transfer).

Results

We found that the EDNRB is the main receptor expressed in IDH1-mutant low-grade glioma and high-grade gliomas cells. Stainings performed on live and fixed cells showed that this receptor is distributed between the membrane and intracellular compartments in glioma cells. By treating low-grade glioma cells with ET-1 and ET-3, we found that these cytokines reduce proliferation in two IDH1-mutant low grade and two non-IDH1 high-grade glioma cell lines whereas no effect was observed in one highgrade IDH1-mutant cell line which barely expresses EDNRB. HTRF showed that stimulation of EDNRB induced Ca2+ intracellular signalling.

Conclusion

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

References: Augustus et al., 2021, Koyama, 2013, 2021, Gulati et al., 2016, 2018 ; Adams et al., 2020, Egidy et al., 2000; Bagnato, A., & Rosanò, L., 2008

P124

Toward understanding formation of intra-tumoral cellular heterogeneity in IDH1-mutant gliomas

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Diffuse low grade gliomas are brain tumors mainly affecting young patients. Most of these tumors have a mutation in IDH1 (isocitrate dehydrogenase 1) gene causing differentiation defects and cell accumulation in the brain. These tumors are classified as astrocytomas or oligodendrogliomas and often progress into high grade gliomas (i.e IDH1-mutant glioblastomas).We and others have shown that IDH1-mutant diffuse low grade gliomas contain 3 types of tumoral cells, resembling astrocytes, oligodendrocytes and neural progenitors, the formation of which is regulated by Notch signaling. (Augustus et al, 2021, Cells, Suvà et al, 2020, Cancer Cell). Despite surgery, radiotherapy and temozolomide treatment, the overall survival of patients remains poor. Furthermore, there are few cellular tools to study Low-Grade Gliomas in vitro. Development of these tools is essential to find markers allowing isolation and targeting of each subpopulation present within tumour. In the lab, we have standardized methods to derive cells lines from patients tumoral resections. We were able by this method to generate a biobank of cell lines from patients affected by grade II, grade III or secondary glioblastomas. We first focused on one cell line named LGG275 showing some characteristic of outer radial glial cells, one main progenitor population of the developing human brain.

We hypothesize that 1) The LGG275 is composed by different subpopulations which can reflect in vitro cellular heterogeneity found in gliomas and 2) within LGG275 cell line, there are cells with stem-like properties allowing the maintenance of heterogeneity in vitro.

To investigate these hypothesises, we performed a single-cell RNA analysis on the LGG275 cell line. We have identified at least three cell subpopulations. As observed on tumours resections, we find an astrocyte-like subpopulation expressing markers such as CRYAB and APOE and an oligodendrocyte-like subpopulation expressing OLIG1 and OLIG2. A third population was constituted cells, expressing proliferation markers such as KI67 and PCNA. We confirmed by immunostaining the expression of these markers in LGG275 cell lines. Single-cell RNA analysis reveals that in these three main subpopulations, some cells express markers found in radial glial cells, such as HOPX, FABP7, and SCL1A3. Unexpectedly, GFAP (known as an astrocyte and radial glial marker) transcript levels were not detected in single-cell RNA sequencing, but the protein was found in many cells.

From this data, our first objective is to isolate each subpopulation. Then, we will study their ability to restore the initial heterogeneous population. Finally, we will identify molecular pathways controlling the fate of these cells.

P125

Development of an intracellular antibody-based degrader system for targeted protein degradation

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In the great complexity of a cellular context, the role of proteins is mainly assigned by the phenotypic analysis of their loss or gain of functions obtained by extinction or overexpression of wild-type or mutant forms. Extinction approaches often allow more powerful analysis of protein function. The abolition of gene expression by genome editing by CRISPR/CAS and reversible RNA interference are effective ways to thereby reduce the expression of a target gene. An alternative approach for inhibiting intracellular proteins consists in inducing their targeted degradation. This is based on genetically encoded binding domains such as single-chain antibodies, functionalized by E3 ubiquitin ligase domains to induce ubiquitinylation of the protein of interest (POI) followed by its proteolysis by the proteasome. Two main classes of protein-based degraders exist, either the tagged or the non-tagged system. The non-tagged system will enable the degradation of endogenous POIs with an anti-POI binder (e.g. an intracellular antibody), while the tagged system will deplete tagged-POIs to assess quickly the degradability of a target. However, the only strategy developed so far is based on GFP tagging of the POI and the use of an anti-GFP intracellular single domain antibody (iDAb). Due to the GFP size, this 28 kDa protein can interfere with proteins' function. Hence, alternative strategies would be required to improve the tag-based degradation with smaller tags. In this context, the main objective of my thesis project is to develop a novel tag-based targeted protein degradation system. Our system includes a new tag added to the POI, the ALFA tag, which is only 2 kDa, and its associated intracellular antibody. We showed that this anti-ALFA antibody linked to well-known E3 ligase domains degrades its target in different cell lines. In a second time, we checked whether we could screened for new and better functional E3 ligase domains. SOCS7 was found to induce degradation when fused to the anti-ALFA and anti-GFP intracellular antibodies. Then, the pair ALFA tag - iDAb ALFA was compared to the GFP - iDAb GFP pair for protein degradation efficiency in different cell lines. Our preliminary data show the degrader iDAb ALFA-SOCS7 is at least as good as the GFP degraders or the other ALFA degraders in the different cell lines. The final aim of the project will be to apply this new degradation approach to the study a kinase from the MAP Kinase pathway (MAPK) whose function in the proliferation of pancreatic cancer was previously demonstrated.

P126
Astrocytes in the Glioblastoma microenvironment

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In addition to the well-known crosstalk between cancer cells and their microenvironment through chemokines/cytokines secretion, tunneling nanotubes (TNT) have recently been shown to favor this crosstalk by allowing the exchange of various cellular cargos including mitochondria. Acquisition of exogenous mitochondria has been shown to result in tumor progression and resistance to therapies in many cancers.

Glioblastomas (GBM), grade IV gliomas, are characterized by their rapid growth and recurrence following therapy. Standard treatment consists of maximum surgical resection followed by radiotherapy and chemotherapy with temozolomide (TMZ). However, resistance rapidly takes place resulting in short patients' survival. Resistance to therapy in GBM is supported by the high heterogeneity and plasticity of the glioblastoma cancer cells and to the presence of a main player: glioblastoma stem cells (GSC). Non-cancerous cells found in the GBM microenvironment, including mesenchymal stem cells (MSC) and astrocytes, can also contribute to this resistance.

Our laboratory has previously shown that MSC mitochondria induce increased TMZ resistance of GSCs through major metabolic changes. MSC mitochondria also modify the transcriptional response of GSCs to TMZ with the regulation of a new set of genes. Because astrocytes are an abundant cell population within the central nervous system, this raises the question of their possible role in glioblastoma progression, through similar mitochondria exchange processes.

In this study, mitochondria transfer between astrocytes and GSCs, following 2D-coculture, have been characterized and quantified by flow cytometry. An organoid model of GSCs has also been developed, in which astrocytes can be introduced. GSCs formed tunneling nanotube-like structures, as observed by confocal microscopy. Mitochondrial transfer from astrocytes to GSCs was also observed in these organoids and quantified by FACS. The following steps will be to determine the effects of astrocyte mitochondria on GSC proliferation and resistance to TMZ chemotherapy.

P127

Oncogenic cooperation in FGF19-driven hepatocellular carcinoma

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Fibroblast Growth Factor 19 (FGF19) is emerging as a possible oncogenic driver and a potential therapeutic target in different cancers and notably in hepatocellular carcinoma (HCC), the main primary liver tumor. Focal amplifications of chromosome locus 11q13, spanning FGF19 and cyclinD1 (CCND1), have been identified among the most frequent amplification events in HCC and are associated with poor prognosis. FGF19 is an ileal postprandial hormone that acts through FGFR receptors to regulate multiple metabolic processes, including bile acid synthesis and glucose homeostasis. Analogs of FGF19, such as Aldafermin (NGM282), have been developed to mimic the hepatoprotective metabolic effects of FGF19 while being theoretically devoid of its oncogenic effects. Much remains to be learned of the oncogenic properties of FGF19, independently of CCND1 co-expression, and it is crucial to assess thoroughly the oncogenicity of FGF19 analogs.

Here, we investigated FGF19 involvement in hepatic carcinogenesis using mouse model of FGF19 overexpression through in vivo stable transfection of hepatocytes. We performed hydrodynamic gene transfer (HGT) to combine FGF19 overexpression with distinct oncogenic events commonly found in HCC, including p53 inactivation, CCND1, c-MYC, RasG12D and □-cateninS33Y overexpression. We show that FGF19 alone has weak oncogenic properties in HCC, but displays strong tumorigenic cooperation with specific oncogenes. We used serial hydrodynamic injections, which give rise to distinct sets of hepatocytes being transfected with different oncogenes, to demonstrate that FGF19 acts in a paracrine fashion. Moreover, stable transfection of hepatocytes with a constitutively active FGFR4 mutant (FGFR4V547L) mimics FGF19 overexpression effects. We performed transcriptomic analyses to investigate the molecular mechanisms involved in the tumorigenic effects of the FGF19/FGFR4 pathway activation. Our results depict FGF19 as a central player of hepatic carcinogenesis. Detailed characterization of its oncogenic effects will be of great interest for the ongoing development of HCC therapeutic approaches based on targeting of the FGF19/FGFR4 pathway.

P128
Vps34 role in PDAC carcinogenesis

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease with poor prognosis. Although the importance of the activating mutation of KRAS, 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. We are particularly interested in the possible involvement of the class III PI3-Kinase, Vps34. This protein has known roles in autophagy, phagocytosis, endocytosis and vesicular trafficking.

Materials and methods

To mimic low Vps34 protein activity, we developed a mouse model combining the KrasG12D mutation with heterozygous inactivation of Vps34 protein in the exocrine pancreas. This conditional genetic targeting is achieved by the expression of a Cre recombinase under the control of the Pdx1 promoter, a transcription factor required for pancreatic organogenesis. The pancreatic pathology of KrasG12D mutated and Vps34 inactivated mice (named KCVps34) is compared to that of KrasG12D mutated mice (named KC).

Results

Experimentally, the KCVps34 mouse cohort shows a drastic decrease in prognosis compared to the KC cohort; the first clinical signs of metastatic spread (cachexia) are observed in mice as young as 4 months of age. From a histological point of view, at 6 months, in the KCVps34 model, we observe an earlier appearance of high grade lesions compared to KC mice, and so in the majority of this cohort, whereas the pancreas of KC mice presents only precancerous lesions. Moreover, these high grade lesions (adenocarcinoma) are aggressive with the detected presence of metastasis to the lungs and liver. Associated with these cancerous lesions, immune infiltrates are observed and the adjacent pancreatic parenchyma shows an accumulation of lipids within the acinar cells.

Discussion

Additional data are needed to understand the signaling pathways involved in Vps34 inactivation that would explain this poor prognosis; in particular, the impact on the metabolic niche of tumor cells in the tumor and in metastatic sites (systemic effect) will be studied. We next aim to understand how Vps34 activity is altered by studying a possible correlation between pesticide exposure and modulations of autophagy by Vps34. Understanding the pathology of PDAC and the molecules involved in its poor prognosis is essential to improve the care of this pathology.

P129

Effects of the mitochondria transfer from the glioblastoma stem cells to the microenvironment cells on the tumor progression

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Cancer cells have the capacity to modify their microenvironment, which can ultimately result in promoting tumor progression and resistance to therapy. Cell behavior in the tumor microenvironment (TME) can be altered in several manners, including altered secretion of cytokines/chemokines and metabolites. These altered concentrations of cytokines and metabolites are expected to activate novel signaling and metabolic pathways in cancer cells and consequently modify their functional properties.

Glioblastoma (GBM) is the most aggressive brain cancer, with short patient survival. The standard treatment for glioblastoma is resection followed by radiotherapy and several cycles of Temozolomide (TMZ) chemotherapy. However, glioblastomas usually recur within a few months. The exact mechanisms underlying this resistance are still partly unknown. GBMs contain self-renewing, tumorigenic cancer stem cells called Glioblastoma stem cells (GSC). GSCs contribute to the recurrence of the disease by generating full GBM tumors, characterized by their high cellular heterogeneity and plasticity. In addition, GBM tumors contain non-cancerous cell types, including mesenchymal stem cells (MSCs), which contribute to GBM progression.

Our laboratory has shown that GSCs and MSCs can exchange mitochondria through tunneling nanotubes. We are now investigating whether GSCs can reprogram MSCs and trigger their secretion of additional metabolites and cytokines. For these identified metabolites and/or cytokines, the next question is to determine their effects on GSC functions.

We have characterized the mitochondria transfer from GSCs to MSCs in cocultures, through different means. Time-lapse imaging (Incucyte) showed highly dynamic interactions between GSCs to MSCs, allowing mitochondria intercellular exchange. This transfer of mitochondria from GSCs to MSCs was also quantified by FACS. Using the Mitoception protocol, which allows the quantitative transfer of pre-isolated mitochondria, we have shown that GSC mitochondria increase the proliferation of MSCs (Cellomics). Metabolites secreted/utilized by MSCs after acquisition of GSC mitochondria were also determined (NMR). We are currently determining the MSC cytokine secretion pattern in these conditions prior to addressing the effects of these altered MSC functions on GSC proliferation, invasion rate and TMZ chemoresistance.

P130

Influence of immunosuppressive myeloid cells on cancer stemness promotion in breast cancer

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Compelling evidence has indicated that cells of myeloid origin represent major components of the complex immunosuppressive tumor microenvironment. These myeloid cells such as tumor-associated macrophages, 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 functions. Indeed, accumulating evidences has demonstrated 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. The role of cancer-induced myeloid cells in resistance to chemotherapy and immunotherapy has also been described. 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) are endowed with the capability to promote stemness features in breast cancer cells in a contact-dependent manner and these interactions confer to cancer cells chemotherapy resistance properties. Moreover, our data provide insights into the ability of mouse-derived MDSC to increase tumorsphere formation. Finally, we confirmed our results with the study of myeloid cells isolated for breast tumor bearing patients.

P131

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 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.

P132

Improving immunomodulatory potential of a tumor targeting monoclonal antibody in melanoma using innovative drug delivery system BEPO

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Cutaneous melanoma is one of the major forms of skin cancer. The treatment of metastatic melanoma has undergone a dramatic transformation over the past decade with the advent of molecular targeted therapy and immunotherapy. Despite these huge advances in melanoma treatments, new combination therapies are needed for patients resisting to targeted and immune treatments, and new strategies should be developed to avoid severe side effects of combinatorial approaches.

Using B16F10 mouse melanoma model we showed that a monoclonal antibody (TA99 mAb) targeting TYRP1 antigen overexpressed in melanoma induces a partial tumor free protection with the development of an endogenous antitumoral immune response. Our objective is now to improve these immunomodulatory effects trough the development of combinatorial approaches between TA99 mAb and conventional BRAF and MEK targeted therapies (vemurafenib and trametinib) or immunomodulatory agents (TLR agonists). We use an innovative delivery system BEPO that is the core technology from MedinCell, which delivers molecules in a controlled manner in order to avoid the strong adverse effects of these small molecules after systemic exposure. Moreover, since the molecules are delivered close to their targets, it may also improve the drug availability and efficiency while decreasing the effective dose compared to systemic exposure.

We first tested several polymers to choose the one giving the best TA99 mAb delivery kinetic. Then we showed that the BEPO formulation does not modify the physical and biological properties of TA99 mAb. We thus tested the biological activity of TA99 mAb in vivo. The subcutaneous administration of BEPO-TA99 formulation close to the B16F10 melanoma tumors displayed an improved control of tumor growth and survival as compared to the intraperitoneal conventional administration route. Moreover, TA99 mAb was recovered in serum 15 days after the BEPO-TA99 subcutaneous injection demonstrating a sustained therapeutic delivery and the presence of TA99 mAb in the general circulation. We are focusing now on formulating vemurafenib and trametinib with BEPO to obtain the best release kinetics and characterize the toxic effects of each molecule individually. Next steps we will consist to study the synergistic effects of BEPO delivery of small molecules with TA99 mAb. Our work will allow us to do the proof of concept, in a solid tumor model, that the use of BEPO technology is able to both increase synergistic effects in these combinatorial therapeutic approaches and circumvent severe toxic sides effects very often observed in clinic.

P133

The cytolethal distending toxin from helicobacter hepaticus modulates the hippo signaling pathway

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We are frequently exposed to infection with genotoxin-producing bacteria, such as cytolethal distending toxin (CDT), a prevalent heterotrimeric toxin among Gram-negative bacteria. CDT causes severe DNA damage in host cells, a well-known risk factor of cancer development and progression. Numerous data point for an etiological role of CDT in carcinogenesis. Indeed, CDT from Helicobacter hepaticus, via its active CdtB subunit, was shown to be involved in the development of murine hepatocarcinoma. We previously showed that CDT modulates cell differentiation and elicits epithelial to mesenchymal transition (EMT), a process by which cells lose their epithelial characteristics in favor of mesenchymal ones, conducive to cell motility. The evolutionarily conserved Hippo signaling pathway is involved in EMT and metastasis. In the present study, we thus investigated the effect of CDT on the Hippo signaling pathway following H. hepaticus CdtB subunit expression. These investigations were performed in vitro on human epithelial cell lines upon ectopic expression of H. hepaticus CdtB and its corresponding mutated CdtB lacking catalytic activity (CdtBMut), to attribute the observed effects specifically to the CdtB. Some results were also confirmed in vivo using xenograft mouse models following H. hepaticus CdtB or CdtBMut expression. In vitro 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 (Yes-associated protein 1) and TAZ (WW Domain Containing Transcription Regulator 1). Increased transcriptional enhanced associated domain (TEAD) activity was shown upon CdtB expression using the TEAD reporter assay. Verteporfin, a compound preventing YAP1/TAZ-TEAD interaction, reduced CdtB-increased nuclear remodeling and CdtB-increased TEAD transcription activity, confirming the involvement of CdtB in the regulation of Hippo signaling pathway. An increase of LATS2, YAP1 and phosphorylated YAP1 was observed in vivo in the CdtB-expressing tumor using the xenograft mouse models. Taken together, these data show that CDT/CdtB activates the Hippo signaling pathway supporting the idea that infection with CDT-producing bacteria can promote cancer development.

P134

Combining experimental and mathematical analysis of cancer adaptive therapy with kinase inhibitors

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Adaptive therapy (AT) aims to control tumour burden by exploiting competition between therapy-resistant and -sensitive cells. We tested this strategy using a lung cancer model of resistance to Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors (EGFR TKIs), such as osimertinib, in the PC-9 NSCLC cell line. From monolayer growth cultures we find that resistants cells are less proliferative but nevertheless retain partial drug sensitivity. Exchange media assays point towards a paracrine signalling mechanism as a contributor to osimertinib resistance. In tumour spheroids, where cell-cell competition is spatially confined, low dose therapy outperforms the maximal tolerated doses (MTD) in both controlling resistance and minimising tumour burden. Our preliminary experiments in mice confirm the benefit of AT *in vivo*. By combining mathematical modelling and Bayesian inference methods, we quantified key parameters of this system to inform planned preclinical and clinical trials of AT. We will use these key parameters to tailor a spatial, agent-based model to simulate and predict *in vitro* spatial growth, and compare these simulations with in-vivo murine data from pre-clinical trials.

P135

In vivo analysis reveals context-specific gain of function phenotype of p53 in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the main primary liver cancer, with poor prognosis and rising incidence. Genetic alterations of the tumor suppressor gene TP53 are among the most frequent events in HCC (40%). p53 inactivation occurs mainly through missense mutations, which alter the p53 wild type function and exert a dominant negative (DN) effect on the wild-type protein. Moreover, it has been proposed that the oncogenic mutations confer novel activities to the p53 protein, defining a Gain-of-Function (GOF) phenotype. Despite many reports supporting the mutant p53 GoF phenotype, the issue remains controversia. One hypothesis that could explain contradictory reports is that the GoF phenotype is both mutant- and tissue-specific. It might also depend on the oncogenic context as well as the stage of tumor development. HCC present a specific spectrum of TP53 mutations, and the question of GoF acquisition in this pathology has not been investigated.

We investigated the functional consequences on hepatic carcinogenesis of eight p53 mutations of interest (V157F, A159P, R175H, R248Q, R248W, R249S, R273C, R273H). We used a mouse model to trigger tumorigenesis via stable transfection of hepatocytes in vivo by hydrodynamic gene delivery (HGD). Expression of each p53 mutant, or genetic inactivation of p53 as control, was combined with c-Myc overexpression, giving rise to hepatic tumors in 3 to 6 weeks. All mutants were not equivalent, with V157F and A159P showing weak oncogenic activity whereas R175H and R249S giving rise to the highest tumor burden. We performed transcriptome profiling of the tumors (RNAseq) and identified two clusters of mutants with very distinct transcriptomes. Analyses are ongoing to pinpoint the mechanistic bases of the differences in phenotypes. In conclusion, our results highlight striking differences in phenotypes of tumors harboring p53 mutants frequently encountered in human hepatocellular carcinoma.

P136

Translational control in Acute Myeloid Leukemia and monocytic differentiation

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Acute myeloid leukemia (AML) is a haematopoiesis malignance, characterized by a massive proliferation and an accumulation of immature myeloid cells (or blasts) in the bone marrow and in blood. We have performed RNA sequencing from both healthy donors and patient blast samples to monitor the expression of mRNA translation initiation factors during normal and pathogenic haematopoiesis. Among them, the two factors eIF4E1 and eIF4E3 attracted our attention. They belong to the eIF4E (eukaryotic translation initiation factor 4E) family of translational initiation factors that regulate cap-dependent mRNA translation. eIF4E1, a well-known factor, plays a role in survival and proliferation in both normal and cancer cells while eIF4E3 is poorly described.

During normal haematopoiesis, we found an increase in eIF4E3 expression particularly during monocyte differentiation, unlike to eIF4E1 that tends to decrease. This is consistent with observations made in AML patient samples, where the differentiation status of leukemic blasts is correlated with more eIF4E3 than eIF4E1 expression. Also, when established leukemic cell lines (HL60 and U937) are engaged into monocyte differentiation, eIF4E3 expression is similarly increased. In addition, the down regulation of eIF4E3 in these two cell lines by stable shRNA transduction reduces their capacity to differentiate into monocytes.

These results highlight a required switch from eIF4E1 to eIF4E3 during monocyte differentiation, suggesting that eIF4E3 may play a specific role in cap-dependent mRNA translation necessary for proper differentiation.

P137

Acquired chemoresistance in pancreatic adenocarcinoma: mechanism implicating the stromal transcription factor Zbtb16 ?

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Pancreatic ductal adenocarcinoma (PDA) is highly lethal. Chemotherapies remain standard protocols even if most patients relapse on treatment. PDA is a stroma-rich tumor, comprising numerous cancer-associated fibroblasts (CAFs). CAFs are recognized as key actors of PDA chemoresistance, even so underlying clinically-targetable mechanisms still need to be identified to set up novel more efficient drug combinations. The goal of our project is to identify and functionally characterize stromal targets involved in PDA relapse under chemotherapy. We developed a murine Patient-Derived Xenograft (PDX) model of relapsed tumors, whereby tumors first 'sensitive' to Gemcitabine (Gem) became, for the majority, 'resistant' by chronic mouse treatment with Gem. Using RNAseq-based bioinformatics, we find that the transcription factor Zbtb16 is uncovered as highly expressed in the stroma of Gem-still 'sensitive' compared to 'resistant' tumors. Zbtb16 is known to regulate transcriptional cell-fate programs. Therefore, we hypothesize that Zbtb16 is expressed in fibroblasts that do not present yet any chemoprotective properties, allowing to many cancer cells to be Gem-sensitive. Progressively, Zbtb16 could be lost upon chronic Gem-treatment, turning initial fibroblasts into chemoprotectives CAFs subtypes, favoring initially Gem-sensitive cancer cells to become Gem-resistant. To test this hypothesis, we exogenously over-expressed Zbtb16 in CAFs in vitro and evidenced that i) they change their identity and ii) they are less prone to be differentiated into described pro-tumoral and chemoprotective CAF subtypes. Moreover, we show that Gem treatment reduces Zbtb16 expression in CAFs. Our perspectives are now to test whether CAFs overexpressing Zbtb16 favor pancreatic cancer cell sensibility to Gem and if so, to find by which molecular mechanisms. Overall, this project will help to understand how Gem reprograms CAF to favor the acquisition of chemoresistance with the hope to therapeutically target such plasticity in PDA patients.

P138

Imiqualine for pancreatic cancer treatment: New inhibitor of microtubule polymerisation ?

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The survival rate for patients with Pancreatic Ductal Adenocarcinoma (PDAC) is dramatically poor with a five-year survival rate less than 10%. The research of new treatments, which could complement the current therapeutic arsenal constitued by Gemcitabine, FOLFIRINOX (fluorouracile, leucovorin, irinotecan, oxaliplatin) and nab-paclitaxel is a major challenge.

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

We characterized EAPB02303 effect on tumor growth both *in-vitro* and *in-vivo*, using a panel of pancreatic cancer cells including cells derived PDX (Patient Derived Xenograft), 3D models with Cancer Associated Fiborblasts and pancreatic xenograft models. Moreover, we looked for synergy with other pancreatic cancer standard treatments and found a potent synergy with paclitaxel.

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

We analyzed mRNAseq data of pancreatic cancer cell lines treated with EAPB02303 at 6h and 24h to identify signaling pathways and key proteins implicated in EAPB02303 effect. These data led us to suspect a direct interaction of EAPB02303 with microtubules.

We finally showed that EAPB02303 1) binds to free tubulin by using Differential Scanning Fluorimetry (DSF), 2) is able to inhibit microtubule formation in a tubulin polymerization assay, 3) inhibits microtubule growh in a cellular context by flow cytometry and immunofluorescence analysis.

All these data suggest that EAPB02303 is a new microtubule-targeting agent, which presents *in-vivo* activity in pancreatic cancer and *in-vitro*synergy with Paclitaxel.

P139

Depletion of a pro-tumour soluble factor from the microenvironment of colorectal cancers with a new type of therapeutic bispecific antibody.

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The transferrin receptor (TfR1) is the main pathway for iron entry into the cell. Tumor cells with higher iron requirements coincide with overexpression of TfR1 at their surface. In the team we have developed a recycling anti-TfR1 monoclonal antibody (Ab) that inhibits tumor growth by iron deprivation. The recycling capacity of this Ab, that binds to TfR1 both at extracellular neutral and intracellular acidic pH, permitted us to design a new type of bispecific antibody (BsAb) targeting TfR1 and a soluble antigen (patent: WO2020104496). Such BsAb would allow tumour-localized neutralisation and removal of protumour factors from the tumour microenvironment by a sweeping mechanism supported by the tumour cells themselves. In this design, the intracellular elimination of the protumour factor requires its release from the BsAb at endosomal acidic pH to ensure its degradation in the lysosomal pathway while the BsAb is recycled to the cell surface to capture a new soluble factor.
Here, we constructed a BsAb to eliminate the pro-tumour cytokine C from the tumour microenvironment via TfR1. The cytokine C is overexpressed in the tumour microenvironment TME) of patients with colorectal cancer (CCR) and has a strong impact in the metastasis process and the immunosuppression.

Results:
The format chosen for the BsAb is a scFv-Fc using knob into hole modifications in the CH3 domains (Merchant et al. Nat Biotechnol. 1998). It was derived from our anti-TfR1 recycling antibody and a neutralizing anti-cytokine C. The BsAb was produced in HEK cells and purified by protein A affinity chromatography.
First, the functionality of the BsAb towards both targets was tested in vitro and compared with both parent bivalent monospecific antibodies (anti-TfR1 and anti-cytokine C). The ability of the BsAb to simultaneous bind both targets was verified by ELISA. The BsAb inhibited cytokine C signaling in a cell-based luciferase reporter gene assay and reduced the viability of the colorectal cancer (CRC) cell lines HT29 (anti-TfR1 IC50 0.96 nM, BsAb IC50 10.5 nM) and SW222 (anti-TfR1 IC50 5.37 nM, BsAb IC50 19.27 nM). Analysis of iron related proteins in treated cells showed interference with iron metabolism upon BsAb and anti-TfR1 treatment. Moreover, the treatment of CRC cell lines with the BsAb induced an increase of TfR1 at the cell surface in vitro compatible with a sustained uptake of the cytokine. These observations indicate that the BsAb has a promising potential to allow pro-tumor cytokine C neutralization and elimination specifically into the TME of TfR1 expressing tumors. However, the anti-cytokine C antibody had a pH independent binding to cytokine C, not suitable for a sweeping effect.
Secondly, to allow the release of the cytokine in the endosomal pathway, the anti-cytokine C paratope was evolved for decreased binding at acidic pH using substitution of selected amino acids near the antibody paratope by histidine (Christian Schröter and al. mAbs 2015). The locations of substitutions were determined by in silico molecular docking. A library of His substitution combinations (from 1 to 9, total 512 variants) was displayed on yeast and sorted by flow cytometry for pH dependent variants. In parallel, monovariants scFv anti-cytokine C (presenting one substitution) were displayed on yeast and tested for cytokine binding at various pH. An Asp substitution by His located in the CDR3 of the VH domain allowed a reduced binding at acidic pH without substantial loss of binding at pH 7.4.

Perspectives:
We are currently validating the sweeping activity of the His-CDR3-BsAb variant in vitro. Further work will focus on testing the biodistribution and therapeutic effect of the sweeping BsAb in vivo in mouse models of CRC to compare it to the non-targeted anti-cytokine C neutralizing Ab. Our study shows that BsAb antibody formats may confer new properties to old targets and hence produce more efficient anti-cancer drugs.

P140
The role of autophagy-inactivated acinar cell in PDAC initiation

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Despite the extensive research on PDAC and the development of various late stage-PDAC models, the 5-years survival rate of patients is below 5% (1). This compels the deflection of the research focus into earlier stages of the PDAC carcinogenesis and the development of prevention and inception strategies. Autophagy is an evolutionarily conserved process which plays an important role in both physiology and pathophysiology (2). In PDAC, the role of autophagy is broadly studied and was described to perform both tumor suppressor and pro-tumor role (3). The Class III PI3K Vps34 is required for autophagy initiation and for the late-stage autophagosome-lysosome fusion (4). Cumulative evidence reinforce the role of microenvironment in the initiation/progression of pancreas cancer (5). Using genetically engineered mice, we found that Vps34 inactivation results in heterogeneously stressed pancreatic acinar and initiate pancreatic inflammatory disease, known as a risk factor for PDAC development. Single-cell RNA-sequencing (scRNA-seq) showed that Vps34 inactivation resulted in a selective loss of a subset of acinar cells with high mitochondrial and autophagy-related genes. Ex vivo, acinar cells with Vps34 inactivation induced Reg family member's specific autophagy-mediated degradation. These data are critical for our ongoing experiments that aim to understand the role of stressed acinar cells as part of the environment in PDAC initiation through co-culturing acinar cells inactivated for Vps34 with the PDAC initiating acinar cells holding the oncogenic KRAS mutation. Finally, the role of the acinar cells with inactivated Vps34 in PDAC initiation will be studied in vivo by crossing mice mutated for the Vps34 inactivation specifically in acinar cell with mice mutated for the oncogenic KRAS.

P141

YAP nuclear translocation is regulated by EGFR activation through PTEN/AKT axis in glioblastomas

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Gliomas are the most common and lethal primary brain tumors in adults. Glioblastomas, the most frequent and aggressive form of gliomas, represent a therapeutic challenge as no curative treatment exists to date and the prognosis remains very poor. Recently, the transcriptional cofactors YAP and TAZ belonging to the Hippo pathway have emerged as a major determinant of malignancy in solid tumors including gliomas. However, the mechanisms involved in its regulation, particularly in brain tumors, remain ill-defined. In glioblastomas, EGFR represents one of the most altered oncogenes to be affected by chromosomal rearrangements, mutations, amplifications and overexpression. In this study, we investigate the potential link between EGFR and the transcriptional cofactors YAP and TAZ by in situ and in vitro approaches. We firstly studied their activation on TMA including 137 patients from different glioma molecular subtypes. We observed that YAP and TAZ nuclear location was highly associated with IDH-wildtype glioblastomas and poor patient outcome. Interestingly, we found an association between EGFR activation and YAP nuclear location in glioblastoma clinical samples suggesting a link between these two markers contrary to its ortholog TAZ. We tested this hypothesis in patient-derived glioblastoma cultures by pharmacological inhibition of EGFR using Gefinitib and showed an increase of S397-YAP phosphorylation associated to a decrease of AKT activation in PTEN-wild-type unlike PTEN-mutated cell lines. Finally, we used PTEN inhibitor bpV(HOpic) to mimic the effect of PTEN mutation and counteract Gefitinib effect in PTEN-wild-type cultures. Altogether, these results support the regulation of YAP by the EGFR-AKT axis in a PTEN-dependent manner.

P142

Connected tumor cells make glioblastoma stronger: from microtubes to metabolic symbiosis

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Glioblastoma (GB) is a common and devastating brain tumor, associated with low median survival, despite standard therapeutic management. Among its major features, GBs are highly angiogenic and exhibit paradoxically an elevated glycolysis. Tumor cells connect *via* physical structures, such as nanotubes. We and others showed that GB cells connect *via* recently-described microtubes. Thrombospondin-1 (TSP1) is a central actor in GB development and invasion (Daubon et al, Nat Comm 2019), and in microtube formation, *via* a TGFb1-Smad3 axis (Joseph et al, NeuroOncology 2022).

Beyond cell-cell connections, metabolic exchanges are also central during tumor development. Lactate symbiosis has been described between glycolytic astrocytes and oxidative neurons, the latter consuming lactate for fueling TCA cycle. We also interrogated if such a lactate symbiosis is used by glycolytic and oxidative GB cells, via lactate shuttle. By using multiple models such as immunostainings on invasive spheroids in 3D or on patient-derived tumors, and spatial transcriptomics data on patient material, we described a regiolocalized expression of lactate dehydrogenase (LDH) A and B in GB, suggesting a metabolic symbiosis. LDHA is more expressed in hypoxic areas, and in few invasive cells, and LDHB is expressed in peripheric and invasive areas. We showed that lactate fuels TCA cycle to sustain the invasion and proliferation of glucose-starved cells. Then, we showed that, under hypoxia, double LDHA/B KO cell growth and invasion were dramatically decreased in comparison to control cells, mainly caused by an increase in apoptosis. Moreover, double impairment of LDHA and B significantly reduced tumor growth and cell invasion and induces a massive increase in mouse survival. Tracing experiments with 13C-Glucose coupled with RNA sequencing revealed how metabolism adapts to these constraints, by modifying electron transport chain subunit expressions. Antiepileptic drug inhibited LDH activity, tumor growth, and invasion, prolonging mouse survival (Guyon et al, EMBO Mol Med 2022). Considered for a long time as metabolic waste, lactate is shown here to play a critical role in GB cell symbiosis.

Another recent study by our group also showed that pyruvate dehydrogenase kinases (PDHKs) have a central role in lactate metabolism regulation, and then in GB development (Larrieu et al, Cancers 2022). These studies highlight tumor cell connectivity using microtubes or metabolite exchanges is crucial for GB development and represents original therapeutic targets.

P143

A 31-plex panel for high-dimensional single cell analysis of murine preclinical models of solid tumors by imaging mass cytometry

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Background: The tumor microenvironment contains numerous cell types in addition of tumor cells such as immune cells, fibroblasts, and endothelial cells. They all interact with each other to form a complex and dynamic ecosystem. To investigate this complex ecosystem at the single-cell level, high dimensional imaging mass cytometry (IMC) has been recently developed. IMC allows to study simultaneously 40 markers and to observe their distribution in the tissue. In addition to anatomical characterization, adapted downstream analyses allow in-depth in situ single-cell analysis, cell clustering and spatial distribution. Spatial analysis allows to evaluate attraction or avoidance between cell clusters identifying cellular communities. Correlated to clinical data, these communities could define new predictive or prognostic signature.
Although many antibodies have been validated for highplex imaging in human tissue samples, none or very few for IMC in mouse formalin-fixed paraffin-embedded (FFPE) tissue samples. Yet, mouse models are important preclinical tools for basic and translational research on treatment resistance mechanisms (primary or acquired), tumor dormancy and recurrence. They enable dissection of complex biological mechanisms through chemical and genetic manipulations. Thus, we developed an original panel of 31 antibodies and one DNA marker for mouse FFPE tissue sections.

Methods: We selected 31 antibodies to explore the TME including immune and tumor cells, fibroblasts, endothelial cells and epithelial cells. Each antibody have been individually tested by IF with the IMC staining conditions, conjugated. Conjugation has been validated on a TMA and titrated. The use of different tissues allowed us to test the robustness of our technique but also its versatility. The full panel has been then teste on a lymphoid tissue (lymph node) and two tumor types, the B16K1 melanoma model and APCD14 gut cancer model. Then, a cell segmentation has been done to generate a single-cell file. We identified manually cells with complex phenotypes to achieve our panel validation. Through supervised and unsupervised analysis, we assessed our analysis pipeline and realized a deep immunophenotyping of tumors.

Results: We validated and applied the panel on mouse tumors. We showed that IMC allowed an accurate spatial visualization of the tissue architecture and the cell distribution. In lymph node, IMC permitted to identify cortical area, paracortical area, follicles, blood vessels and high endothelial venules. Cell segmentation and subsequent single cell analysis permitted us to explore deeply the TME and identify rare cells presenting a complex phenotype such as the different T cell subset (Th1, Th2 and Th17) in the gut. Unsupervised approaches revealed the strong intratumoral heterogeneity in both immune and tumor cells. Single cell data from IMC keep the spatial coordinate of the cells. This allowed us to measure the distribution of a immune cells in the tumor, from the core to the periphery. Furthermore, we also identified significant cell-cell interactions or avoidance in the tissues identifying spatial signature of tissular features such as Peyer's patch, tumor and healthy tissue.

Conclusion: We developed with the support of the GSO-Emergence grant a 31-plex panel for mouse tumor exploration by imaging mass cytometry. We demonstrated the great benefit that IMC brings to preclinical models including tumor architecture visualization, biomarker distribution, in-depth immunophenotyping and cell interactions. IMC allows to compare tissues and tumors to identify characteristic signatures. The use of this technology represent a great onset for translational research. Validating a panel for mouse samples would extend and optimize the use of these models in onco-immunology.

P144

SLAP promotes UBE3C-dependent ubiquitination of mLST8 to control mTORC2 signaling and to limit tumorigenicity in colon cancer cells

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Deregulation of receptor-tyrosine kinase (RTK) signaling is tightly connected with carcinogenesis. Small adaptor proteins emerged as a new class of regulatory proteins that can exert tumor suppressive functions by targeting several components of the oncogenic RTK signaling cascades. Such as the Src-like adaptor protein (SLAP) which shares strong structure similarity with the Src family kinases but is devoid of catalytic activity. Through recruiting various E3 ubiquitin ligases, SLAP can destabilize RTKs, associated components or downstream substrates to fine tune cell signaling in immune and cancer cells. We previously reported that SLAP is frequently down-regulated in colon cancer and displays important anti-oncogenic functions. However, the associated molecular mechanisms are poorly decrypted. Importantly, we identified a novel mechanism behind the SLAP tumor suppressive functions which is dependent on the targeting of the serine/threonine kinase and metastasis inducer mTORC2. By proteomics, we found that SLAP strongly interacts with most components of the mTORC2 complex (i.e. mTOR, Rictor, mLST8, Sin1, Telo2 and Tti1) and showed that SLAP specifically and negatively regulates mTORC2 signaling to limit invasion and anchorage-independent cell growth of colon cancer cells. Mechanistically, SLAP promotes mTORC2 complex disassembly by inducing the ubiquitination of the regulatory subunit mLST8 via the E3 ubiquitin ligase UBE3C. Finally, we showed that the level of SLAP expression in colon cancer cells is inversely correlated with the sensitivity to mTOR catalytic inhibitors. Collectively, these data support a new important mechanism by which SLAP controls cancer cell signaling and unveil SLAP as a potential biomarker of response to catalytic mTOR inhibitors in colon cancer cells.

P145

Upregulated flotillins and sphingosine kinase 2 derail AXL vesicular traffic to promote epithelial-mesenchymal transition

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Altered endocytosis and vesicular trafficking are major players during tumorigenesis. Flotillin overexpression, a feature observed in many invasive tumors and identified as a marker of poor prognosis, induces a deregulated endocytic and trafficking pathway called upregulated flotillin-induced trafficking (UFIT). Here, we found that in non-tumoral mammary epithelial cells, induction of the UFIT pathway promotes epithelial-to-mesenchymal transition (EMT) and accelerates the endocytosis of several transmembrane receptors, including AXL, in flotillin-positive late endosomes. AXL overexpression, frequently observed in cancer cells, is linked to EMT and metastasis formation. In flotillin-overexpressing non-tumoral mammary epithelial cells and in invasive breast carcinoma cells, we found that the UFIT pathway- mediated AXL endocytosis allows its stabilization and depends on sphingosine kinase 2, a lipid kinase recruited in flotillin-rich plasma membrane domains and endosomes. Thus, the deregulation of vesicular trafficking following flotillin upregulation, and through sphingosine kinase 2, emerges as a new mechanism of AXL overexpression and EMT-inducing signaling pathway activation.

P146
Modelling gene regulation for drug target identification

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Oxaliplatin is a platinum chemotherapy administered as first-line treatment for patients with colorectal cancer. Inside cells, oxaliplatin interacts with DNA to form inter- and intra-strand bridges, which interrupt DNA synthesis and result in cell death. Cellular adaptive responses play a role in cancer cell resistance to chemotherapy agents. Untangling this response could provide precious information for selecting targeted treatments to complement chemotherapy. This requires the identification of the signaling pathways that drive the response of the cell to the treatment. In this context, our project aims to identify the signaling cascade(s) by which oxaliplatin acts on the transcription. We developed for this a machine learning approach that trains a statistical model to predict whether a gene is differentially expressed or not in response to oxaliplatin.

RNA sequencing was performed on HCT-116 cells treated or not with oxaliplatin for 24 hours. The genes were classified in two classes according to whether their expression increased or not in the presence of the drug. Based on the assumption that a large part of the instructions for gene regulation lies at the DNA level, our method investigates the regulatory elements that can be found in the regulatory sequences (promoters) of the differentially expressed genes. In the model, each gene is described by a vector of scores that reflect the affinity of a large number of transcription factors for its promoter sequence. For this, the Position Weight Matrices (PWMs) of the JASPAR database were used to scan the promoter sequence of each gene. We also add in this description vector, several variables that resume the nucleotide environment of each gene. Then, a logistic regression model predicting the class of the genes (i.e. differentially expressed or not) on the basis of the description vector was trained. The problem is difficult because the data are very unbalanced (103 positive vs 15497 negative genes). To reduce the dimensions and help the model, two strategies were implemented. First, we provide a priori information using KEGG, WikiPathways and STRING databases. More precisely, one model per pathway was trained using only the nucleotide environment and the transcription factors targeted by the pathway. Second, a specific learning algorithm based on LASSO penalty [Tibshirani, 1996] was used to automatically identify the most important variables of each model.

This approach has several interests. First, the predictor can integrate different features and mechanisms of gene regulation into one model. Second, by comparing the accuracy of the models built on the different pathways, we can identify the most likely signaling cascade(s) used by the cell in response to oxaliplatin. Third, the LASSO penalty minimizes the number of predictive variables, 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.

Our first results highlight the importance of a small set of signalling pathways linked to the P53 transcription factor family, which have been shown to control specific cell death programmes in response to acute DNA damage.

P147

Unveiling the Proteome and Transcriptome of Stress Granules and P-Bodies in Human T Lymphocytes

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Stress granules (SGs) and processing bodies (PBs) are membraneless cytoplasmic assemblies regulating mRNAs under environmental stress such as viral infections, neurological disorders, or cancer. Upon antigen stimulation, T lymphocytes mediate their immune functions under regulatory mechanisms involving SGs and PBs. However, the impact of T cell activation on such complexes, in term of formation, constitution and relationship remains unknown. Here, by combining proteomic, transcriptomic and immunofluorescence approaches, we simultaneously characterized the SGs and PBs from primary human T lymphocytes pre- and post-stimulation. The proteomes and transcriptomes of SGs and PBs were identified, unveiling an unanticipated molecular and functional complementarity. Notwithstanding, these granules keep distinct spatial organizations and abilities to interact with mRNAs. This comprehensive characterization of the RNP granule proteomic and transcriptomic landscapes provides a unique resource for future investigations on SGs and PBs in T lymphocytes.

P148

The aminoglycoside streptomycin triggers mitochondria-dependant ferroptotic cell death of tumor initiating cells

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Compelling evidence suggests that tumor initiating cells (TIC) are the roots of current shortcomings in advanced and metastatic cancer treatment. TIC represents a minor subpopulation of tumor cells endowed with self-renewal and multi-lineage differentiation capacity, which can disseminate and seed metastasis in distant organ. For that reason, targeting TIC has become a major goal to design new therapeutic routes that may prevent tumor relapse and metastasis. We focused our attention on streptomycin (SM), a potent bactericidal antibiotic widely used across the globe to prevent cell contamination in cell culture and generally administered for the treatment of individuals with moderate to severe infections such as tuberculosis. Our work identified **SM as a new molecule capable of targeting non-adherent TIC** from colon and breast cancer cell lines by inducing **mitochondria-dependent ferroptosis**. Ferroptosis is a form of cell death characterized by intracellular iron dependence, increased reactive oxygen (ROS) production and aberrant lipid metabolism. SM treatment recapitulates ferroptosis hallmarks and leads to **profound alterations in mitochondrial morphology**, such as swelling and cristae enlargement, coupled with hyperpolarization of mitochondrial membrane potential and production of mitochondrial ROS. At the molecular level, the aldehyde group present on the streptose moiety of SM is essential for this mechanism to occur. As such, the mere **reduction of SM into dihydrostreptomycin abolishes its effect on TICs** while preserving bactericidal activity. In the course of this study, we uncovered a **transcriptional program dedicated to counteract oxidative stress**. Remarkably, **this inducible program was restricted to eight genes** whose upregulation enabled a fistful of TICs to survive IC50 SM concentration and initiate spheres despite severally altered mitochondria. This study reveals a new mechanism of action of SM that could help **comprehend the molecular mechanisms behind TIC adaptation to inhospitable environment** and pave the way for new treatment of advanced cancers, in particular grade three that are susceptible to spread to distant organs.

Posters – Axis 2 “Genome Dynamics and Cancer”

P201

Molecular characterization of the combined chemotherapy of SUV4-20h epigenetic enzymes inhibitor with Topoisomerase II poisons in metastatic prostate cancer

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Despite the long-term survival in localized prostate cancer (PCa), this disease is still a major cause of death notably because its advanced forms remain largely incurable even after intensive multimodal therapy. The search for new therapeutic targets and strategy is therefore essential. In last few years, pathologists revealed that the progression of prostate cancer and its resistance to current therapy often coincide with chromatin and epigenetic alterations, a facet that could be exploited for identification and development of new therapeutic strategy. In this regard, we discovered that a subset of prostate cancer patients display an up-regulation of the epigenetic enzymes SUV4-20H1 and SUV4-20H2, two methyltransferases responsible for the di- and tri-methylation of histone H4 at lysine 20 (H4K20me2/3). We found that the upregulation of SUV4-20H2, but not of SUV4-20H1, is associated with a poor prognosis and the appearance of metastases in patients, suggesting an important role of this enzyme in cancer progression. We hypothesized that SUV4-20h enzymes and H4K20 methylation marks could constitute predictive markers of advanced forms of PCa and might be exploited as new targets to improve therapeutic strategy. Consistent with this last hypothesis, we demonstrated that the pharmacological inhibition of SUV4-20H1/2 enzymes and the complete loss of H4K20me2/3 states impair replication fork progression and chromatin compaction but, surprisingly, prostate cancer cell survival and proliferation were not affected. Nevertheless, the inhibition of SUV4-2H1 and SUV4-20H2 also affects DNA repair mechanisms, which creates a specific synthetic lethality with innocuous concentrations of topoisomerase II (TOPO2) poisons used for cancer treatment. Yet, this new drug synergy between inhibitors targeting SUV4-20h and TOPO2 enzymes is not observed with other DNA damaging agents, suggesting a specific interplay between these chromatin-associated enzymes in genome stability and repair. We will present here our last data and discuss the mechanisms by which the inhibition of SUV4-20h enzymes could specifically impair DNA repair pathways upon the poisoning of TOPO2 in metastatic prostate cancer cells. Altogether, our results show that the targeting SUV4-20h enzymes and H4K20me2/3 methylation dramatically improves the treatment responses with TOPO2 poisons and might constitute a novel promising drug combination for cancer treatment.

P202

Role of transcription 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, re-proliferate 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

Defining the landscape of circular RNAs in neuroblastoma unveils a global suppressive function of MYCN

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Background

Circular RNAs (circRNAs), a class of regulatory RNAs originating from alternative splicing, are highly abundant in neural tissues and were shown to regulate gene expression e.g. by interacting with microRNAs and RNA-binding proteins. While cancer-driving functions have been identified for single circRNAs, how they are controlled and globally modulate gene expression in cancer is not well understood. Neuroblastoma is an embryonal cancer and one of the prime diseases responsible for cancer-related death in children.

Aims

Here, we delineate the complex expression patterns of circRNAs in cancer and their functional role for cancer-progression in neuroblastoma

Methods

Deep whole-transcriptome sequencing was used to analyze samples from 104 primary neuroblastomas covering all risk groups. Published datasets from other cancer entities and healthy tissues served as control. An inducible overexpression system of the MYCN oncogene, which defines a subset of high-risk neuroblastoma cases, was employed to assess the specific effects of MYCN on circRNA expression. Hierarchical clustering analysis of circRNA and expression of RNA-binding proteins was performed to identify global regulators of circRNA biogenesis. The mode of action of a candidate circRNA derived from the ARID1A tumor suppressor gene in neuroblastoma cell biology was further validated and characterized in vitro. circARID1A knockdown models were created and RNA sequencing performed to investigate molecular changes. An RNA pulldown assay, bioinformatics motif analysis and mass spectrometry were performed to identify interaction partners of circARID1A.

Results

We identified 5,203 circRNAs generated from 2,302 genes. Hierarchical clustering and differential expression analysis revealed that MYCN-amplified neuroblastoma samples had the lowest expression of circRNAs. Mechanistically we demonstrate that this global negative regulation is dependent on the DHX9 RNA helicase, which is controlled by MYCN. We detected similar mechanisms in shaping circRNA expression in the pediatric cancer medulloblastoma implying a general MYCN effect. Comparisons to other cancers identified 25 circRNAs, including circARID1A, which are specifically higher expressed in neuroblastoma. Transcribed from the ARID1A tumor suppressor gene, circARID1A, was validated in neuroblastoma cell lines and its localization in the cytoplasm shown. Specific knockdown revealed that circARID1A promotes cell growth, survival and proliferation in neuroblastoma cells, while not affecting ARID1A mRNA. Mechanistically, we identified by RNA pulldown and subsequent mass spectrometry that circARID1A exerts its function by direct interaction with the KHSRP RNA-binding protein, to control TP53 signaling.

Conclusion

In summary, we here extend knowledge of the neuroblastoma transcriptome to the noncoding RNA class of circRNAs. We demonstrate that circRNAs are globally controlled by activity of the oncogene MYCN in concert with the RNA helicase DHX9 in neuroblastoma and other childhood malignancies. Furthermore, we outline a function for circARID1A, which is specifically upregulated in neuroblastoma tumor tissues and acts in a tumor-promoting manner in neuroblastoma cell lines, independent of linear mRNA transcripts. Our study offers mechanistic insights into the regulation of circRNAs in high-risk neuroblastoma as well as exemplifies and highlights the importance of this class of noncoding RNAs for neuroblastoma cell maintenance and survival and presents new angles for therapy design to tackle this malignancy.

P204

Analysis of the prognostic interest of sortilin_dependent molecular profile in lung cancer

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Epidermal growth factor receptor (EGFR) activating mutations are one of the most common oncogenic events in non-small cell lung cancer (NSCLC), which has made EGFR an important therapeutic target for the treatment of these cancers. Inhibition by tyrosine kinase inhibitors (TKIs) has generated considerable tumor responses. However, cancer cells progressively acquire resistance to these TKIs, leading to progression and relapse. Several mechanisms are involved in this resistance process, including nuclear localization of EGFR, which is associated with a significant increase in local recurrence and decrease patient survival.

Our previous works have highlighted the involvement of sortilin in the intracellular trafficking of EGFR and have shown the involvement of sortilin in the internalization of EGFR and the attenuation of it signaling. Recently we observed that sortilin counteracts EGFR's transcriptional program through its interaction with chromatin upstream of the transcription starting site of EGF response genes. While the doubling time of SORT1 KO cells remains greater than that of the parental cell line, transient expression of mutant EGFR results in even greater proliferation. Sortilin restoration abrogates the dominance of EGFR mutants on proliferation, suggesting that the expression of mutant EGFR in a sortilin-free genetic background may have cellular advantages. Because sortilin expression decreased with clinical stage, we performed RNA-Seq analysis to analyze differentially expressed genes. We observed that 2 histones were downregulated in 3 SORT1 KO clones. In pathological models with adenocarcinoma cell lines harboring EGFR mutants insensitive to 1st and 2nd generation TKIs, sortilin overexpression increases the expression of these histones and decreases cell proliferation. As histones are actively involved in chromatin packaging and genome integrity, these results suggest that the co-occurring loss of sortilin in EGFR mutant tumors would participate in genome complexity and disease progression.

To better understand the possible involvement of sortilin in genome regulation and its specific interaction at the upstream regions of EGFR target genes, the putative binding of sortilin with other nuclear proteins such as transcription factors is being investigated.

P205

Exploring Sensitivity to Replicative Stress in BRCA1 deficient Triple Negative Breast Cancer

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In most Triple Negative Breast Cancer (TNBC), chemotherapy is the only systemic treatment and sustained remissions in advanced TNBC are rare. About 30% of TNBC tumors are BRCA1 defective. These tumors show Homologous Recombination DNA repair Deficiency (HRD) and increased sensitivity to genotoxic drugs. We hypothesize that BRCA-defective TNBC are highly sensitive to replicative stress inducing drugs, which could open therapeutic perspectives. Our results show that BRCA1-deficient (BRCA1-Def) TNBC, as well as BRCA1-Def ovarian cancer cell lines were more sensitive to Gemcitabine, a well-known replication poison.

Gemcitabine treatment induced massive cell death in BRCA1-Def cells compared with their BRCA1 WT counterpart. Noticeably, increased cell death of BRCA1-Def cells was associated with mediocre replicative stress management. Indeed, up to 80% of BRCA1-Def cells displayed persistent gH2AX staining even 48h after washing off Gemcitabine, whereas at the same time point staining had decreased significantly in the BRCA1-WT. We also noted that Gemcitabine treated BRCA1-Def cells showed a persistent imbalance between RPA32-positive and gH2Ax-positive cells, suggesting unresolve replication stress in these cells and that a substantial fraction of the cells were suffering a replication catastrophe as described by Toledo & al (2012). Furthermore, an important fraction of gH2AX+ cells displayed pan-nuclear staining. Numbers of pan-nuclear gH2AX-positive cells steadily increased over time in Gemcitabine treated BRCA1-Def cells, while they decreased in the BRCA1-WT counterpart. Interestingly, nearly 90% of gH2AX pan-nuclear cells were completely negative for RPA32 and showed a strong BrdU staining in non-denaturing conditions, indicating an important accumulation of single stranded DNA specifically in these cells. Noticeably, an important number of cells with pan-nuclear gH2Ax staining were also negative for RAD51 foci, but were positive for both 53BP1 and FANCD2 foci. The opposite landscape was observed in the BRCA1-WT cells in which gH2AX pan-nuclear cells were RAD51+ and 53BP1- and FANCD2-. These results indicate an acute replicative stress response in the BRCA1-Def context that induces replication forks arrest and possibly collapse leading to massive DNA Double Strand Breaks (DSB). To test this hypothesis, we performed Pulse Field Gel Electrophoresis experiments that clearly showed important accumulation of DSBs in the BRCA1-Def cells 48h after Gemcitabine release. We could show that these massive DSBs did not result from an apoptotic process as cells treated jointly with Gemcitabine and the apoptosis inhibitor Z-VAD-FMK still presented the same amount of DSBs, thus, suggesting DSBs resulted from an accumulation of unprotected ssDNA. Next we tested whether the accumulation of ssDNA resulted from over-resection caused by uncontrolled activity of the exonuclease MRE11 in BRCA-Def cell and applied a joint treatment of Gemcitabine and Mirin. As a matter of fact, inhibition of MRE11 with Mirin resulted in a specific decrease of the number of cells presenting the pan-nuclear gH2AX staining. Finally, we noted that BRCA1-Def cells were more prone to mitotic catastrophes as shown by their accumulation of micronuclei. Noticeably, micro-nuclei showed double positive BrdU and pan nuclear gH2AX staining indicating that they corresponded to fragmentation of nuclei with elevated ssDNA content. We have also been able to show that the hypersensitivity of BRCA1-Def cancers to acute replication stress is reproducible in vivo in PDX models, which displayed a pan-nuclear gH2AX staining profiles, in accordance with their Gemcitabine response status. Hence, we propose that pan-nuclear gH2Ax staining could be a marker of replication catastrophe in treated tumors and could possibly be considered as a biomarker of the response to replicative stress.

P206

Mechanism for inverted-repeat recombination induced by a replication fork barrier

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Replication stress and abundant repetitive sequences have emerged as primary conditions underlying genomic instability in eukaryotes. To gain insight into the mechanism of recombination between repeated sequences in the context of replication stress, we used a prokaryotic Tus/Ter barrier designed to induce transient replication fork stalling near inverted repeats in the budding yeast genome. Our study reveals that the replication fork block stimulates a unique recombination pathway dependent on Rad51 strand invasion and Rad52-Rad59 strand annealing activities, Mph1/Rad5 fork remodelers, Mre11/Exo1/Dna2 resection machineries, Rad1-Rad10 nuclease and DNA polymerase δ. Physical analysis of the replication-associated recombinants revealed that half are associated with an inversion of sequence between the repeats. Based on our extensive genetic characterization, we propose a model for recombination of closely linked repeats that can robustly generate chromosome rearrangements.

P207

New insights into the mechanisms keeping the main DNA double-break sensor Ku from spreading into chromatin

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The most deleterious lesion of DNA is the DNA double-strand break (DSB). When misrepaired or unrepaired, DSBs can lead to genomic instabilities and ultimately cell death. The first responder to DSB is the Ku complex, a heterodimer formed by the two subunits Ku70 and Ku80 stably interdigitated in a ring-shaped structure. Ku encircles DNA at each broken extremity to protect DNA ends and initiates the repair of the break by a pathway called Non-Homologous End-Joining. In vitro studies showed that multiple Ku molecules slide on DNA from a free end under non-limiting conditions of Ku concentration. Considering that Ku is a highly abundant protein in the cell nucleus, multiple Ku heterodimers would be expected to slide similarly onto chromatin from DNA ends in cells. However, previous work established that, in cells, Ku translocation is limited to an average of one molecule per DNA end, implying that an unknown regulatory mechanism operates at DNA ends. Using super-resolution microscopy and cell fractionation assays, we reveal here that a protein involved in DNA repair is responsible for blocking Ku entry intro chromatin. Inhibition of neddylation, a process promoting the ubiquitylation and removal of Ku, further exacerbates Ku accumulation in its absence. Moreover, our results suggest that abnormal Ku loading on DNA affects cellular processes at the break vicinity. Altogether, our findings support a model in which this DNA repair factor plays a major role in limiting Ku entry on chromatin to protect key cellular processes from deleterious consequences of abnormal Ku loading.

P208

TopBP1 condensation as a potential therapeutic target in colon cancer

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Human Topoisomerase IIβ binding protein 1 (TopBP1) is a scaffold protein containing nine BRCA1 carboxyl terminal (BRCT) protein-protein interaction motifs. It is involved in the initiation of DNA replication, DNA repair, transcription regulation, and checkpoint activation. TopBP1 forms nuclear condensates that act as a molecular switch that amplifies ATR activity promoting the activation of checkpoint effector kinase CHK1. ATR activity is crucial for cancer cells to tolerate the intrinsically high level of DNA lesions and impediments that block the progression of replication forks. Thus, ATR inhibitors are currently under clinical trials, often in combination with chemotherapies. In addition, TopBP1 has been involved in resistance to oxaliplatin in gastric cancers. The weak interactions that hold TopBP1 condensates together are highly sensitive to changes in the properties of the cellular milieu, suggesting that small molecules may alter the formation of TopBP1 condensates. To test this hypothesis, we developed here a high throughput screening system for modulators of TopBP1 condensation. We identified FDA-approved drugs that inhibit TopBP1 condensation, block the activation of ATR/CHK1 signaling, and sensitize colon cancer cells to chemotherapeutic agents.

P209

Functional interaction between Ku and its protein partners

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When unrepaired or mis-repaired, DNA double strand breaks (DSBs) can lead to cell death or to cancer-prone chromosomal aberrations. In vertebrates, the majority of DSBs with two ends are repaired by the classical Non-Homologous End-Joining pathway (c-NHEJ). The c-NHEJ reaction is initiated by the detection and protection of the DNA ends by the ring-shaped Ku70-Ku80 heterodimer (Ku). Ku has also a central role for the recruitment of c-NHEJ factors bearing ligase, nuclease, kinase and polymerase activities. Several of these factors possess Ku Binding Motifs (called KBMs) defined as small motifs of 10-20 conserved amino acids that are necessary for the direct interaction of the protein with Ku. At least four classes of KBMs have been defined (A-, X-, P- and B-KBM) and found in several partners of Ku like APLF, WRN, XLF, PAXX, MRI or PolX family DNA polymerases (Frit et al, 2019). Our aim is to study the functional interactions between Ku and some of these partners through the phenotypic characterization of mutants at the interface between Ku and the respective partners. However, Ku is essential in human cells. For the purpose of functional evaluation of mutations in Ku, we have constructed different human cell lines allowing conditional depletion of Ku70 or Ku80 using a degron-based inducible degradation of Ku70 and/or Ku80 subunits. As the two Ku subunits stabilize each other, the latter technique enables for the first time a complete loss of expression of the Ku70/Ku80 heterodimer within a few hours. These cellular tools further allow the expression of a Ku heterodimer mutated on either or even both Ku subunits. Evaluation of the functional impact of the loss of interaction with a Ku partner in several dedicated assays will be presented.

Frit P, Ropars V, Modesti M, Charbonnier JB, Calsou P (2019) Plugged into the Ku-DNA hub: The NHEJ network. Prog Biophys Mol Biol 147: 62-76

P210

Identification of a new form of the PDCD4 tumor suppressor resulting from an alternative splicing in acute myeloid leukemia

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PDCD4 (Programmed cell death 4) is a tumor suppressor involved in many cellular processes. It has been discovered as an induced gene during apoptosis and, later, it has been described as an mRNA translation inhibitor. Thanks to its roles in mRNA translation, PDCD4 inhibits cell proliferation and induces apoptosis, making it a good candidate tumour suppressor.

The different functional domains composing PDCD4 protein are essential for its tumor suppressor activity. Its N-terminal part allows direct binding to mRNA secondary structures, thus inhibiting RNA scanning by the ribosome. This domain is also involved in binding to IRESes (Internal ribosome entry sites) contained in several mRNAs encoding anti-apoptotic proteins. Therefore, PDCD4 inhibits expression of proteins from BCL and IAP families, otherwise involved in cancer cell survival. The rest of PDCD4 protein is composed of a tandem of two MA3 protein-protein interacting domains able to sequester mRNA translation initiation factors such as eIF4A, eIF4G and PABP, thus avoiding the formation of protein complexes, essential for cap-dependent mRNA translation initiation.

PDCD4 is often down-regulated in cancer cells. Studying this protein in AML (acute myeloid leukemia) samples, we identified a new PDCD4 form expressed in 15-20% of AML patients. This form is encoded by a spliced variant of PDCD4 mRNA in which exon 2 is skipped. As the start codon of PDCD4 protein is located in the second exon, with this spliced variant, the protein starts at the next in frame initiator codon in exon 6. This leads to the expression of a shorter PDCD4 protein devoid of its N-terminal part. Losing this important functional domain, the short PDCD4 protein is probably less efficient as a tumor suppressor. We found that the level of this short PDCD4 form correlates with expression of hematopoietic stem cell markers and diminishes in favour of the full-length form during myeloid differentiation. This short PDCD4 protein could also be ineffective on the translational repression of anti-apoptotic factors.

P211

Regulation of RNA Polymerase II transcription during quiescence and cell cycle re-entry

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Quiescence is a reversible state in which cells remain metabolically active without dividing while preserving their capacity to re-enter the cell cycle. The ability to alternate between a quiescent and proliferative state is central to the response to unfavourable environmental conditions of single-cell organisms. In multicellular eukaryotes, quiescent cells are present throughout these organisms and play a role in development and differentiation as well as in tissue homeostasis and renewal. In addition, quiescent cells have also been identified as contributors to tumorigenesis, metastasis, and resistance to therapy. However, despite the importance of this critical state, how cells maintain quiescence and their capacity to proliferate remains an open question.

Quiescent, or G0, cells in different organisms share features such as reductions in cell size, metabolic activity and gene expression. Many of the genes and pathways that modulate G0 are conserved from yeast to human, and studies in the fission yeast*S. pombe* have been critical for our understanding of this state. In fission yeast, quiescence is induced when cells are starved of nitrogen, and cells can persist in this state for long periods of time before condition allow for cell cycle re-entry. We have found that compared to proliferating cells, quiescent cells show changes in their profiles of RNA polymerase (Pol) II binding along transcriptional units. Moreover, G0 cells display distinct modification patterns of the Pol II carboxy-terminal domain (CTD), which points to a specific mode of transcriptional control in quiescence. To investigate the alterations in transcription that result from these differences in Pol II localization and CTD modifications between proliferating and G0 cells, we performed precision run-on sequencing (PRO-seq) to map the localization of active RNA polymerases. We will also assess RNA synthesis by transient transcriptome sequencing (TT-seq) to complement our PRO-seq findings. These analyses will allow us to obtain RNA synthesis and degradation rates as well as calculate the duration and strength of Pol II pausing. In addition, quiescent cells re-entering the cell cycle showed unexpected changes in Pol II occupancy that suggest a coordination between DNA replication and gene expression. Altogether, these studies will provide an understanding of the distinctive regulation of the transcription cycle that may be critical for the maintenance of the quiescent state.
As quiescence is a property of diverse cells in the human body, from stem cells to memory T cells, the results of our study will contribute to our comprehension of human health. They may also have implications for pathological contexts in which quiescent states play critical roles, such as cancer, degenerative diseases and microbial infections.

P212
Ki-67 is not essential for tumourigenesis in melanoma

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The vertebrate nuclear protein Ki-67 is widely used as cell proliferation biomarker in cancer histopathology as its expression levels correlate with the proliferative state of the cells. While widely assumed to have roles in cell proliferation, we have previously shown that this is not the case, but Ki-67 is nevertheless involved in tumor initiation, progression and metastasis in breast cancer models. In this study we analyze the roles of Ki-67 in a mouse melanoma model. We show that, as expected, Ki-67 is dispensable for proliferation of melanoma cells, but Ki-67 mutant melanoma cells can form tumours efficiently in immunocompromised mice. Thus, either low residual Ki-67 levels of mutant cells are sufficient in this tumour type, or Ki-67 expression is dispensable in certain cancers. This may have important implications for understanding differences in mechanisms of tumourigenesis in different tissues and for clinical analyses of tumour samples, where cell proliferation can occur in the absence of Ki-67.

P213

G-quadruplex regulators are potential molecular targets for cancer

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G-quadruplexes (G4s) are single-stranded and dynamic DNA structures alternative to B-DNA. Recent studies suggest that genomic G4s are associated with important nuclear processes, such as transcription and replication, and the growth of cancer cells. Furthermore, G4s are over-represented in promoters of human oncogenes which makes them strong candidates as molecular targets for cancer. Since G4s are over-represented in mammalian genomes, this implies that they must play an important role in genomic regulation. However, they are also known as a source of genomic instability, that can lead to cancer whenever G4s are unresolved. Several nuclear helicases are known to resolve G4s in vitro such as WRN, BLM and RecQ1 helicases. RecQ1 overexpression has been involved in Mutiple Myeloma cancer and it has been shown that cancerous cells become addicted to the presence of the Helicase. I have disrupted several G4 regulators, including RecQ1, in cancerous models using siRNAs. I investigated G4 formation, using a novel approach developed in the lab called G4access that allows to characterize G4s in the context of open chromatin. I complemented these analyses with transcriptome dynamics. I found important features of disruption at promoters and other genomic sites carrying G4 structures. I will discuss my results and their implication for the implication of G4 regulation at promoters in homeostasis and cancer.

P214

The E3 ubiquitin ligase TRIP12 induces the formation of heterochromatin altering gene expression and DNA damage repair independently of its catalytic activity

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TRIP12 is a nuclear HECT-type E3 ubiquitin ligase that is overexpressed in numerous cancers. TRIP12 is involved in several nuclear functions via its catalytic domain. Indeed, TRIP12 is involved in pancreatic carcinogenesis by controlling the stability of PTF1a, an essential transcription factor for pancreatic homeostasis. TRIP12 regulates the chromatin remodelling complexes SWI/SNF and PRC1 by inducing the degradation of BAF57 and ASXL1, respectively. It is also involved in DNA damage repair by targeting RNF168 and parPARP1. Recently, we identified in the N-terminal extremity of TRIP12 an intrinsically disordered region (IDR) which is responsible for TRIP12 interaction with the chromatin. However, the consequences of TRIP12 overexpression observed in cancers on chromatin homeostasis and the involvement of its IDR in this process remain largely unknown.

First, we performed BioID experiments to establish an exhaustive mapping of TRIP12 protein partners. We identified 328 statistically enriched proteins, among them, already known substrates such ASXL1 or BAF57. In silico functional network analysis revealed that TRIP12 partners are massively involved in chromatin organization and histone modifications which are in favor of a global function of TRIP12 on chromatin organization. Second, we evaluated the effects of a TRIP12 overexpression on global chromatin organization by high-resolution microscopy. Interestingly, we observed that TRIP12 expression modifies the organization of the genome by forming chromatin condensates in a dose-dependent manner and independently of its catalytic activity. Using a series of TRIP12 deletion constructs, we identified the IDR as the domain responsible of the chromatin condensates formation. Moreover, we proved that the chromatin condensates are enriched in heterochromatin marks such as HP1a, EZH2, H2AK119Ub and H3K27me3. By using a nanobody coupled-degrader and half-bleach FRAP experiments, we demonstrated that the formation of these chromatin condensates is reversible and governed by polymer-polymer phase separation.

In parallel, we measured the functional consequences of TRIP12 induced-chromatin condensates on biological processes such as cell cycle progression, transcription, DNA replication, genome accessibility and DNA damage response. By live cell microscopy and immunofluorescence, we demonstrated that these chromatin condensates impair cell cycle progression and global transcription but do not affect DNA replication. By ATAC-seq approaches, we demonstrated that the global genome accessibility is drastically modified in response to IDR-expression. Finally, it is well known that chromatin compaction regulates DNA damage repair efficacy. Interestingly, in TRIP12-transfected cells, we observed a drastic inhibition of NHEJ pathway effectors (53BP1 and MDC1) after irradiation in a dose dependent manner and independently of its catalytic activity.

Altogether, our results reveal a new dynamic role for TRIP12 on heterochromatin homeostasis independently of its catalytic activity and through its IDR which alters major biological processes such as transcription and DNA damage pathways. Therefore, TRIP12 overexpression could dramatically modify the genome organisation/expression of cancer cells and sensitize them to DNA damage-inducing chemotherapies.

P215

Evolutionary gene age is linked to variability and chromatin 3D structure in cell differentiation and cancer

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The human genome is composed of genes that appeared at different evolutionary ages for 3.5 billion years. Those genes have progressively been integrated across time, they acquired new functions and made species genomes more and more sophisticated. Evolutionary scientists have been able to precisely estimate current human gene ages by studying duplication events through time. Genes of specific age classes were found to preferentially interact in protein-protein networks. Whether genes with the same evolutionary age are associated within the genome, have similar expression or share a same chromatin structure is still not well characterized.

Inspired by the atavistic theory of cancer, which relates malignancy to the expression of evolutionary ancient phenotypes, we investigate whether cell differentiation and cancer can alter the associations between gene age and other (epi)genomic characteristics.

We therefore investigate whether genes with different evolutionary ages could show specific epigenome properties, expression regulation, variability and location in 3D chromatin structures that can be potentially altered in cancer and during differentiation.

In our preliminary results, using data integration and network approaches on published epigenomic datasets, we identify specific characteristics of genes of specific ages that are lost during differentiation and reacquired during oncogenic transformation.

P216

Chromatin accessibility dynamics during DNA Double-Strand break repair

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DNA Double-Strand Breaks (DSB) are the most deleterious DNA lesions and many human diseases associates with DSB repair defects. In eukaryotes, DNA is organized into chromatin, which tightly regulates its accessibility and therefore impacts all aspects of DNA metabolism, including DSB repair. Indeed, chromatin can be extensively modified following DSB, allowing for the recruitment of specific factors involved in the DNA Damage response (DDR). Furthermore, the chromatin context which is present before damage occurs is crucial to determine how a given DSB will be repaired.

In order to understand how chromatin structure is modified during DSB repair, we apply high resolution next-generation sequencing methods such as ChIP-seq or ATAC-seq using a DSB induction system generating multiple sequence-specific DSB in human cells (named DIvA for DSB Induced via AsiSI). We observed an increased accessibility at the vicinity of enzymatically-induced DSB. Furthermore, this increased accessibility shows striking similarity with the binding profiles for ssDNA binding protein such as RAD51, suggesting a link between DNA end resection and local chromatin accessibility. We are currently applying single-cell ATAC-seq methods in order to characterize the extent of chromatin accessibility and potentially end resection at the level of individual DSBs in single cells.

P217

Impact of single-nucleotide polymorphisms associated with increased lung cancer risk on 3D genome organization and gene expression

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Genome-wide association studies (GWAS) on large cohorts of patients and healthy individuals have identified numerous statistical associations between single-nucleotide polymorphisms (SNPs) and various diseases, including many cancers. However, more than 90% of SNPs that possess disease-associated variants are located in non-coding regions of the genome. Therefore, these studies rarely provide clues about the functional mechanisms underlying this statistical association and the increased risk of developing cancers. One possible explanation is that some genetic susceptibility sites, and their immediate genomic environment, may contribute by their mutation to a change in the 3D spatial organization of the genome, in particular at the borders of "Topologically-Associating Domains" (TADs), in the cell type(s) affected by cancer. This change may in turn induce a pathological deregulation of gene expression. Our recent bioinformatics analyses strengthen this hypothesis by showing that, for some cancers, SNPs that possess disease-associated variants are over-represented at TAD borders (Jablonski *et al.*, 2022 *Human Genomics* 16:2).

We have identified 157 SNPs associated with human lung cancer (Experimental Factor Ontology: EFO_0001071: Lung cancer carcinoma; EFO_0000571: Lung adenocarcinoma; EFO_0000708: Squamous cell lung cancer) that are located in, or near (less than 20kb), TAD borders. Among these 157 SNPs, 44 (28%) are located in a 7.68 Mb region on chromosome 6 (hg38, chr6:25684378-33360927). We are focusing specifically on two SNPs of this region (rs-13218875 and rs-3131856), both located in TAD borders, for which the disease-associated variant is linked to a very high risk of developing lung cancers. These two SNP variants are indeed very infrequently found in the population (Minor Allele Frequency MAF<5%) but are nevertheless strongly associated with the risk of developing lung cancer (Odds Ratio OR>1.13 for the above-mentioned EFO). Using the 3C-qPCR method, we are characterizing the exact position of the TAD borders relative to these SNPs in the IMR-90 cell line (human fetal lung fibroblasts) and we are also very accurately quantifying their ability to prevent inter-TADs contacts. We will then use the CRISPR/Cas9 technology to insert in the IMR-90 cells the disease-associated variants of these two genomic sites, and their immediate genomic environment, in order to assess the consequences of these variants on 3D chromatin organization, inter-TAD contact frequencies and gene expression. Our results should thus provide new insights to better understand how the disease-associated variants of SNPs might impact gene expression by altering chromatin 3D organization and how this might in turn favors genetic susceptibility to lung cancer, and possibly other cancers.

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

P301

Sensitizing the tumor microenvironment to immune checkpoint therapy trough monoclonal antibody-based therapeutic combinations in pancreatic cancer

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Pancreatic duct adenocarcinoma (PDAC) is one of the most lethal solid tumors, with an extremely unfavorable prognosis. The complex tumor microenvironment (TME) is responsible for the failure of many clinical trials including combination chemotherapies, targeted therapies or immunotherapies and there is a real need to develop new effective clinical strategies against this disease. This TME is characterized by an extremely low ratio of neoplastic to stromal tissue (< 20%). Cancer-associated fibroblasts (CAFs) make up the vast majority of this stroma and constitute an heterogeneous population with essentially pro-tumor characteristics. Moreover, PDAC tumors are poorly infiltrated by T cells, and the majority of immune cells present at the tumor site, such as macrophages (type 2), MDSCs and regulatory T cells (Tregs), are immunosuppressive.

This cellular context leads to the failure of clinical trials using immune checkpoint inhibitors in PDAC. The objective of this project is to develop a combinatorial approach using a monoclonal antibody that targets the microenvironment combined with interleukin-15 (IL15) or with conventional chemotherapies (Gemcitabine or Folfirinox) in order to reactivate the tumor microenvironment and obtain higher response rates to the immune checkpoint inhibitor programmed cell death 1 (anti-PD-1). We thus develop three-dimensional in vitro spheroid models 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. Different 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, also described in patients, are one of the trademarks of pancreatic cancer.

In parallel we have shown that, upon 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 that IL15 modify 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 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 steps will be to find the best synergistic mechanisms in our combination therapies based on monoclonal antibodies or IL15 combined with different chemotherapies (FOLFIRINOX, Gemcitabine) to promote and increase the infiltration of immune effector cells and sensitize the PDAC microenvironment to anti-PD1 treatments.

P302

Evaluation of a Metformin treatment on CRC cell lines and on a patients' cohort exhibiting different stages

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According to the WHO, colorectal cancer (CRC) is the 3rd most frequently diagnosed cancer in men after lung and prostate cancer, and the second most common after breast cancer for women. Indeed, despite improved diagnostic and screening tools, CRC is still deadly: its incidence has even recently increased in younger people (under 50) in high-income countries, and often recurs, even when diagnosed at early stages. It develops in 5 stages according to the TNM (Tumor Node Metastasis) classification (stages 0 to IV). In stage 1, only the mucosa is affected, whereas the muscularis is affected in stage 2, the stage 3 corresponds to lymph nodes invasion and stage 4 is characterized by the presence of metastases.

The epithelial-mesenchymal transition (EMT) is one of the supporting principles for the spread of metastasis. It is mainly recognized by the increase in mesenchymal markers and cell movement and is a critical step occurring during embryonic development and cancer cell spread. Loss of epithelial markers such as E-cadherin enables EMT and correlates with CRC aggressiveness (1). So, new therapies able to counteract this mechanism are needed. Metformin is an antidiabetic drug used in the treatment of type 2 diabetes. EMT inhibitory effect of metformin has been studied in several tumors, such as gastric cancer; however, its effects on colorectal cancer remains unknown. (2)

The aim of this study is to examine the effects of Metformin on the inhibition of EMT-related genes and on migration and invasion of the colorectal cancer cell line (HCT-116 and SW-620) as well as on a cohort of 23 patients, belonging to different tumors' stages. We focused on E-cadherin and Sortilin expressions, the last one being described in our lab as a poor prognosis marker in CRC (3). In order to study the effect of glucose on metformin-mediated EMT inhibition in vitro, all experiments were performed in two different glucose concentrated media, similar to fasting blood glucose (7.8 mM) and hyperglycemic conditions (17.5 mM). For the ex vivo experiments, the patient cohort consists of stage 1, 2 and 3 patients with CRC or with CRC combined with diabetes-Metformin treatment.

First results seem to show that Metformin has a beneficial effect especially preventing the evolution of early stages of colorectal cancer. Indeed, we were able to observe, on cellular models, a reduced cleavage of E-cadherin (that occurs during EMT) upon Metformin treatment in initial stages, as well as a weak Sortilin expression. In accordance, the migration capacities of cells (scratch test) were also reduced, independently of glucose concentration. Similarly, the effect of Metformin evaluated ex vivo on patients tumors seems to be beneficial in early-stage of CRC. These results need to be confirmed with further experiments.

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(3) Blondy S., et al. J Cell Mol Med 2021. doi: 10.1111/jcmm.15752. Epub 2020

P303

Synergistic effect of FOLFIRINOX with an ATR inhibitor on pancreatic tumor cells and its microenvironment

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Pancreatic Ductal Adenocarcinoma (PDAC) is an extremely aggressive disease. There is a clear need of new strategies and new researches to treat and diagnose these patients. Regarding treatments, surgery is possible in only 20% of cases, and the chemotherapeutic molecule Gemcitabine is unfortunately lacking a good response rate. Recently, a new polychemotherapy oxaliplatin-based (FOLFIRINOX), which is a combination of 4 drugs: oxaliplatin, irinotecan, 5-fluorouracil and leucovorin, has been approved. It has showed a significant increase of the overall survival in patients compared to gemcitabine, but associated with more toxicity and still limited efficiency. This resistance to chemotherapy can come from the stroma that represents up to 90% of the tumor mass, therefore the impact of the chemotherapies on the microenvironment can be a key to increase the efficiency of these treatments. Most chemotherapies induce their toxicity by provoking DNA damages and replicative stress, leading to the activation of DNA repair pathways. That is why our research project proposes to find a synergistic association of FOLFIRINOX with a specific inhibitor of DNA damage repair - Ataxia Telangiectasia and Rad3 related inhibitor (ATRi) - to increase the efficiency of the chemotherapy while reducing its toxicity. Moreover, in order to be as close as possible to the tumor models observed in the clinic, the impact of our combination is studied in vitro in 3D co-culture models of tumor cells associated with cells from the microenvironment and more particularly cancer-associated fibroblasts (CAFs). These co-culture models make it possible to study the effectiveness of the FOLFIRINOX association with ATRi on each population as well as the signaling pathways impacted in response to the treatment.

We demonstrated a synergistic effect of the association in vitro (2D and 3D) independently of the KRAS, ATM, TP53, BRCA1/2 mutation status in several pancreatic models (ATCC and derived from PDX) and in co-culture with CAFs. We observed chemoresistance from the CAF and a protection of the tumor cells in co-culture. Higher DNA damage were observed in tumor cells treated with FOLFIRINOX combined with ATRi compared to FOLFIRINOX alone. These results were associated with a decrease of DNA damage repair pathways leading to apoptosis. In vivo, the association FOLFIRINOX with ATRi significantly inhibits the tumor growth compared to each treatment alone and no toxicity was observed in both immunodeficient (PDX models) and immunocompetent orthotopic model. Furthermore, we were able to observe more immune infiltration in tumors treated with the association compared to the chemotherapy alone. The localization and the nature of infiltrating immune cells are now under investigation.

To conclude, our work shows that FOLFIRINOX associated with an ATRi is highly synergistic in vitro in our co-culture models and in vivo where it also induces more apoptosis, less DNA damage repair and more immune infiltrate. These results show that this combination could be a new therapeutic strategy in order to increase the survival of patients with PDAC for whom only a few solutions have been found until now and that is why this cancer represents a major challenge of public health today.

P304

Allosteric Modulators of Cyclin Dependent Kinases for Cancer Therapeutics

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Protein kinases (PKs) constitute one of the major classes of pharmacological targets for cancer therapeutics. Although a large number of ATP-pocket binding inhibitors have been developed, they suffer limitations, including poor selectivity and emergence of resistance. Efforts to develop new classes of drugs targeting the conformational plasticity or essential interactions with partners are believed to offer promising perspectives for therapeutics (Cohen & Alessi ACS Chem Biol 2013; Fang et al. ACS Chem Biol 2013 ; Guarnera & Berezovsky Curr. Op.Struct.Biol. 2016; Tong & Seeliger ACS Chem Biol 2015; Leroux & Biondi Trends Biochem Sci 2020).

Cyclin-dependent kinases (CDKs) are frequently hyperactivated in human cancers and constitute attractive pharmacological targets, for which several ATP-competitive inhibitors have recently been approved by the FDA for clinical use (Abemaciclib, Ribociclib, Palbociclib). However these drugs are not effective in monotherapy and have been reported to induce compensation and emergence of resistance (Herrera-Abreu et al. Cancer Res. 2016; Haines et al. Oncotarget 2018). With the aim of targeting the conformational activation of CDKs, we have developed a fluorescent conformational biosensor technology that discriminates against ATP-pocket binding inhibitors, which we have implemented to screen several libraries of small molecules and successfully identify allosteric modulators of CDK2, CDK4 and CDK5. CDKCONF biosensors are engineered through incorporation of environmentally-sensitive dyes at strategic positions within the CDK scaffold, thereby reporting on conformational changes of the activation loop (Prével et al. Eur. J.Med.Chem.2014, Pellerano et al. Biotechnol.J. 2017, Peyresstre et al. Frontiers Chem. 2020).

Thanks to CDKCONF technology, we have identified CDK2 inhibitors that dock onto the T-loop of CDK2 and compete with substrate binding, block cells in S phase and G2 and potently inhibit cancer cell proliferation (Pellerano et al 2017). We have also identified CDK4 inhibitors that inhibit proliferation of a large panel of cancer cell lines (NCI60 panel) with IC50 values in the same range as Abemaciclib, prevent RB phosphorylation, and inhibit a signaling pathway involving CDK4 in G2/M, rather than in G1 phase like ATP inhibitors of CDK4 (SATT maturation project; MATWIN Award 2019). More recently, we identified new families of CDK5 inhibitors, which we have characterized in U87 glioblastoma cells and in A549 lung cancer cells. Two of these, ethaverine and papaverine, are cases of drug repositioning, since these drugs were previously identified as phosphodiesterase inhibitors. Cell extract thermodenaturation (CETSA) studies confirmed target engagement in cells and their reduced efficacy observed following CDK5 siRNA treatment further confirmed their selectivity. Whilst these compounds inhibit proliferation of A549 cells with IC50s in the same range as the ATP-competitive CDK inhibitor Roscovitine, we further found they inhibited migration. Moreover, combination of these allosteric drugs with Roscovitine lead to enhanced, additive inhibition of proliferation (Laure et al. Cancers, submitted).

Taken together our studies highlight the originality and relevance of conformational biosensors for identifying highly selective allosteric inhibitors of CDKs. This approach also provides means of repositioning and repurposing drugs for cancer therapeutics. Last but not least our work reveals that combinatorial treatment of allosteric drugs with ATP-competitive inhibitors can enhance inhibition of the same target, thereby reducing the overall concentration of drugs required for treatment and potentially preventing emergence of resistance.

P305

Cancer Stem Cell glycosylation markers: A promising biomarker for prognosis and disease progression

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Lung cancer is one of the leading causes of cancer deaths worldwide. Despite scientific advances, its diagnosis and the patient management are still difficult due to the lack of biomarkers to predict tumor aggressiveness and the risk of recurrence. This is even pronounced in early stages leading to a recurrence rate up to 15% at 2 years.
However, growing evidence supports that cancer stem cells (CSCs) are responsible for cancer initiation, progression, aggressiveness and also therapeutic resistance. Thus, CSCs might constitute useful prognosis biomarkers to lung cancer patient monitoring and to prevent recurrences.

In this context, this work focuses on specific and early detection of Cancer Stem Cells (CSCs) using a new diagnostic and prognostic approach based on the recognition of specific CSCs glycosylation patterns. An immunohistochemistry (IHC) kit, called LungSTEM and based on a mix of biotinylated plants was developed by Carcidiag Biotechnologies. This mix specifically detects CSCs through (over)expressed glycan patterns. This first study aims to demonstrate the potential of this new tool for efficiently detecting and sorting CSCs from a heterogeneous tumor cell population. In a second step, we analyzed the clinical significance of the lung STEM kit from a Non-Small cell Lung Cancer patient cohort by establishing the link between the detection of CSC with Immunohistochemistry in Tissue microarray and overall survival. So, we demonstrated through two different cell sortings (Magnetic or fluorescence-activated cell sorting - MACS and FACS) on A549 cell line that the fraction sorted using the Mix is significantly enriched in CSCs compared to CD133 sorted-fraction.

We also evaluated the capacity of Mix sorted-CSCs to induce tumorigenicity in collaboration with the Functional Genomics Institute (IGF-CNRS of Montpellier). Finally, the detection of CSCs using the mix could be correlated with the patient survival from a retrospective study based on 235 patients from a cohort from Lyon civil hospices.

Altogether these results confirm the clinical significance of this specific mix as a biomarker for detecting CSCs and predicting tumor aggressiveness at early stages. These promising results suggest that this new kit based on a specific mix able to detect CSCs biomarkers is of prime interest to improve patient management determining the prognosis value regarding therapeutic response in lung cancer patients.

P306

Role of Furin in Colon Cancer Stem Cells Phenotype in KRAS and BRAF-Mutated Colon Tumors

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Found in respectively 50% and 10% of colorectal cancer (CRC) patients, KRAS and BRAF gene-inactivating mutations mediate colon cancer initiation through cancer stem cells (CSCs) activation. CSCs are involved in tumor progression, metastasis induction, chemotherapy resistance, and tumor relapse. **Proprotein convertases (PCs)** are known to regulate the malignant phenotype of colon cancer cells by different mechanisms, but their effects on cancer stem cells (CSCs) have been less widely investigated.

Here, we studied the P**Cs expression in colon CSCs, and the effect of their inhibition**by using general PC inhibitors α1-PDX or decanoyl-RVKR-chloromethylketone (CMK) on colon CSCs markers, growth, survival, and invasion in three-dimensional spheroid cultures.

Moreover, Furin convertase was reported to be a pro-oncogenic driver in KRAS and BRAF-driven colorectal cancer2. We evaluated the**specific repression of Furin in KRAS or BRAF mutant CRC cell lines** and wild-type KRAS and BRAF on the expression of the stemness markers and global PCs activity.

P307

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

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Brain metastases (BM) from colorectal cancer (CRC) are associated with a poor prognosis. Cancer stem cells derived from patients with BM (BM-CSC) from breast and lung cancer have already been described, but those from CRC have not yet been identified. In this study, we identified and characterized BM-CSC from CRC patients (BM-CSC-CRC).

BM-CSC-CRCs were obtained by mechanical dissociation of patient's tumor and cancer stem cells selection by appropriate culture conditions. BM-CSC-CRCs were characterized in vitro and in vivo by performing clonogenic and limiting-dilution assays, as well as immunofluorescence and Western-blot analyses. A chicken chorioallantoic membrane (CAM) model and xenograft experiments using BALB/c-nude mice were performed to study BM-CSC-CRCs phenotype.

Four patient-derived CSC (BM-CSC-CRC1 to BM-CSC-CRC4) were obtained. These cells formed metaspheres and contained tumor- initiating cells with self-renewal properties. The BM-CSC-CRC lines expressed stem cell surface markers (CD44v6, CD44, ALDH1, CD133, Lgr5 and EpCAM) in serum-free media and CRC markers (CK19, CK20 and CDX-2) in fetal bovine serum-enriched media. The CAM model demonstrated invasive and migratory capabilities of these BM-CSC-CRCs. The phenotype of the tumor mice intracranial xenotransplantation of BM-CSC-CRCs tumorspheres adequately recapitulated the original patient BM.

For the first time we successfully characterized BM-CSC from CRC patients. These promising BM-CSC-CRC cell lines will be a useful model to understand dissemination of CSC in brain and identify future therapeutic targets.

P308

Therapeutic efficiency of Fc-engineered human anti-cathepsin D antibody in mono and combotherapy in triple negative breast cancer

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Triple-negative breast cancer (TNBC) defined by the absence of estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 overexpression and/or amplification, accounts for 15-20% of all breast cancer (BC) cases. Resistance to systemic treatment is common in TNBC so new treatments are required. The aspartyl protease cathepsin D (cath-D), an independent marker of poor prognosis in BC, is overexpressed and hypersecreted into the tumor microenvironment. Recently our team has shown that cath-D is a tumor-specific extracellular target in TNBC suitable for antibody-based therapy (*Ashraf, Mansouri et al., JITC, 2019, 7:29*) and that the co-expression of cath-D and AR (androgen receptor) is an independent bad prognostic factor for overall survival in TNBC (*Mansouri, Alcaraz et al., Cancers, 2020,12(5):1244*).

The objective of this project is to develop a targeted therapy in TNBC using an optimized anti-cath-D antibody. The therapeutic efficacy of the anti-cath-D F1 antibody was studied in vivo in athymic nude mice (Foxn1nu) xenografted with TNBC (MDA-MB-231) cell line or PDX (Patient-derived xenografts). F1 antibody significantly reduces tumor growth of TNBC cells and PDX in nude mice and exhibits immunomodulatory activity with natural killer cell activation and tumor depletion of myeloid cells (*patent WO/2016/188911; Ashraf, Mansouri et al., JITC, 2019, 7:29*).

The mechanism of action of therapeutic antibodies is known to involve both binding to the target and immune cell recruitment by Fc part (crystallizable fragment). Here we mutated Fc-part F1 (F1Fc+ antibody) to augment its affinity for Fcγ receptors and C1q complement protein leading to increased ADCC (antibody-dependent cellular cytotoxicity), CDC (complement-dependent cytotoxicity) and ADCP (antibody-dependent cellular phagocytosis).

We showed in vitro that F1Fc+ antibody activates degranulation and cytotoxic activity of natural killer (NK) cells and lysis of TNBC cells via the ADCC mechanism. In addition, the therapeutic efficacy of the F1Fc+ antibody was improved over that of the non-mutated Fc F1 antibody in a TNBC model xenografted in nude mice. Recruitment, maturation and activation of NK cells have been analyzed by immunophenotyping in TNBC tumor xenografts. Besides, combination treatment strategies with this optimized antibody are being studied, particularly with chemotherapy, with promising results.

In this study, we showed the benefit of an Fc-improved antibody to target an extracellular protease, the cathepsin D, in TNBC. Based on these encouraging in vivo results, we hope that antibody-based targeting of cath-D may represent an attractive avenue for therapeutic applications in TNBC.

P309

Platinum-based chemotherapy induce inflammation via a non-canonical STING pathway independent on cGAS and interferon production

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Upper Tract Urothelial Carcinomas (UTUC) are extremely aggressive tumors of ureter or renal pelvis. UTUC present less tumor mutational burden and low tumor immune infiltrate compared to bladder cancer. Despite this they are treated with the same protocol than bladder cancer with more than 50% of relapses justifying the need of new therapeutic options. To improve patient care, we suggest stimulating the immune system by platinum-based chemotherapies, Cisplatin-Gemcitabine (CisGem) or Carboplatin-Gemcitabine (CarboGem) in order to potentiate the effect of an anti-PD-L1, the Durvalumab. To that, we conduct an in vitro project that aims to determine if chemotherapies could transform cold tumor into a hot tumor, and if so by which mechanisms?

Using 3 UTUC cell lines (UM-UC-14,UCC3 and UCC17) we have evaluated the cytotoxicity effects of the chemotherapies combinations in 2D and 3D cell cultures. We have assessed their potential (i) to induce DNA damage using image cytometry, (ii) to induce PD-L1 expression using flow cytometry, (iii) to induce immune cell death using ELISA kits, (iv) to activate the cGAS/STING pathway using qPCR and Western-Blot, and finally (v) to attract immune cells by using heterotypic spheroids model (tumoral cells+PBMCs).

Our results demonstrate that CisGem and CarboGem present synergistic effects in UTUC spheroid cultures. These treatments also induce DNA damage pathway demonstrated by an increase of γH2AX, P-ATM, P-CHK1 and P-CHK2 positive cells. We found an increase of PD-L1 membrane expression after treatment in UTUC cell lines. RNA Seq analyse indicates that the major pathways upregulated by these combinations are inflammatory pathways (TNF-α signaling via NFκB, interferon alpha response, inflammatory response, interferon gamma response) without induction of IFN I. We could observe an immune cell death induction demonstrated by an increase of ATP and HMGB1 release and calreticulin translocation.And finally, we showed STING pathway activation, independent of cGAS or interferon production but dependent on ATM and ATF3 . On the other hand, we have created heterotypic spheroids composed of UTUC cancer cell lines and immune cells, and we showed that CisGem and CarboGem increase the percentage of immune infiltration in the heterotypic tumor spheroids and enhance chemotherapy effect.

These results indicate that the combination of platinum salts + gemcitabine induces inflammatory pathways via a non-canonical STING pathway independent on cGAS and interferon production. These combinations induce an upregulation of PD-L1 expression and allow immune cell attraction at the tumor. All these data support that a combination CisGem or CarboGem with an anti-PD-L1 will be efficient for UTUC patients.

P310

Repression of protein maturation inhibits PD-1 expression and enhances tumor clearance and tils: virtual ligand screen-ing and drug repurposing approach

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Immune checkpoints, such as programmed death-1 (PD-1) are involved in the regulation of T cell effector function, are now exploited for the treatment of various solid and hematologic cancer. However, although therapies targeting PD-1 were clinically effective in various preclinical models and cancer patients, several patients with solid tumors are still refractory to these treatments. Indeed, solid tumors evade anti-cancer immune control by establishing immune privileged niches within the tumor microenvironment that reduce proliferation, viability, and/or activity of cytotoxic T lymphocytes (CTL). Interestingly, a wide range of proteins involved in the expression of PD-1 and CTL function require proteolytic activation by the proprotein convertases (known as PCs). Using general protein-based inhibitors of the PCs we previously reported the implication of the PCs in PD-1 expression and T cell exhaustion. In the current study we identified small molecule convertase inhibitors through virtual ligand screening and drug repurposing approach that inhibit the activity of the convertases. Using organoids culture, we found that some of these molecules were able to repress cancer cells viability, proliferation and invasion. These molecules were also able to mediate potent repression of PD-1 expression on T cells activated by CD3. In vivo, subcutaneous inoculation of mice with syngeneic cancer cells revealed their anti-tumoral efficacy that associated increased intratumoral T cell infiltration in the developed tumors. The treated mice showed improved overall survival while compared to controls. These and other findings highlight the potential use of PC inhibitors to increase the anti-tumoral immune response and could act as novel immunotherapeutic approach in cancer used alone or as adjunct therapy.

P311
Programming lactic acid bacteria for cancer therapy

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

Our work aimed to engineer a colorectal and probiotic *Lactobacillus* strain as a new chassis for cold tumor therapy. As precision engineering of LAB (lactic acid bacteria) is currently limited by the lack of tools enabling reliable control of gene expression, a part of this project aims at building a collection of well-characterized regulatory elements to control transcription, translation and secretion levels. In parallel, We are optimizing the production of cytotoxic (Azurin, cytolysine A) and immunomodulatory proteins (VHHa-PDL1) in our chassis *Lactobacillus gasseri.*Ultimately, bacterial therapeutic activity will be controlled by biosensors responding to signals from the tumor microenvironment and external trigger combined together in biological logic gates. In order to test, improve and validate our recombinant strains, we combining in vitro spheroid-based screening with animal models infection and therapeutic tests.

P312

Foldamers mimicking the B-DNA surface as a new class of Topoisomerase I inhibitors

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DNA mimicry has been the subject of intensive research and resulted in the development of DNA analogues such as PNAs and LNAs. However, there are also examples of proteins that imitate the substrates of DNA binding proteins. These proteins are involved in multiple cellular mechanisms such as chromatin assembly, DNA repair, transcription or recombination. Their mimetic nature relies on structural and/or charge distribution analogies with respect to the DNA double helix, allowing them to interfere with other DNA-binding proteins and modulate the biological processes in which they are involved.

We previously characterized a new class of DNA-surface mimic molecules constituted by repetitions of dimeric units of 8-amino-2-quinolinecarboxylic acid (Q) and 8-aminomethyl-2-quinolinecarboxylic acid (mQ) whose helical folding can mimic a B-DNA molecule (Nat Chem. 2018;10:511-518). Similarly to B-DNA, these mimics displayed both a minor and a major groove, their width being able to be modulated depending on the dimers and of the nature of their side chains. We first investigated the potential effects of these mimics on the activity of a series of DNA interacting enzymes in vitro. We found that they could inhibit, in a relative selective manner, the catalytic activity of DNA topoisomerase I (Top1), whereas they had no effect on the activity of DNA polymerases or DNAses. We also showed that inhibition of Top1-mediated relaxation of supercoiled DNA plasmid increased with the length of the DNA mimics and that the extent of inhibition could be modulated by modifying the nature and the charge of DNA mimics' side chains.

Top1 is the main target of camptothecin (CPT) derivatives such as topotecan or irinotecan, that are routinely used in the clinic for the treatment of colon, ovarian and lung cancers. They poison Top1 by inhibiting the religation step of the reaction, leading to the generation of lethal replication-mediated DNA double-strand breaks. Using specific DNA oligonucleotides, we found that, conversely to CPT, DNA mimics inhibited the cleavage step of the Top1 reaction in vitro, probably by preventing the binding of the enzyme to its DNA substrate, a mechanism referred to as catalytic inhibition. We also found that co-incubation of DNA mimics with CPT had an additive effect on the inhibition of Top1-mediated relaxation of supercoiled DNA, further confirming a differential mechanism of action.

Because transfection of DNA mimics could inhibit the growth of various cancer cell lines, we further investigated whether Top1 could play a role in this cytotoxicity. We found that Top1 knock-down in OVCAR4 ovarian cancer cells resulted in decreased sensitivity to the (mQQ4)8 DNA mimic as compared to OVCAR4 control cells, suggesting that Top1 is a target of DNA mimics in cells and is involved in their cytotoxic effects. We showed that, conversely to CPT, transfection of HCT116 cells with the (mQQ4)8 DNA mimic was not associated with an increase in γH2AX, suggesting that Top1 inhibition was not due to the trapping of Top1 complexes. This is in accordance with preliminary results showing that in HeLa cells, (mQQ4)8 had no effect on the level of Top1-DNA complexes induced by CPT. Interestingly, we also showed that several SN38-resistant HCT116 cell clones characterized by specific Top1 mutations were still sensitive to the (mQQ4)8 DNA mimic.

Together our results show that DNA mimics can be considered as a new class of antiproliferative agents targeting Top1 via a catalytic inhibition of the enzyme, a mechanism that differs from Top1 poisons. Further studies would be required to identify the DNA mimics structural features that are essential for Top1 inhibition in order to generate more potent derivatives that could be used as potential alternatives to counteract resistance to CPT derivatives used in the clinic.

P313

TREM1+ CD163+ myeloid cells are potent immunosuppressive cells and associate with poor survival in human liver cancer

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Background. Hepatocellular carcinoma (HCC) is an inflammation-associated cancer and is among the deadliest cancers worldwide. Despite well-known risk factors, i.e. chronic viral infection with hepatitis B virus (HBV) primarily in Asia and HCV in western countries, excessive alcohol consumption and the metabolic syndrome-associated non-alcoholic steatohepatitis (NASH), HCC is diagnosed late in most patients (Llovet et al., 2021). The landscape of clinical trials for the treatment of advanced HCC has recently shifted to the field of immunotherapy and therapeutic options now includes the immune checkpoint inhibitors (ICI) nivolumab and pembrolizumab, and since 2020, the combination therapies (atezolizumab/bevacizumab and nivolumab/ipilimumab) (Finn et al., 2020). However, despite significant therapeutic advance with ICI, ~75% of patients do not respond to these immunotherapies for unclear reasons (Giraud et al., 2021). Recently, a meta-analysis of three randomized phase III clinical trials administering ICI to patients with advanced HCC showed a superior efficacy of immunotherapies in virally-infected patients compared to NASH-affected patients with HCC (Pfister et al., 2021). This suggests that the tumor microenvironment (TME) of HCC is an important determinant of therapeutic success and highlight the urgent need to further explore human liver-specific immunity towards the identification of theranostic immune biomarkers for patients' stratification and novel immunotherapies.

Expansion of suppressive myeloid cells is a hallmark of chronic inflammation and cancer. Their heterogeneity in HCC is not fully resolved and might underlie immunotherapy resistance. Several studies have employed single cell analyses, including single cell RNA sequencing (scRNA-seq) and mass cytometry, to characterize the cellular landscapes of HCC. However, the bulk of these studies included all liver cells, limiting the granularity of the analysis.

Objective. In this study, we setup to discriminate and localize human liver-specific innate immunity cells to improve the stratification and the treatment of patients with HCC.

Methods. Here we implemented scRNA-seq on purified CD45+panTCRαβ-CD19- cells freshly isolated from tumoral and juxta-tumoral tissues from 10 patients with HCC of different etiologies, and performed spatial transcriptomics (10x Genomics) to map their localization. We validated our results by multiplex immunofluorescence, by functional analyses performed on*ex-vivo* FACS-sorted cells co-cultures, on a mouse model of HCC, and by computational analyses of published HCC data sets.

Results. We report a high-resolution atlas of innate immunity cells (around 100,000 transcriptomes) in HCC and unravel a strong myeloid bias in NK cell differentiation and a remarkable myeloid cell heterogeneity. In particular, we identify three phenotypically distinct myeloid-derived suppressor cells (MDSC) populations, including polymorphonuclear MDSC, monocytic MDSC and a distinct population expressing a variety of myeloid lineage-affiliated genes and selectively marked by elevated expression of triggering receptor expressed by myeloid cells-1 (TREM1) in conjunction with CD163. We show that TREM1+CD163+ MDSC are the most potent immunosuppressive subset *ex vivo* and expand in models of liver inflammation and fibrosis *in vivo*. A specific gene signature defining TREM1+CD163+ MDSC correlate with poor patient survival in HCC and response to immune checkpoint blockade in different cancers. Accordingly, TREM1+CD163+ MDSC correlate with signatures of other myeloid cells with pro-tumoral activities, including TREM2+SPP1+ tumor-associated macrophages and VSIG4+ monocytic DCs. We further show that TREM1+CD163+ MDSC are high producers of TGFβ and spatially localize at liver fibrotic lesions in close association with scar-associated profibrogenic fibroblasts.

Conclusion. Collectively, our data support for a myeloid subset-targeted immunotherapies to treat HCC.

P314

Profiling the Non-Small Cell Lung Cancer (NSCLC) microenvironment across disease stages

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Lung cancer is the leading cause of cancer death worldwide, with a survival rate of less than 50% after 5 years. Despite great progress in cancer therapeutics, we do not know why some patients react well to cancer therapies while others do not and progress or undergo recurrence. Given cancer's complex and heterogeneous nature, effective early diagnosis and treatment(s) should be tailored from a personalized medicine perspective. Here, we propose a computational immunology approach using bulk transcriptomics to identify patient tumor phenotypic profiles (which include genes, transcription factors, specific cell types, and their spatial pattern in the microenvironment) associated with the disease stage.

We performed computational analyses (including differential expression, pathway, cell-type deconvolution, weighted gene correlation network analysis, independent component analysis, and transcription factor estimation) on a bulk RNAseq dataset composed of 62 non-small cell lung cancer (NSCLC) primary tumor samples at varying stages of the disease associated with comprehensive clinical information about the patients.

Cell-type deconvolution methods were employed to identify the presence of specific cell types in each stage of the disease. An unsupervised clustering analysis based on these scores identified groups of high and low immune infiltration (mostly high B and T cells vs. high cancer and NK cells). Estimation of transcription factors activities based on the expression of their targets allowed us to identify a subgroup of regulators related to immune response and tumor aggressiveness inside each cluster. Our results indicate that patients can be either grouped by TFs that dominate their transcriptome implying specific processes, such as high or low proliferation, in close association to stage, or they can be grouped by immune infiltration characteristics that are not so clearly associated with stage.

These findings can give us new insights toward better patient stratification and potentially suggest which early-stage patients are more likely to progress. Through this analysis, we have started identifying immunological and transcriptomics profiles in LUAD that can be used to determine which patients can elicit an immune response, potentially aided by immune checkpoint blockers, while others are progressing toward immune evasion. Our results are currently being validated in an independent cohort.

P315

Unravelling the role of early dissemination in colorectal cancer

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Context: Most of the mortality attributed to cancer is due to metastasis; however, the mechanisms involved remain poorly understood. The literature mainly describes the late stages of tumorigenesis, ignoring early dissemination particularly in colorectal cancer. To overcome this lack, we have generated an inducible mouse model in which specifically in the intestinal epithelium the cells are fluorescently labeled with TdTomato, and simultaneously intestinal tumorigenesis is induced by a partial deletion of APC, known as the gatekeeper gene in CRC. This mouse model, thanks to the expression of tdTomato allowed us to detect early disseminated cells (eDTC) in the liver, the main distant organ for colorectal cancer metastases. The impact of eDTCs in the liver was assessed using CyTOF/hyperion and immunolabelling and we could detect a strong enrichment of macrophages and neutrophils suggesting a microenvironmental remodelling. Moreover, at the systemic level, a cytokine profile (M-SCF, SDF1, CXCL2, Timp1) was observed, which could be involved in this massive enrichment of myeloid cells (macrophages).

Objectives: Our aims are to decipher the mechanisms explaining the enrichment of macrophages in response to early dissemination and to validate some of our results, on blood samples from patients with intestinal polyps.

Materials and methods: Detection of eDTC has been possible by using imunolabelling. Identification of enriched macrophage subpopulations was first performed by RT-qPCR on livers mice and further characterization have then been performed by single cell RNA-sequencing. To identify genes and cytokines that are essential for macrophage subset enriched in the liver, co-culture assay of intestinal polyp cells purified from mice with HoxB8-derived macrophages were carried out; the macrophage phenotype was determined by RT-qPCR. The cytokine profile of patient plasmas was performed using an ELISA assay.

Results: The qPCR results obtained on the livers of APC mutated mice suggest an overall polarization towards an M2-like immunosuppressive phenotype. HoxB8-derived macrophages cultured for 2 days in the presence of intestinal polyps adopt a state of polarization mimicking macrophages in the liver of mice with mutated APC. Plasma TIMP-1 and M-CSF levels were elevated in patients with adenomas compared to healthy donors; however, SDF-1 and CXCL2 levels in plasma were patient dependent.

Conclusion: this project demonstrated for the very first time that not only tumor dissemination occurs much earlier than previously believed in intestinal tumorigenesis but also that at very early stages, concomitant with the presence of early disseminated cells in the liver, drastic remodeling is induced, including strong macrophage enrichment. In addition, macrophages tend to have an M2-like immunosuppressive, which strongly suggests that this early dissemination has a causal role in the establishment of a premetastatic niche to promote late colonization.

P316

Sensitization of pancreatic cancer to radiotherapy and chemotherapy by proprotein convertase inhibition.

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The **pancreatic ductal adenocarcinoma (PDAC)** is a major health issue with a 5-year relative survival rate of only 6%. This aggressiveness is mainly due to a late diagnosis and a lack of curative option with a resistance to most conventional treatment (chemotherapy and radiotherapy).

Interestingly, the activation of the majority of the signaling pathways involved in the initiation and progression of pancreatic cancer is mediated by various protein precursors that require proteolytic activation by a family of nine enzymes called **proprotein convertases (PCs)**. Consequently, deregulation in the expression and activity of proprotein convertases (PCs) is associated with pathological conditions and they are known to behave as oncogenes in various types of cancer.

Indeed, we have found that four members of PC family are predominantly expressed in PDAC tissues while compared to noncancerous tissues. Therefore, due to their aberrant activity in PDAC, the inhibition of PCs, by siRNA or chemical inhibitors, in combination with conventional treatments have been tested in vitro on 2D adherent cancer cells as well as in 3D pancreatic tumorsphere. In both cases, PCs inhibition sensitizes pancreatic cancer cells to current chemotherapy and radiotherapy. The combination treatment shows a reduction of the different tumorigenic properties of pancreatic cancer, including cell growth, motility and survival.

These and other findings highlight that PCs inhibition, in combination with the current first line treatment, might be beneficial for treatment of PDAC and could improve the current therapeutic status of pancreatic cancer patients.

P317

BTC, Biological Tissue Collection of pre-clinical models to rationalize biomedical research

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Since its creation in 2008, the RHEM core facility (Réseau d'Histologie Expérimentale de Montpellier) has ensured, through an original software developed under contract (Laboratory Information Management System, LIMS), the traceability of all samples from preclinical models generated by Montpellier researchers (> 100 000 paraffin blocks). By improving this software and developing a e-tracking system, the RHEM wants to create an experimental biobank (i.e., the "Biological Tissue Collection" or BTC) similar to human biobanks. The BTC consists in a web portal, which can be used by the whole international scientific community to consult the database. Using this portal, any scientist will be able to interrogate the BTC according to various criteria (species, genotype, mutated gene, age, sex, organ) and to request histological slides from the selected blocks.

This BTC aims to enable researchers to (1) promote ethical and responsible research, in line with the 3Rs rule, by replacing animal experiments with the use of histological slides from blocks that have already been generated and grouped together in a valuable biological collection, (2) save time and money by avoiding replicating experiments that have already been carried out, thereby reducing the number of used animals, and (3) encourage scientific collaboration through the sharing of biological material.

This BTC is currently being tested and will open to the public in 2023. Initially, it will allow to query 27% of the tissue database, i.e. more than 30,000 blocks. 99% of those are from rodents representing 365 different genotypes. This portal will in the future new versions to allow the integration of animal models which has received humans/mice cells graft or humans' tissues (Patient Derived Tumor Xenograft models) and also models of treatments induced pathology.

This public sharing tool will provide researchers new opportunities to study biomarker expression in tissues from rodent models of human cancers which are difficult to access or expensive. This BTC will help research teams to improve their general 3Rs strategy.

P318

Helicobacter pylori induces hepatic 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 liver where different strains were found. In mice, *Helicobacter hepaticus* colonizes the liver leading to chronic active hepatitis and hepatocellular carcinoma. However, whether human strains of *H. pylori* can induce liver lesions remains unknown. This study evaluated the consequences of mice infection with different strains of gastric Helicobacters on their liver. In this double-blind study, histopathological analysis of HES-stained paraffin-embedded liver tissue sections were scored for inflammation and other lesions. Mice infected with *H. pylori* were found to develop liver inflammation and steatosis, known precursor lesions of liver carcinogenesis. Understanding the impact of *H. pylori* infection on extra-gastric lesions could help in fine prevent the emergence of other digestive-track related diseases.

P319

The Effect of Benzodiazepine and Benzodiazepine-related Drugs on Survival after Surgery for Colorectal Cancer

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Introduction: Benzodiazepine, usually used for depression, was shown to increase the plasma brain-derived neurotrophic factor (BDNF) that may improve the survival of colorectal cancer (CRC) patients. The aim of this study was to evaluate retrospectively the associations between benzodiazepine and benzodiazepine-related drugs (BZRD) use and overall survival (OS) or disease-free survival (DFS) in patients operated for CRC.

Methods: It was a retrospective cohort study. It included patients who underwent surgery for CRC at Limoges' University Hospital between 2010 and 2019. Data on the characteristics of patients, CRC, comorbidities and drug exposure were collected from the electronic medical records. Patients were divided into two groups, benzodiazepine users and non-users. All patients were followed for five years after surgery for CRC. The outcomes were overall survival (OS) and disease-free survival (DFS). All cases of CRC recurrence were confirmed by computed tomography (CT) scan or magnetic resonance imaging (MRI) and verified by biopsy. Multivariate analysis using the Cox model was performed to adjust various confounding factors (age, sex, body mass index (BMI), tumor site, cancer stage, Charlson comorbidity index, benzodiazepine/BZRD use, propensity score, antipsychotic drugs, antidepressant drugs) and all statistical analyses were done with IBM SPSS Statistics 22.

Results: In total, 512 patients were included in this study. A third of patients were treated with benzodiazepine (33.4%). Univariate survival analysis using the Kaplan-Meier method and comparing benzodiazepine/BZRD users and non-users showed no statistically significant differences in 5-year OS (64.4±4.4% vs. 68.1±3.0% respectively, p = 0.69) and 5-year DFS (53.9±4.4% vs. 55.3±3.1% respectively, p=0.52) in patients operated for CRC. After adjustment to confounding factors, the use of benzodiazepine/BZRD was not associated with OS and DFS. Using further adjustment for propensity score, multivariate analysis provides similar results (aHR=1.10, 95%CI: 0.71-1.73 and aHR=1.01, 95%CI: 0.70-1.46 respectively).

Conclusion: the use of benzodiazepine doesn't seem to be associated with improved survival in patients operated for CRC.

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

P401

Survie après un cancer du sein selon le dépistage organisé ou individuel et les inégalités sociales

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Introduction : La survie nette du cancer du sein a souvent été étudiée selon les inégalités sociales et le dépistage. En effet, de nombreuses études ont mis en évidence que les femmes qui participent à un dépistage ont une survie plus élevée que celles qui n'y participent pas. Toutefois, la plupart des études prennent en compte uniquement la pratique du dépistage organisé (DO). Or, parmi les femmes qui ne réalisent pas le DO, certaines réalisent un dépistage individuel (DI) aussi appelé opportuniste. En France, la pratique du DI reste difficile à identifier et donc à évaluer. L'objectif de cette étude était donc d'identifier la pratique du dépistage individuel et d'estimer la survie nette après un cancer du sein selon la participation au DO, DI ou aucun dépistage et selon les inégalités sociales.

Méthode : A partir des données de 3 registres des cancers, nous avons identifié les femmes de 50 à 74ans, diagnostiquées avec un cancer du sein entre 2009 et 2015, résidant dans les départements de Gironde, Côte d'Or et Loire-Atlantique-Vendée. Grâce à un croisement avec les données des structures de gestion des dépistages des départements, nous avons pu identifier les femmes qui avaient participé au DO dans les 2 ans avant leur diagnostic de cancer. Un second croisement avec les données du Système National des Données de Santé (SNDS) a permis d'identifier, parmi les femmes qui n'avaient pas réalisé le DO, si elles avaient bénéficié d'une surveillance mammographique ou non. Ainsi, 3 groupes de femmes ont été établis selon leur pratique de dépistage : celles qui ont réalisé un DO, celles qui ont eu une surveillance, que nous avons assimilé au DI et celles qui n'ont pas eu de dépistage avant leur diagnostic de cancer. Le niveau de défavorisation a été pris en compte, grâce à l'indice de défavorisation «European Deprivation Index», disponible à partir des adresses de résidence des femmes. Nous avons décrit, pour chacun des 3 groupes, les variables relatives aux patientes et aux tumeurs et nous avons estimé la survie nette à 5 ans dans chacun des groupes et selon les quintiles de défavorisation à partir de la méthode de Pohar-Perme.

Résultats : Parmi les 14 208 femmes avec un diagnostic de cancer du sein incluses dans l'étude, 75 % avaient participé au DO; 10 % avaient réalisé un DI et 15 % n'avait pas réalisé de dépistage avant leur diagnostic de cancer. Parmi les groupes de femmes dépistées (DO et DI), la probabilité de survie nette à 5 ans était plus élevée chez les femmes qui avaient bénéficié d'un DO que celle ayant réalisé un DI avec respectivement une probabilité de 97,0% (IC95% [96,5; 97,6]) et 94,1 % (IC95% [92,5; 95,8]). Dans le groupe de femmes sans surveillance, la probabilité de survie à 5 ans était significativement inférieure aux 2 autres groupes (78,1 %; IC95% [76,1; 80,2]). Cette différence de survie nette observée entre les groupes de femmes qui avaient réalisé un dépistage (DO et DI) comparé à celles non dépistées était d'autant plus importante que le niveau de défavorisation était élevé.

Conclusion : Cette étude est l'une des premières à identifier la surveillance mammographique, utilisée comme proxy du dépistage individuel, pour étudier la survie de femmes ayant un cancer du sein. Il s'agit d'un élément important dans l'évaluation du dépistage organisé du cancer du sein en France. Cette étude montre également que les femmes sans dépistage ont une perte importante de survie après le diagnostic de leur cancer comparé à celles qui participent à un DO ou un DI et ce d'autant plus dans les zones défavorisées. Comprendre les raisons de cette non-participation au dépistage dans ce groupe de femme sera une étape essentielle pour mieux cibler les campagnes de prévention.

P402

Les déterminants du sevrage de l'alcool et du tabac dans le cas des cancers VADS : quels enseignements pour une future prise en charge ?

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Contexte scientifique

Le tabagisme et l'alcool sont les deux premiers facteurs de risque de cancer en France. Après un diagnostic de cancer, le sevrage est primordial car la poursuite de consommation entraîne une multitude de risques de santé, ainsi qu'une réduction de la qualité de vie. Cependant, certains patients échouent au sevrage. Des facteurs sociodémographiques peuvent expliquer la consommation d'une personne durant son cancer tandis que des processus psychologiques comme l'image du corps, les stratégies de coping, la détresse psychologique expliquent le vécu d'un cancer. A ce jour, aucune étude n'explique les mécanismes du sevrage par la mise en place de ces stratégies psychologiques déterminées par la présence de certains traits de personnalité ou autres facteurs biopsychosociaux.

Objectifs et méthode

L'objectif principal est d'expliquer les déterminants psychosociaux du sevrage, de la rechute, ou encore du refus suite à un diagnostic de cancer VADS. L'objectif secondaire sera d'expliquer les déterminants psychosociaux de la qualité de vie de ces mêmes patients. Une étude longitudinale et mixte est proposée à un échantillon issu de l'Oncopole. Les données seront recueillies sur quatre temps, à partir du diagnostic, sur une durée de 1 an. La recherche s'appuyant sur le modèle Transactionnel Intégratif Multifactoriel (TIM), nous évaluerons l'ensemble des antécédents et des modérateurs, ainsi que la qualité de vie à l'aide d'outils validés et d'entretiens semi-directifs.

Résultats attendus

Nous attendons de cette étude la mise en évidence de déterminants et de profils psychologiques expliquant le sevrage avec un diagnostic de cancer. Leur identification nous permettra de mettre en place des protocoles adaptés en fonction des profils lors de la prise en charge du sevrage. De plus, une meilleure compréhension des motivations de ce dernier permettra de déterminer les moments à risque des rechutes et ainsi axer une prise en charge plus intensive sur ces moments.

P403

Factor influencing the smoking judgement of women diagnosed with lung cancer

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Introduction : Lung cancer is a deadly disease that is strongly stigmatised by smoking. Reducing stigma would benefit patients, but requires identifying factors that impact stigma. The purpose of this study was to analyze how people consider the behaviours of smokers with lung cancer acceptable.

Methods : This study uses a quantitative method to identify variables involved in one's judgement, based on a comprehensive combination of scenarios rated by participants on an acceptability scale. Scenarios were built using factors putatively influencing participants' judgment of a woman character diagnosed with lung cancer, including smoking habits, type of diagnosis/prognosis and post-diagnosis smoking behaviour. Two different samples were studied: 132 community individuals and 126 health professionals. Data were analysed using within-subject factorial ANOVA and t-tests.

Results : In both samples, post-diagnosis behaviour had a major effect, as smoking cessation was more acceptable than other smoking behaviours. Other factors, including diagnosis and smoking habits had significant influences on the judgment, with small to medium effect sizes. Diagnosis had a stronger effect size when interacting with post-diagnosis behaviour, as the acceptability by health professionals of continuing smoking was almost doubled when the character had advanced- rather early-stage cancer.

Conclusion : The lower the smoking behaviour, the better the acceptability. However, advanced cancer stage attenuates the poor acceptability of smoking in heath professionals, and, to a lower extent, in the community, as participants' attitudes were more permissive when the patient had advanced cancer. This may corroborate the higher distress recently reported among patients with early-stage lung cancer.

P404

Développement et évaluation d'une action de prévention pour les collégiens autour l'alimentation durable basé sur un supermarché virtuel

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Au moins 40 % des cancers sont liés à des comportements ou à des facteurs environnementaux sur lesquels on peut agir. La prévention est donc une priorité pour lutter contre les cancers, et en particulier la prévention nutritionnelle. Il n'est plus à démontrer que les comportements de consommation sont largement influencés par le marketing, la publicité, ou encore les pressions extérieures (pairs, famille, milieu social…), comme le signale entre autre une expertise collective INSERM de 2017.
Le comportement alimentaire est aussi très fortement influencé par les motivations de choix alimentaire (e.g. l'importance de choisir des produits bons pour ma santé, favorable à l'environnement…). Cette motivation de choix dépend de facteurs environnementaux comme le marketing et les médias, l'accessibilité et la disponibilité alimentaire ; de facteurs sociaux tels que la culture, l'influence des parents et des pairs ; mais aussi individuels (e.g. auto-efficacité, normes sociales perçues, connaissances…). Les facteurs influençant les motivations alimentaires n'ont de surcroit pas le même sens et le même poids selon les âges de la vie.
Comme le montre une revue de la littérature sur les interventions visant à modifier l'alimentation des jeunes, l'école est un lieu idéal pour promouvoir l'alimentation saine. Il est donc pertinent de concevoir des outils pédagogiques adaptés aux adolescents dans l'objectif de développer leur capacité à choisir des aliments de manière consciente et éclairée pour une alimentation durable. Aussi, il est important de cibler les jeunes au plus tôt car nos patterns alimentaires à l'âge adulte sont largement influencés par nos habitudes de l'enfance.

Nous avons donc souhaité développer une intervention ancrée théoriquement autour de l'alimentation durable pour les collégiens. Pour cela, nous avons utilisé dans un premier temps sur les travaux de Lamboy (2017) qui à développé une méthodologie pour développer des actions de prévention en partant de la littérature scientifique et de l'expérience des acteurs de terrain. Une revue de la littérature à été conduite afin de cibler les principaux déterminants qui influencent les motivations alimentaires des adolescents. Après une sélection des plus pertinents en équipe pluridisciplinaire incluant des acteurs de terrain, nous avons pu dégager un modèle théorique.
Après avoir ciblé les déterminants, nous nous sommes appuyés sur les travaux de Carey et al, (2019) basés sur les techniques de changement de comportement (BCT) et le modèle du COM-B de Michie et al, 2011. Chaque variable cible de notre modèle théorique à été ciblée par plusieurs BCT adaptées pour l'impacter.

L'intervention Epidaure Market'2 comporte 3 séances menées en autonomie par l'enseignant de sciences. Ces séances sont articulées autour d'une simulation de supermarché en ligne dans laquelle les jeunes font des achats alimentaires selon différents scénarios. La première séance sert de découverte du jeu et cible principalement les stratégies marketing. La seconde permet d'approfondir les connaissances en nutrition et sur l'impact environnemental de notre alimentation tout en faisant un travail pair à pair dans l'apport de connaissances. Un travail à la maison permet ensuite d'amener les connaissances au domicile afin de trouver certains produits dans les placards et de toucher les familles. Enfin, une dernière séance permet de faire le bilan et de comparer les produits achetés entre la première et la dernière séance.

L'objectif actuel du travail est d'évaluer l'efficacité de l'intervention. Pour cela 24 classes (dont 12 contrôles) seront sélectionnés pour tester avant l'intervention puis un et six mois après les différentes variables du modèle. Nous utiliserons plusieurs questionnaires (motivations alimentaires, connaissances en nutrition…), des tâches expérimentales (choix entre 2 produits) et une tâche d'oculométrie (données objectives sur les informations utilisées pour faire un choix).

P405

Sensibilisation au dépistage et à la prévention des cancers auprès des professionnels et résidents des établissements médico-sociaux en Occitanie

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ONCODEFI

INTRODUCTION

Chez les personnes qui ont une déficience intellectuelle (PDI), c'est-à-dire un trouble cognitif et adaptatif apparus à la naissance ou avant l'âge de 18 ans, le dépistage des cancers est une priorité. Elles représentent environ 2,5 % de la population et participent peu au dépistage organisé des cancers. Pourtant, elles développent autant de cancers que les personnes sans handicap cognitif, notamment du sein (aussi fréquent) et du côlon-rectum (plus fréquent) qu'en population générale. Mais ils sont diagnostiqués à un stade nettement plus avancé. Il est important de mettre en place les actions de sensibilisation au dépistage des cancers ; d'une part auprès de leurs accompagnants institutionnels, mais aussi auprès des PDI qui manifestent beaucoup d'intérêt pour ces questions de santé. Les capacités de compréhension des PDI sont largement sous-estimées, leurs capacités d'apprentissage encore mal connues.

METHODE

En 2018, avec le soutien de l'ARS Occitanie, ONCODEFI a mis en place le dispositif ISCaO (Infirmiers de Soutien à la prise en charge du Cancer chez les personnes avec déficience intellectuelle en Occitanie) dont l'une des missions est d'informer sur la prévention et le dépistage des cancers. Deux infirmiers délivrent des sensibilisations dans les institutions médico-sociales (IMS). L'équipe ISCaO a créé, en collaboration avec des personnes porteuses de troubles cognitifs, des outils pédagogiques spécialement adaptés aux PDI. Ce sont des documents en langage aux normes européennes "Facile à Lire et à Comprendre" (FALC): diaporamas, livret, fiche-résumé. Les supports sont utilisables par les accompagnants pour aborder ces sujets. Les réunions destinées aux PDI sont faites en plus petits groupes pour faciliter l'apprentissage. Un questionnaire de connaissance et de satisfaction a été élaboré pour les aidants des IMS. Un questionnaire spécialement adapté est aussi préparé pour estimer le gain en apprentissage par les PDI. Ils sont actuellement en cours de soumission.

RESULTATS

À ce jour des IMS de 11 des 13 départements d'Occitanie ont reçu au moins une sensibilisation. ISCaO a conduit 70 réunions en présentiel auprès de 1128 aidants professionnels des IMS, auxquels ont été expliqués la fréquence des cancers chez les PDI, les signes pouvant les révéler et leur dépistage. Lors de 24 rencontres 304 PDI ont reçu avec beaucoup d'intérêt une information centrée sur le dépistage et la prévention. Les résultats du recueil des questionnaires seront disponibles à l'automne. Ces démarches ont été freinées par la crise sanitaire en 2020, durant laquelle les IMS étaient mobilisées sur d'autres actions.

CONCLUSION

Une action d'information sur le dépistage des cancers, dirigée à la fois vers le personnel d'encadrement des IMS et vers les personnes avec déficience intellectuelle est développée depuis 3 années en Occitanie. Elle a pour but de réduire le nombre de diagnostics tardifs chez ces personnes pour lesquelles le traitement du cancer est particulièrement difficile.

APPRECIATION GENERALE Le dispositif national pour favoriser le dépistage organisé des cancers du sein et du colon-rectum est particulièrement pertinent car il cible les cancers qui sont les plus fréquents chez les personnes avec déficience intellectuelle.

P406

Aide aux soins du cancer pour les personnes déficientes intellectuelles en institution

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ONCODEFI

INTRODUCTION

Les personnes avec déficience intellectuelle (DI) avancent en âge. Elles sont autant touchées par les cancers que la population générale. Les équipes médico-sociales (EMSO) qui sont confrontées au cancer d'un résident DI sont souvent en difficulté. Le traitement de ces cancers fait intervenir deux mondes qui ne se connaissent pas (le secteur médico-social du retard mental, et le secteur médical de la cancérologie) et qui involontairement s'ignorent. Il en résulte d'importants dysfonctionnements dans le parcours de soins, qui sont responsables de souffrances accrues pour les patients. L'accompagnement d'un patient porteur d'un cancer est une tâche hautement technique et difficile qui prend au dépourvu les EMSO. Elles ont difficilement accès aux spécialistes du cancer. L'association ONCODEFI, dédiée à la prise en soin optimale des personnes DI atteintes de cancer a mis en place l'étude pilote ARII (Action-Recherche Infirmière-institutions) soutenue par Malakoff-Méderic Handicap, pour soutenir les institutions qui sont confrontées à un résident porteur d'un cancer et aider aux soins du patient en 2016-2017; puis le dispositif ISCaO (Infirmiers de soutien à la prise en soin des Cancers chez les personnes avec déficience intellectuelle en Occitanie) soutenu par L'ARS-Occitanie débuté en 2018.

MATERIEL ET METHODE

L'équipe ARII impliquait une infirmière (0,5 ETP) pour les années 2016-2017 en Hérault. Le dispositif ISCaO comporte deux infirmiers (1,5 ETP) sur les treize départements d'Occitanie. L'association est surtout contactée par les EMSO et propose une rencontre en présentiel ou par visioconférence/téléphone pour prendre connaissance du contexte. Le temps d'analyse est essentiel pour cerner la demande et le besoin de l'établissement, pour clarifier la situation du patient: le stade de sa maladie, sa capacité à supporter le traitement. ISCaO présente les différentes options, met en contact les EMSO avec les acteurs oncologiques et propose un suivi si nécessaire pour chaque étape difficile, notamment pour les établissements dépourvus de médecin et infirmières.

RESULTATS

Au total 48 accompagnements qui ont duré entre 2 heures, pour résoudre un problème ponctuel, et trois ans et demi pour un résident encore sous traitement ont été menés sur 11 départements. Les 41 cancers validés ont été découverts, pour 21 d'entre eux à un stade avancé ou très avancé. L'équipe ISCaO a aidé les EMSO en les orientant vers les interlocuteurs adéquats, en fournissant les outils et supports de communication, en apaisant les tensions psychologiques dans les équipes, les malaises et la culpabilité. Elle a permis aux éducateurs d'être en meilleure position pour accompagner le patient vers sa guérison, ou dans sa fin de vie tout en tenant compte de son entourage dans l'institution. Les EMSO se sont dites prêtes, et mieux préparées à envisager la survenue d'un cancer pour d'autres résidents.

APPRECIATION GENERALE.

Ce dispositif d'aide aux professionnels du secteur médico-social permet d'offrir un soutien à des patients très vulnérables et à leur entourage dans les institutions.

P407

Participation au dépistage du cancer du sein et influence des autres dépistages - étude chez des femmes de 56 ans dans 4 départements français

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Introduction : Aujourd'hui les femmes de plus de 50 ans sont soumises à 3 types de dépistage organisé (DO) de cancer en France, à savoir celui du cancer du sein, du colon-rectum et depuis peu du col de l'utérus. Cependant les taux de participation aux DO du cancer du sein et du CCR ne sont pas optimaux et sont bien en dessous des taux recommandés au niveau européen. Ainsi, l'objectif de ce travail est de 1) décrire la participation au DO du cancer du sein chez des femmes de 56 ans et l'influence de cette participation sur les 2 autres dépistages ; 2) de décrire les raisons de non-participation au DO du cancer du sein et 3) décrire les raisons d'une participation au dépistage du cancer du sein avant 50 ans.

Méthode : Un questionnaire a été envoyé à des femmes de 56 ans résidant dans 4 départements français : Gironde, Orne, Calvados et Ille-et-Vilaine. Les questionnaires ont permis d'identifier le comportement des femmes vis-à-vis des 3 invitations au DO du cancer du sein dont elles avaient bénéficié depuis leurs 50 ans. Trois groupes de femmes ont ainsi été déterminé selon le nombre de participation au DO du cancer du sein (3 participations, 1 ou 2 et 0). Nous avons ensuite décrit les réponses aux questions quantitatives et qualitatives.

Résultats : Parmi les 4634 femmes ont répondu au questionnaire, 76% avaient réalisé les 3 DO du cancer du sein, 16% avaient une participation irrégulière et 7% n'avaient participé à aucune invitation. Nous avons constaté que la participation irrégulière au DO du cancer du sein allait de pair avec celles du cancer colorectal. Les femmes qui ont participé aux 3 DO du cancer du sein avaient effectué plus de frottis que les 2 autres groupes. Parmi les participantes irrégulières ou non-participantes, beaucoup ont subi un dépistage individuel, souvent initié avant l'âge de 50 ans. Les raisons de non-participation étaient principalement des raisons médicales ou liées à la participation à un dépistage individuel.

Conclusion : Il n'y a pas d'opposition fondamentale à la participation au dépistage du cancer du sein. Toutefois, il demeure de la plus haute importance que les femmes soient mieux informées sur le dépistage et ses avantages.

P408

Interventions par un professionnel de santé de la Protection Maternelle et Infantile auprès des jeunes (filles et garçons) scolarisés en Maisons Familiales Rurales à l'aide d'une bande dessinée pour améliorer la couverture vaccinale pour le vaccin anti-papillomavirus

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Projet financé par la LNCC (AAP recherche en prévention 2021)

La vaccination anti-papillomavirus humain (HPV) a fait la preuve de son efficacité pour prévenir les infections par le HPV et les lésions génitales précancéreuses, mais la couverture vaccinale reste insuffisante. La HAS recommande une vaccination pour tous les adolescents âgés de 11 à 14 ans, mais il existe un fort besoin de rattrapage pour tous les adolescents âgés de 15 à 19 ans. Aucune recherche interventionnelle n'a été menée dans un contexte français auprès d'adolescents pour évaluer l'efficacité d'actions multimodales sur leurs attitudes à l'égard de la vaccination. Les Maisons Familiales Rurales (MFR) qui accueillent les adolescents en milieu rural offrent un cadre propice au déploiement de cette intervention.

L'objectif principal de la recherche est de montrer qu'une intervention conduite par des professionnels de santé et complétée par la distribution d'une bande dessinée (BD) améliore la couverture vaccinale anti-HPV chez les adolescents de 15 à 19 ans, garçons et filles. Les objectifs secondaires portent sur leur intention de se faire vacciner, la mise en évidence des freins et des leviers à cette vaccination et l'analyse du processus.

Cette recherche a été élaborée en lien avec les acteurs de terrain : l'encadrement des MFR, les parents souvent très impliqués en MFR, les professionnels de santé de la Protection Maternelle Infantile (PMI), et un auteur-illustrateur de BD. Il s'agit de proposer de nouveaux messages qui intéressent garçons et filles. Les MFR de 3 départements de Nouvelle-Aquitaine pourront inclure environ 1350 adolescents de 15 à 19 ans des pour lesquels un schéma en 3 doses (M0, M2, M6) est recommandé. La randomisation affectera 3 groupes de classes :
- intervention selon les modalités usuelles (groupe contrôle)
- intervention standardisée par un professionnel de PMI
- intervention standardisée par un professionnel de PMI et une BD.

Cette recherche sera menée en 3 phases consécutives :
- Phase 1 = intervention auprès des adolescents suivie de la remise de questionnaires avant l'intervention puis entre 15 jours et un mois après.
- Phase 2 = 6 à 8 mois après l'intervention, un nouveau questionnaire sera remis aux adolescents qui auront participé à la 1ère phase de l'étude et auprès de leurs parents, puis des discussions de groupe seront menées avec des adolescents, leurs parents et les responsables des MFR pour évaluer l'intervention (selon un plan séquentiel à méthodes mixtes).
- Au cours de 3ème phase, l'accent sera mis sur l'évaluation de l'intervention avec tous les acteurs concernés, ainsi que sur l'évaluation du processus pour valider un cadre général.

La connaissance est une condition préalable importante pour le changement de comportement. L'intervention standardisée sera basée sur des modèles validés, le modèle des croyances relatives à la santé (HBM) et la théorie du comportement planifié (TPB). Dans le HBM, la sensibilité perçue, la sévérité, les leviers et les freins, ainsi que l'auto-efficacité prédisent les comportements individuels. Ce modèle est d'autant plus pertinent qu'il est souvent associé aux comportements de prévention tels que la vaccination. Selon la TPB, l'intention, motivée par les attitudes, les normes perçues et la perception du contrôle, est le facteur le plus important lié au changement de comportement.

Si l'intervention s'avère efficace, nous proposons d'analyser les conditions de sa transférabilité. Une adaptation numérique de la BD pourrait être proposée ainsi que sa diffusion pour accompagner l'information aux élèves. Au-delà de la couverture vaccinale, le bénéfice escompté est une diminution de l'incidence des cancers HPV induits.

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P409

Projet SANITOSMO: développements d'un casque multi-sensoriel Odeur-Musique-Vidéo et d'un kit de stick inhalateurs odorisés pour des applications en oncologie

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Le projet SANITOSMO vise à favoriser l'émergence d'une action pluridisciplinaire et transversale « Odeurs & Santé » au sein de Toulouse INP, intégrant le thème prioritaire « Santé, bien être et bien vieillir » de la Stratégie Régionale de l'Innovation de la région Occitanie.
Si la chimiothérapie est une source de stress durant le traitement pour les patients, elle génère également, pour plus de 30% d'entre eux, une diminution de leurs capacités cognitives, notamment de type anosmie.
Le projet a donc pour objectifs d'une part le développement d'un casque connecté multi-sensoriel pour des diffusions synchronisées d'Odeurs-Vidéo-Musique visant à diminuer le stress des patients lors de séances de chimiothérapie et d'autre part, celui de la creation de kits sensoriels pour lutter contre le Chemofog/Chemobrain,

1) Mise au point du casque connecté multi-sensoriel NOVA pour des diffusions synchronisées d'Odeurs (supports odorisés) + Vidéo (tablette ou casque de Réalité Virtuelle) + Audio (musique déstressante) pour diminuer le stress des patients lors de séances de chimiothérapie :
* extrusion/filage de plastique odorisé au moyen d'huiles essentielles/poudres d'épices
* impression 3D des fils odorisés pour produire des embouts odorisés
* caractérisation et évaluation de la remanence des odeurs des embouts imprimés
* sélection des types de musiques desstressantes (type musique de la nature)
* sélection des vidéos classiques (type parcours en campagne) et VR (Virtual Reality) desstressantes
* conception du prototype de casque multi-sensoriel NOVA

2) Développement du kit sensorial CHEMONOSE+ pout lutter contre le Chemofog / Chemobrain:
* sélection d'huiles essentielles et de formulations naturelles illustrants à la fois les 5 continents (diversité culturale) et le référentiel olfactif Le Champ des Odeurs (diversité olfactive)
* sélection du support de diffusion (stick inhalateur avec support en coton) et optimisation de la concentration des senteurs (rémanence temporelle)
* développement du packaging et fabrication d'une pré-série de kits de 12 senteurs au TRL 7

3) Réalisation d'essais à l'Hôpital de Jour du Service d' Oncologie Clinique du CHU Toulouse Rangueil :
* tests d'évaluation sensorielle du casque NOVA (3 odeurs, 3 musiques, 3 vidéos, 2 vidéos VR) avec prise des constantes (pouls, pression sanguine) avant-durant-après la chimiothérapie
* tests d'évaluation sensorielle du kit sensoriel optimisé (12 senteurs) avec QCM (reconnaissance des odeurs) en post-chimiothérapie (chambre, domicile)

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Le cancer broncho-pulmonaire dans l'Hérault 1995-2019 : tendances par âge, sexe, stade et histologie

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Registre des Tumeurs de l'Hérault

Contexte
En France, en 2018, le cancer broncho-pulmonaire est le troisième cancer par sa fréquence et la première cause de mortalité par cancer, tous sexes confondus.
L'objectif principal de cette étude est d'analyser les tendances de l'incidence des cancers broncho-pulmonaires dans l'Hérault selon le sexe,l'âge, les types histologiques et les stades au diagnostic.

Méthodes. Nous avons inclus dans l'étude tous les cancers broncho-pulmonaires diagnostiqués entre le 01/01/1995 et le 31/12/2019 chez les personnes habitant le département de l'Hérault.
Toutes les données ont été extraites de la base de données du registre des tumeurs de l'Hérault. Les évolutions de l'incidence par sexe, âge, type histologique et stade au diagnostic ont été analysées à l'aide des taux moyens d'évolution annuel (TEM) et de leurs intervalles de confiance estimés par des modèles de régression log-linéaires.

Résultats
L'étude a concerné 16 008 patients dont 27,8% de femmes. Les tumeurs étaient représentées majoritairement par des adénocarcinomes (44,8%) et 43,8% étaient à un stade IV au diagnostic.
Sur la période 1995-2019, le taux d'incidence standardisé augmente de manière significative chez la femme avec un TEM de +6.4% [5.7% ; 7%] et de manière moins rapide chez l'homme avec un TEM de +0.9% [0.7% ; 1.1%].
Chez l'homme, l'incidence augmente significativement après 60 ans (+2,5%/an chez les 60-74 ans et + 1,2%/an chez les plus de 75 ans), mais diminue chez les plus jeunes (-0,7%/an chez les 45-59 ans et - 1,9%/an chez les 25-44 ans). Chez la femme, l'incidence augmente fortement dès l'âge de 45 ans (+5,7% chez les 45-59 ans, +7,6%/an chez les 60-74 ans et chez les plus de 75 ans). Pour les hommes, durant la même période, les taux d'incidence des carcinomes épidermoïdes et des carcinomes à petites cellules ont diminué de manière significative alors que les taux des adénocarcinomes ont augmenté rapidement de manière significative pour les deux sexes. Chez l'homme, les taux d'incidence des stades I, II, IV augmentent de manière significative alors que ceux des stades III ont tendance à diminuer au cours du temps. Les taux d'incidence chez la femme augmentent rapidement pour tous les stades : stade I +10.9% [9% ; 12.9%], stade IV +6.6% [5.5% ; 7.7%].

Conclusion
Cette étude en population confirme les spécificités du cancer broncho-pulmonaire : légère augmentation d'incidence au cours du temps chez les hommes; une forte augmentation chez les femmes dès l'âge de 45 ans; augmentation de l'incidence des adénocarcinomes pour les deux sexes; augmentation pour tous les stades mais de manière plus rapide pour les stades I et IV chez les femmes. Ces résultats soulèvent la question d'éventuelles différences de susceptibilité entre les femmes et les hommes par rapport aux facteurs de risque mais aussi la place d'un dépistage du cancer broncho-pulmonaire.

Posters – Axis 5 “Health Technologies”

P501

Targeting of folate receptor beta expressing TAMs with vectorized magnetic nanoparticles for anticancer therapies

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Tumor-associated macrophages (TAMSs) are well known as protecting tumor cells against apoptosis, immune attacks and therapies. Elimination or deactivation of these pro-tumoral TAMs remains a challenge in cancer therapies. Several ways of TAMs targeting exist, however they are not specific, potentially leading to serious adverse effects.
We produced and patented a monoclonal antibody called 6-25 capable to specifically recognize the nurse-like cells (NLCs), a TAMs from chronic lymphocytic leukemia (CLL), , and TAMs from solid cancers. We showed that the 6-25 antibody recognizes the folate receptor beta (FRβ) at the surface of these cells and that it is internalized without toxicity for these cells. The FRβ is also expressed by the M2 monocytes-derived macrophages (M2-MDMSs) but not by the M1 monocytes-derived macrophages (M1-MDMSs) or other myeloid cells. Interestingly, this receptor is expressed by some acute myeloid leukemia cell lines, including MV4-11 which can be used as an *in vitro*model.

The goal of the project is to produce a tool that specifically targets and kills pro-tumoral TAMs 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 following the localized application of a high frequency alternating magnetic field (AMF) to a tumor containing magnetic nanoparticles (MNP), leading to cell death.

Thus, we chose to functionalize iron oxide MNP with the 6-25 mAb (MNP-6-25) as a specific tool to target cells expressing the FRβ. Iron oxide MNP are highly biocompatible and non-toxic (rapid degradation with iron cations recycling), which allows their combination with conventional therapies. The MNP are first PEGylated, then linked to a fluorophore, the Cyanine 5. The antibodies, 6-25 or IgG control, are then coupled to the MNP via a heterobifunctional PEG spacer, thanks to a Michael reaction leading to a thioether covalent bond.
For this study, we used as cellular models, M2-MDMs and NLC as expressing the FRβ at their surface, and the M1-MDMs as negative control without FRβ at their surface. M2-MDMs and M1-MDMs were obtained by the culture of monocytes from healthy donors in the presence of appropriate cytokines cocktails.

First, we showed that the MNP-6-25 were not toxic toward M1-MDMs and M2-MDMs in a concentration range from 2 to 64 µg/ml following incubation for 72h. Then, we obtained a maximum binding of MNP-6-25 to M2-MDMs at 48h of incubation at the concentration of 8µg/ml. Subsequently, we showed that MNP-6-25 have a high binding specificity for M2-MDMs but not for M1-MDMs. Finally, we demonstrated by confocal microscopy imaging that MNP-6-25 were internalized in M2-MDMs with a lysosomal co-localization.

In the perspective, we plan to evaluate efficacy and specificity of MNP-6-25 in killing FRβ expressing macrophages upon application of magnetic field *in vitro*. Then, we will have to show that MNP-6-25 are able to target and facilitate depletion of TAMs in an *in vivo* model.

P502

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.

P503

Origin of Fe ions in ROS production induced in magnetic hyperthermia anti-cancer nanotherapy: release from iron oxide nanoparticles or not?

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The first and critical reaction in magnetic hyperthermia to kill cancer cells is the catalysis of ROS (reactive oxygen species) production. We previously showed that it is possible to specifically deliver iron oxide magnetic nanoparticles (IONPs) in the lysosomes of cancer cells and eradicate them by targeted intra-lysosomal magnetic hyperthermia via the application of a high frequency alternating magnetic field (AMF) without macroscopic temperature elevation [1,2]. The mechanism involves a local temperature elevation at the IONPs surface which enhances the ROS production through the Fenton reaction ($Fe^{2+}+H_2O_2 \rightarrow Fe^{3+}+OH^-+OH^\bullet$); ROS then peroxide the proteins and lipids of the lysosomal membrane, inducing its permeabilization and leading to lysosomal enzymes release and cell death [2]. Fe ions, critical to produce ROS in magnetic hyperthermia, were assumed to be released by IONPs.

To verify this hypothesis, we developed PEGylated IONPs constituted by iron oxide multi-cores called "NanoFlowers" (NF) presenting or not a SiO2 shell (NF@SiO_2), the later preventing Fe release from IONPs. As expected, NF@PEG released Fe ions and produced ROS production in vitro, in acidic medium mimicking lysosome upon AMF exposure, whereas NF@SiO_2@PEG did not. We then conducted magnetic hyperthermia experiments in cells. To increase the IONPs accumulation in cancer cells overexpressing the CCK2 receptor (MiaPaca2-CCK2), both PEGylated NF and NF@SiO_2 were decorated with gastrin molecules. Both IONPs internalized and accumulated in the lysosomes of MiaPaca2-CCK2 cancer cells up to level of 4 pg/cell (NMR relaxometry). Surprisingly, the NF@SiO_2@PEG@Gastrin increased the ROS production (2.8±0.4 vs 3.8±0.9-fold), induced lysosome permeabilization (79.6±2.9 vs 72.0±14.4 %) and cell death (32.2±1.6 vs 35.3±5.9 %), and slowed down the proliferation (47.3±4.7 vs 53.4±6.9 %) of MiaPaca2-CCK2 cells with the same efficacy than NF@PEG@Gastrin, upon AMF application. This shows that magnetic hyperthermia is also efficient to induce ROS production and kill cells even in absence of Fe release from IONPs. So, does magnetic hyperthermia involve the endogenous Fe ions? To answer this question, we performed magnetic hyperthermia experiments in presence of Ferristatin-II, an iron uptake inhibitor. 50 µM Ferristatin-II incubation for 18h, preventing the iron uptake by MiaPaca2-CCK2 cells, blocked the ROS production in magnetic hyperthermia induced with NF@SiO_2@PEG@Gastrin, demonstrating that endogenous Fe is necessary to produce ROS during magnetic hyperthermia with NF@SiO2@PEG@Gastrin. This study elucidates the origin of Fe ions involved in ROS production during magnetic hyperthermia: Fe ions can be released from IONPs, but also they can originate from the endogenous iron pool.

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P504

3D cell models to study liver physiopathology : 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. Modeling NASH disease and HCC carcinogenesis is crucial to better understand underlying molecular pathways and find new therapeutic targets. 2D cellular models are easy to manipulate but are too distant from the complexity of the pathophysiology of the liver. Existing in vivo mouse models of NASH do not recapitulate the whole spectrum of the human pathology. Moreover, we have to think about reducing experimentation on mouse models in accordance with the 3R strategy.

3D cell models have seen a great breakthrough since twenty years to answer this challenge, going from monocellular spheroids to complex organ-on-chip. 3D cell models enable a better modeling of the disease thanks to 3D cell interaction, better cell differentiation and function. Additionally, 3D models are suitable for complex multicellular models and allow to recapitulate a suitable microenvironment. 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 new 3D cell models 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.

We use primary human hepatocytes (PHH) and the HepaRG® cell line that are grown either in normal conditions or in a culture medium enriched in fatty acids and LPS in order to induce NASH phenotype. Our 3D cell models are based on two processes : spheroids and cell encapsulation technology in 3D alginate capsules thanks to VOXCELL platform facilities.

P505

Targeted thermal or mechanical nanotherapy of pancreatic adenocarcinoma

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Magnetic nanoparticles (MNPs) are already widely used in nanomedicine, particularly as MRI contrast agents or in magnetic hyperthermia therapy. The first clinical trial using nanotherapy was conduct in 2011 to treat high-grade brain tumors. Currently, the efficacy of nanotherapy combined with radiotherapy is investigated as a new treatment against prostate cancer. However, the benefit on life expectancy remains negligible and neither radiotherapy nor magnetic hyperthermia can distinguish between normal and cancerous tissues, responsible of adverse effects. Our strategy is based on the vectorization of iron oxide magnetic nanoparticles called **NanoFlowers** (NFs) capable of recognizing targeted cells and therefore specifically treating cancerous tissue thought the application of an external magnetic field, minimizing damage to healthy tissue. Under a **high frequency magnetic field** (AMF) exposure, the heat of NFs will specifically eradicate these cells, without macroscopic temperature elevation. Therefore, the rotation of the NFs under a **low frequency rotating magnetic field** (RMF) application generates mechanical forces leading to cell destruction. As a proof of concept, we have chosen a model of **pancreatic adenocarcinoma** (PDAC), a cancer with a very poor prognosis. The therapeutic failure is especially due to the development of multidrug resistance resulting from many mechanisms such as the lysosomal sequestration of chemotherapies. Moreover, tumor microenvironment plays a critical role in the development of PDAC resistance. By secreting extracellular matrix proteins, Cancer-Associated Fibroblasts (CAFs) create a physical barrier that limits the penetration and the efficacy of treatments (chemotherapy and radiotherapy). PDAC cancer cells and CAFs can overexpress the type 2 cholecystokinin (CCK2) receptor that is internalized after its activation. The graft of a specific agonist of the CCK2 receptor, the Gastrin, at the Nanoflower surface (NF@Gastrin) allows their accumulation into the lysosomes of pancreatic cancer cells and CAFs overexpressing the CCK2 receptor. The RMF (1Hz, 40 mT) or AMF (275 kHz, 30 mT) application kills up to 45% of cancer cells and CAFs that have internalized NF@Gastrin, slows down their proliferation without affecting cells lacking the nanoparticles,. We showed that these two strategies also inhibit cell migration and stimulate the expression of Damage-Associated Molecular Pattern (DAMP) proteins such as Calreticulin and HSP70, well known to induce an immunogenic anti-tumoral response. Current studies are performed in order to determine the impact of these two strategies, consisting in thermal or mechanical energy delivery through magnetic nanoparticles excited by an external magnetic field, on spheroids & preclinical in vivo models.

P506

Bifunctional chemical tools for the targeted degradation of Furin

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Proprotein convertases (**PCs**) are a family of 9 serine proteases involved in the processing of cellular protein precursors by triggering their activation, inactivation or functional changes. Amongst them, the **Furin**protease cleaves proteins just downstream of the specific basic amino acid target sequence Arg-Xaa-(Arg/Lys)-Arg. This enzyme is ubiquitously distributed in human tissues and plays important physiological functions, but also contributes to numerous diseases.[1,2] For instance, it has been found that the viral Spike glycoprotein, which mediates the **SARS-CoV-2** entry into host cells, harbors the consensus Furin recognition motif. Inhibition of the Furin activity blocks SARS-CoV-2 virus entry and virus replication, therefore potential antiviral agents for infection and pathogenesis can be developed.[3]

In this project, we aim at developing bifunctional molecules that could be used as innovative chemicals tools to induce a Furin **targeted degradation** through recruitment to the cellular quality control machinery.[4] We will report here the design and synthesis of a small library of bifunctional molecules, containing either a **Hydrophobic Tag**(**HyT**) or an E3 ligase recruiter (**PROTAC**: **PROteolysis Targeting Chimeras**) connected to a Furin inhibitor. The first results regarding their activity will be discussed as well.

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P507

TranSipedia: a novel framework for large scale RNAseq data analysis with applications in cancer from research to diagnosis

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Driven by myriads of projects, public RNA-seq databases are exploding. To date, over 850,000 RNA-seq are deposited on SRA for human. This huge body of publicly available RNA-seq libraries is a precious resource to identify specific transcriptional events. The challenges lie in the complexity of RNA biological content and the exponential increase in data volume. We want to make RNA-seq data easily accessible providing a better capture of the whole transcriptome complexity, in the context of human health applications. New computational methods that perform indexing of k-mers across huge datasets constitute interesting solutions to interrogate "Omics data" at a large scale from dataset collections. Here, we developed TranSipedia, a new framework based on k-mer approach, constructed with several modules: 1/ **The RNA-seq indexing step** constructed with Reindeer (REad Index for abuNDancE quERy; Marchet et al., 2020), a novel computational method that serves as an efficient platform to request all transcribed information, 2/ **a module to generate k-mers as signature** of transcripts (Kmerator; Riquier et al, 2021), 3/ **a supporting web site** to facilitate the queries easily shared by biologists (TranSipedia, https://transipedia.montp.inserm.fr).

Reindeer performs indexing of k-mers and records their counts across a large collection of datasets. Interestingly it associates k-mers to their counts instead of only recording the presence/absence of k-mers as frequently done in previous works. Moreover, Reindeer provides an ultra-fast performance in the query process while indexing several thousands of RNA-seq. One of the great advantages of indexing raw data is also that it integrates reference-free and annotation-free approaches. For applications where gene expression level is required, the k-mer count must be sufficiently sensitive and representative to be applicable. The quantitative accuracy with k-mers counts from Reindeer indexed datasets was compared to classical quantitative methods like Kallisto. Secondly, the design k-mer module uses Kmerator, a tool developed to construct specific k-mers, already avalailable on github. Thirdly, the website is also available to facilitate index queries by the biologists with sequences on fasta file format. The Transipedia platform now includes several thousands of datasets from public and private collections mainly from acute myeloid leukemia (AML) for cancer applications. We indexed the whole CCLE cohort representing 1019 RNA-seq samples for a total of 10 To and indexes from Leucegene, BEAT-AML, SRA and TCGA collections represented with more than 1000 RNA-seq samples for AML application. Concerning biological applications, we already requested in selected public datasets biomarker tissue specificity as well as tumor specific signatures comparing normal/tumor, for simple and useful medical usage. In perspectives, based on data structures such as k-mer features, diagnosis applications are in developpement. Moreover, Machine learning models could be used to search for signatures and explore better diagnostic and prognosis models.

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Estimating spatial distribution of oxygen and hypoxia in tumor microenvironment: a mechanistic approach

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Being a hallmark of several solid tumors, hypoxia - a state of reduced level of tissue oxygen tension and a result of aberrant vasculature - leads to several alterations in the tumor microenvironment. Hypoxic regions of neoplasm are prone to be more resistant towards radiation therapy than compared to well oxygenated ones (A. L. Harris 2002). Furthermore, hypoxia and its mediators influence multiple signaling pathways and gene regulation to promote neovascularization, invasion, migration, adhesion, metastasis, and phenotypic switches (D. S. Widmer et al. 2013, A. Tameemi et al., 2019). Hence hypoxia is one of the leading factors which contributes towards intratumor heterogeneity and resistance against treatments, these two features being particularly important and common in many invasive tumors including melanoma (B. Bedogni et al. 2009, D'Aguanno et al. 2021). Estimation of accurate hypoxia profile would be key for better prognosis and design of more efficient treatment approaches. Mathematical modeling has been proven a useful tool to understand and predict such complex dynamics. Several computational and mathematical models have been proposed to describe tissue oxygenation, however the majority of them are restricted to synthetic data and qualitative results, lacking application to and connection with real tumor tissues and experimental results.

We propose mechanistic modeling frameworks, which are driven by experimental data, to explain and mimic oxygen-hypoxia dynamics. The data is in the form of tissue scans of Patient Derived Xenograft (PDX) of breast, ovarian and pancreatic as well as human melanoma tumors. These scans of tumor tissue slices are immunohistochemical stained with CD31 -cluster of differentiation 31, marking the presence of endothelial cells- and CAIX- carbonic anhydrase IX, regulated by the hypoxia-inducible factor (HIF) 1, is an intrinsic marker of tumor hypoxia - markers. Keeping the data availability in mind, the distribution of oxygen is described by a reaction-diffusion partial differential equation with the source term incorporating the contribution from blood vessel density (obtained from CD31 staining) for the 2D model and from the vasculature architecture and the geometry of each blood vessel (reconstructed from several 2D tissue slices) for the 3D model. Next, hypoxia is modeled from the obtained oxygen distribution using an algebraic equation. The further steps include estimation of parameters and validation. The obtained parameters demonstrate biological relevance. 3D reconstruction, which is underway, is required for obtaining 3D profiles of oxygen and hypoxia. This requirement leads to another aspect of this work consisting in quantification of the error made when 2D models are used instead of more realistic 3D models. This is important since the 3D reconstruction is not always feasible, especially for patient tissue samples. A framework to quantify this approximation error would be essential for evaluating the hypoxia profile for clinical applications. Future work involves development of a general framework, applicable to most of the solid tumors, to estimate oxygen and hypoxia distribution based on the 3D reconstruction of blood vessels as well as for the 2D case with an error bound due to the approximation.

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Gas vesicles: contrast agents for ultrasound imaging of cancer therapeutic bacteria

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In recent years, bacteria have been engineered to detect and treat several pathologies in vivo, including infections, metabolic disorders, and cancer. Using the tools of synthetic biology, bacteria can be genetically engineered to recognize, colonize, and proliferate specifically into the tumor microenvironment and finally produce in situ therapeutic molecules in a controlled manner.

One advantage of using bacteria as delivery vehicles is to counter the side effects of systematically administrated chemo and immunotherapeutic treatments. In addition, in situ, tunable synthesis and delivery of therapeutic molecules can improve treatment efficacy, and synergistically complement existing therapeutic approaches.

To monitor the biodistribution of bacterial therapeutics, strong and reliable reporter genes compatible with in vivo tracking are desirable. "Acoustic bacteria", producing gas vesicles detectable via ultrasound imaging, can be used as live reporters of the tumor microenvironment.

Our goal is to design bacteria that can trigger gas vesicles production only when and where desired thanks to specific sensing and logic circuits. We have engineered bacteria that can detect specific body locations and biomarkers of the tumor microenvironment. Our goal is now to connect these sensors to the production gas vesicles.

Ultrasonic imaging will then be used to detect these cells turned into smart and active contrast agents.

In this way, we will monitor the location, density, and ultimately finely control the activity of cancer therapeutic bacteria.

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Cell types and states autodetection properties of SeedCell deconvolution software

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Context and problem statement

Many partial cell deconvolution or gene signature methods allow to quantify the presence of cell types in a tissue or a tumor from bulk-RNAseq data. Some techniques such as CIBERSORTX, Bisque or MUSIC use the transcriptome at the single cell level (scRNA-seq) to define specific gene signatures of the cell types to be searched. These approaches suffer from important preconditions in the choice of cells and gene signatures. Some optimization processes lead to the loss of information about the existing cell states for some cell types.

Objective of the SeedCell software

The goal is to take into account a large set of input signatures and to perform autodetection of the cell types that are present, to define the main signature and the secondary signatures that may be specific to cell states. The relative quantification of cell types in a sample is performed for each selected signature.

Algorithm

The algorithm is based on 3 main innovations. (1) The autodetection module takes into account large signatures mainly from scRNAseq studies to extract highly correlated gene cores (seeds). We worked with the PangloaDB and MSigDB databases. (2) A graph mining reconciliation method is used to select a primary signature per cell type and secondary signatures of cell states if needed. (3) The calculated z-score takes into account the weighted expression of the genes present in several signatures by the expression level of the other genes of the signatures sharing the gene.
The software offers two possibilities of intervention to the user: 1) to adjust the number and the nature of the cell types detected by the first module and 2) in the choice of the signatures before editing the score matrix

Results

The sensitivity and specificity of the SeedCell software were measured by comparison with the Bisque and CIBERSORT software, among others. TCGA data on colorectal cancer and bulk RNAseq datasets of blood samples were used to compare the results obtained with clinical and biological data

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Regressive Modular Response Analysis

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MRA (Modular Response Analysis) is a method used to infer biological networks. From a set of independent perturbations applied on network nodes [1], it is possible to compute the connectivity between every pair of nodes. It is well-known that classical MRA is sensitive to measurement noise and perturbation intensity. One of the most important questions about MRA concerns discovered edges meaning. We have developed a new approach linking MRA and multiple linear regression. Connectivity coefficients estimation is equivalent to reckon regression parameters as confidence intervals. We have confirmed this approach successfully by comparing it with classical MRA, in the case of an "in silico" six nodes Map kinases network. One important MRA's application, coupled to regression, is to identify null edges, notably concerning gene networks, which are often sparse. Many regression methods have been compared: multiple regression, "Lasso", "threshold regression", applied to "in-silico" gene networks stemming from Dream Challenge 4. Results have shown a correct error rate for 10 and 100 genes networks. Interesting results have already been achieved [2], [3].

[1] Boris N Kholodenko et al. Untangling the wires: a strategy to trace functional interactions in signaling and genenetworks. Proc Natl Acad Sci, U S A, 99, 2841-12846, 2002.
[2] https://www.iscb.org/ismb2022
[3] https://www.biorxiv.org/content/10.1101/2022.08.11.503572v1

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Seadra: a fully customizable and user-friendly annotation software

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Nowadays, Artificial Intelligence (A.I.) is becoming a central tool in medicine to help practitioners to better understand the huge and growing amount of data they are facing. Recent approaches are more and more based on Machine Learning (ML) to automatically extract patterns from annotated data. However, building datasets, the basis of any ML algorithms, is a necessary preliminary task that takes a tremendous amount of time from medical experts. In this paper, we present a new efficient and flexible software, Seadra, dedicated to annotating medical multimodal data (images, medical information, etc.). We illustrate its use in the field of oral cancer detection as it presents all required types of data for this initial application. Oral cancer kills around 180,000 people each year in the world even though survival rates are between 75% and 90% if diagnosed early. As with many diseases, the lack of specialists and their poor geographical distribution reduces the survival rates of patients because they are often diagnosed too late. In this case, as in many others, AI-based methods could be beneficial to propose a first diagnostic with the objectives to save time for pathologists and prioritize their appointments with higher risks of cancer.

Seadra is a fully customizable and user-friendly annotation application developed within the CRCT-IRIT team. It is meant to allow quick labeling of data for any data-driven project. One of its main advantages over other annotation tools is that it has been designed to be compatible with a graphical tablet, which allows gains in both speed and precision of the annotation. The idea behind Seadra is that, when starting a new annotation project, an interactive window is displayed to the user where they can choose all the annotation types they want (segmentation of the image, single or multi-choice tags, comment section) and the labels to associate with those annotations.
Then, the user will be able to label their data (image or other) with a tag section and various drawing tools: ellipsis, rectangle, and polygon.
Once this is done, the annotations are saved in a json file named with a defined pattern using the name of the image, so that a link can easily be made.
An annotation project on Seadra is a simple folder that can be copied to another computer in order to be used on it without further set up needed.

We have conducted a study to measure the potential gain obtained using Seadra compared to another democratized annotation tool, we chose QuPath for this purpose. We have built three sets of 10 images each and annotated them with three different methods: first with QuPath, then with Seadra but with mouse and keyboard, and finally with Seadra and the graphical tablet.
Moreover, even though it is not quantified, Seadra appeared easier to adapt to for the pathologist and they also told that the use of a graphical tablet allowed them to identify more features than they could identify without it.

In the end, Seadra is a new annotation software that will help with the fast creation of well-annotated datasets to allow new AI-based projects to be developed. We believe that, thanks to its flexibility and the fact that it is easy to use, this application can be used in a lot of projects, mainly in the healthcare field where the time of the experts is a particularly valuable resource.

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BiNGO-3C: Binary Encoding of Genomic Sequences for Optimal Compression, Comparison and Clustering

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The vast amount of data generated by sequencing technologies gave rise to the need for faster and adequate algorithms. Statistical methods for the comparison of biological sequences based on word-counts such as k-mer have shown great potential in this context. However, the characters-based representation of DNA and the redundancy of common words leave much to be desired in terms of memory and complexity optimization. Here, we upgrade the traditional words-count approach for sequence comparison by introducing a new representation of DNA sequences for minimal time and memory requirements.

To reduce the memory and time consumption in our approach, we start by splitting the long DNA sequence into smaller words of size s and a gap h, as in any other sliding-window algorithm. We then change the representation of the words from DNA bases (A, C, G and T) to a 2-bit binary format (00, 01, 10 and 11) respectively, then to the numeric value corresponding to the resulting binary string. This step significantly reduces the storage-memory required, as well as the computation time for the next steps. Finally, the set of numeric values are compared to each other and clustered using Jaccard-Similarity Index to compute a similarity score.

Compared to characters, numbers are processed much faster, as they can be held in smaller registers in most programming languages. In fact, combining the sliding-window approach and the binary representation of data allowed for a significant reduction in the amount of storage space required and, as a result, the processing time needed.

We apply our method to various types of genomic datasets with different evolutionary-scales to demonstrate that it can consistently spot genomic changes, classify related samples and minimize processing time and memory. Finally we implemented BiNGO-3C in open-source with a dedicated JavaScript web-server and Python Notebooks to offer a more comprehensive ready-to-use solution (github repo: https://github.com/mouneem/BiNGO-3C).

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Fluorescent Peptide Biosensors Reporters of Kinase Activities: profiling signatures in human tumour biopsies through a multiplex approach for cancer diagnostics

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Protein kinases (PK) are frequently hyperactivated in many human cancers thereby constituting relevant biomarkers and attractive pharmacological targets for anticancer therapy (Fleuren et al. Nat Rev Cancer 2016; Roskoski R. Jr. pharmacol. Res. 2021; Cohen et al. Nat Rev Drug Discovery 2021). Although these biomarkers may be detected through antigenic, proteomic, transcriptomic or genetic approaches, there are currently no approaches that report on their functional activity for diagnostic purposes. In order to monitor the kinase activities of cyclin-dependent kinases (CDKs), we have developped a toolbox of fluorescent peptide biosensors through conjugation of environmentally-sensitive probes to synthetic peptides derived from CDK substrates (Morris M.C. Life 2022a, European J Organic Chem 2022b). Specifically, we have engineered a CDK4-specific biosensor which enables quantification CDK4 hyperactivity in skin cancer cell lines, biopsies and melanoma xenografts (Prével et al. Biosens. Bioelectron. 2016; Gonzalez-Vera et al. Chem. Commun. 2017; Henri et al. Br. J. Dermatol. 2019), a CDK6 biosensor which was implemented to compare CDK6 and CDK4 activities in lung cancer (Soamalala et al. ChemBioChem 2020), a CDK5-selective biosensor for neuronal disorders such as glioblastoma (Peyressatre et al. Frontiers Chemistry 2020), and a CDK1 biosensor conjugated to carbon nanotubes for in vivo imaging in tumour xenografts in mice (Tilmaciu et al. Small 2021). These synthetic biosensors offer straightforward means of quantifying differences in kinase activities between healthy and cancer cells, and of sensing alterations in response to therapeutics.

With the aim of implementing CDKACT technology to profile CDK activity signatures in biopsies in human tumours, we developed a multiplex approach combining four different biosensors and established a protocol for standardized and calibrated quantification of CDK activities. In collaboration with the CRB-CHU Montpellier, we characterized the CDK activity profiles from 40 lung adenocarcinoma and 40 lymphoma samples and performed Western blots to determine CDK expression levels in parallel. We further correlated our results with age and sex of patients, as well as with the genetic and immunohistochemical characterization of the biopsies performed by the CHU (Royet et al. in preparation). This study shows that CDKACT biosensing technology provides new and complementary information relative to current genetic and immunohistochemical characterization of tumour biopsies, enabling further stratification of patients and potential to develop a diagnostics approach based on kinase activity profiling using synthetic fluorescent biosensors.

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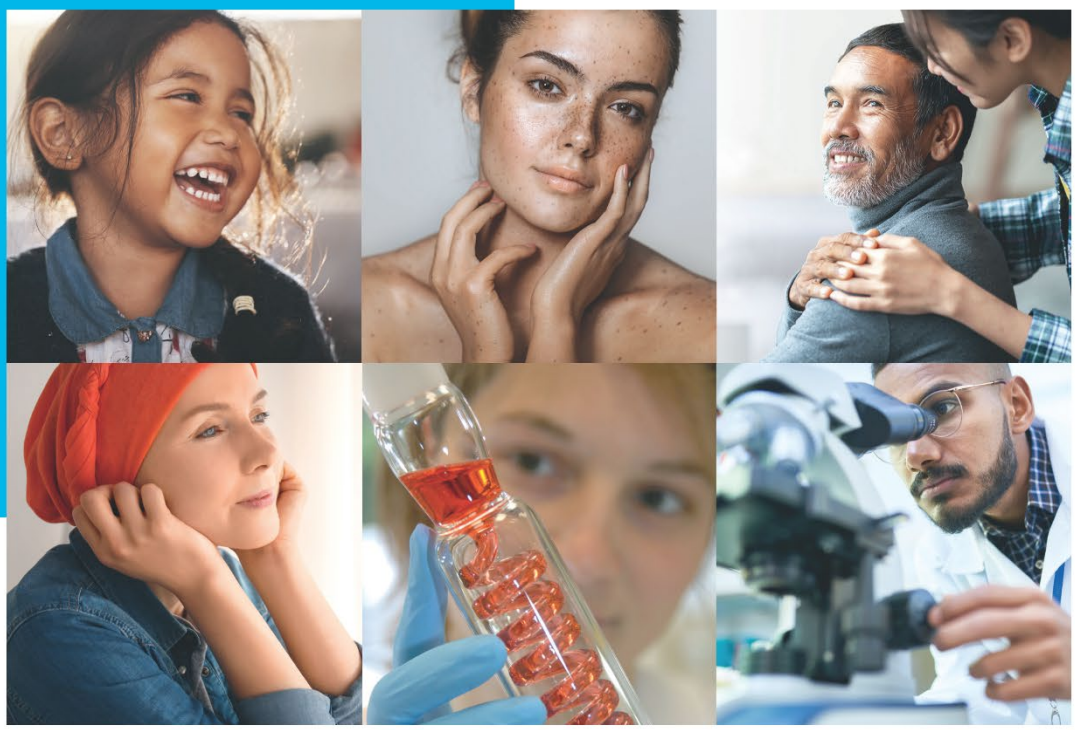

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