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INSTITUT NATIONAL DU CANCER

APPEL À PROJETS 2023

Projets libres de Recherche : Biologie et Sciences du Cancer

PLBIO-2023

Lettre d'intention / Letter of Intent

DATE LIMITE DE SOUMISSION : 2 novembre 2022 16h00

La lettre d'intention doit être rédigée en anglais / *The letter of Intent should be written in English*

N° du dossier : Veuillez indiquer le n° de dossier attribué par le portail PROJETS (Menu "Dépôt de projets") /*Project number*	**PLBIO23-053**
Coordonnateur du projet (NOM, Prénom) / *Project coordinator (NAME, First name):*	**CREMER Isabelle**
Organisme d'appartenance du coordonnateur : *Affiliated institution*	**INSERM - Sorbonne Université - Université de Paris**
Titre du projet :	Décrypter les mécanismes cellulaires et moléculaires conduisant à l'exclusion immunitaire dans le cancer du poumon : IMMUNEX
Project title *:*	Deciphering the cellular and molecular mechanisms leading to immune exclusion in lung cancer: IMMUNEX
Durée prévue du projet / *Scheduled duration of the project* (36 ou 48 mois/36 or 48 months) :	36 months

Dans le cadre d'un AAP de l'INCa / In the frame of a call for proposals of INCa

X 1ère soumission du projet */ 1st submission of the project*

☐ Soumission(s) antérieure(s) du projet / *Previous submission(s) of the project*

En cas de soumission(s) antérieure(s) du projet, veuillez préciser les principales modifications apportées au projet / *In case of previous submission(s), please indicate the main changes made to the project*

Projet scientifique / Scientific project

Résumé scientifique du projet (Max. 2000 caractères espaces compris – Arial taille 11)
• Contexte scientifique Le cancer du poumon non à petites cellules (CPNPC) est un cancer de mauvais pronostic avec un microenvironnement (TME) complexe et hétérogène, qui contient des cellules immunitaires, notamment des cellules T et B organisées ou non en structures lymphoïdes tertiaires (TLS), des fibroblastes et des capillaires sanguins et lymphatiques. Une forte densité de TLS est associée à une bonne survie et à une réponse positive à l'immunothérapie. De plus, la composition et la structure du TME influencent la migration des cellules T et B dans la tumeur. Nous supposons que l'infiltration et l'organisation des effecteurs immunitaires en TLS dépendent des caractéristiques cellulaires et moléculaires du TME. • Objectifs du projet et brève description des méthodes qui seront employées pour les atteindre Notre objectif est de déterminer les mécanismes cellulaires et les voies fonctionnelles conduisant à l'absence ou au défaut d'une réponse immunitaire organisée dans le CPNPC et d'identifier des stratégies pour permettre l'infiltration des cellules immunitaires dans des tumeurs qui en sont dépourvues. Nous proposons trois tâches interdépendantes: (i) classification cellulaire et moléculaire des patients à l'aide de la transcriptomique spatiale, définition de la signature du TME associée au profil d'infiltration immunitaire et identification de cibles candidates clés ; (ii) évaluation fonctionnelle de la migration des cellules immunitaires dans le TME à l'aide de sphéroïdes 3D et de fragments tumoraux dérivés de patients (PDTFs) et (iii) identification des mécanismes moléculaires impliqués dans l'exclusion immunitaire en se concentrant sur des cibles candidates clés, à l'aide de l'invalidation CRISPR/Cas9 et de tests fonctionnels dans des modèles 3D hétérotypiques. • Résultats attendus Ce projet mettra en évidence les mécanismes responsables de l'exclusion immunitaire et/ou du manque d'organisation dans le TME, ainsi que les molécules à cibler dans le TME pour induire une réponse immunitaire intra-tumorale. Cela pourrait également permettre d'identifier de nouvelles cibles thérapeutiques pour améliorer la réponse à l'immunothérapie chez les patients ayant un pronostic défavorable.

Scientific abstract (Max. 2000 characters– Font size Arial 11)

- Scientific background

Non-small-cell lung cancer (NSCLC) is a poor prognosis cancer with a complex and heterogeneous microenvironment (TME), which contains immune cells, including T and B cells organized or not in tertiary lymphoid structures (TLS), cancer-associated fibroblasts, and blood and lymphatic capillaries. Patients with a high density of TLS have a good clinical outcome, unlike those with no immune infiltration or absence of TLS. Our consortium has accumulated evidence showing that TLS are needed to provide objective response to immunotherapy, and that the TME composition and structure influence T cell migration in the tumor. We hypothesize that the immune effector infiltration and organization in TLS depend on the TME features.

- Project objectives and brief description of the methods which will be used to achieve them

We aim to determine the cellular mechanisms and functional pathways leading to immune exclusion or defective organization in lung cancer and to identify strategies to warm up these “immune cold” tumors with organized immune microenvironment. We propose 3 inter-related tasks: (i) Cellular and molecular classification of patients using spatial transcriptomics, and definition of TME signature associated with the pattern of immune infiltrate through the identification of key target candidates; (ii) Functional assessment of immune cell migration into the TME using 3D lung tumor patients spheroids and patients-derived tumor fragments (PDTF) and (iii) Deciphering of the molecular mechanisms involved in immune exclusion using CRISPR/cas9 invalidation of key target candidates and functional assays in heterotypic 3D models.

- Expected results

Upon completion, this project will highlight the mechanisms responsible for immune exclusion and/or lack of organization in TLS, and the molecules to be targeted in the TME to “warm up” tumors. This may also provide identification of novel therapeutic targets in poorly immunotherapy-responding patients with poor prognosis.

(Veuillez copier-coller le même résumé dans la rubrique résumé du portail PROJETS/ Please copy-paste this summary in the PROJECTS portal)

Project description (Max. 3 pages[1], Arial 11)

Issue, hypothesis and research work's main objective(s), expected results and impact:

Issues: The tumor microenvironment (TME) of non-small cell lung carcinoma (NSCLC) is highly complex and heterogeneous, and its composition strongly influences clinical outcome and response to treatments. Besides tumor cells, the TME comprises cancer-associated fibroblasts (CAFs), endothelial cells, extracellular matrix, and subsets of immune cells localized in the stroma or in the tumor nest (Altorki, 2019).

The complexity of the TME arises from multiple events that occur at different cellular levels: clonal and sub-clonal tumor cells differentially evolve during tumor progression (Jamal-Hanjani, 2017), tumor cells express the calreticulin (CRT) immunogenic cell death molecule at variable levels (Fucikova, 2016), subsets of fibroblasts or endothelial cells have different phenotypes and functions (Goveia, 2020; Hu, 2021; Grout, 2022), and immune cells or soluble immune mediators such as molecules belonging to complement system may perform pro- or anti-tumoral functions, depending on the context (Roumenina, 2019; Daugan, 2021).

The immune cell infiltrates in lung TME have been extensively studied and well characterized: T and B lymphocytes with mature dendritic cells (mDCs) are organized in tertiary lymphoid structures (TLS), leading to a coordinated antitumoral immune response which is associated with a good prognosis (Dieu-Nosjean, 2008; Goc, 2014; Germain, 2014). TLS maturation is characterized by the presence of follicular helper T cells (Tfh) and germinal centers, and their presence is correlated with good response to immunotherapy in many solid tumors, including ccRCC and NSCLC (Fridman, 2019). **On the contrary, patients with no immune cell infiltration ("immune cold"), with immune infiltration but no contact with tumor cells ("immune excluded") and/or no proper organization in TLS in the TME (Fig.1) have a poor prognosis. Indeed, understanding how the cellular and molecular components of the TME influence the immune cell infiltration/organization is a major issue to solve, to be able to design novel therapeutic targets that would promote T and B cell recruitment in the tumor bed.**

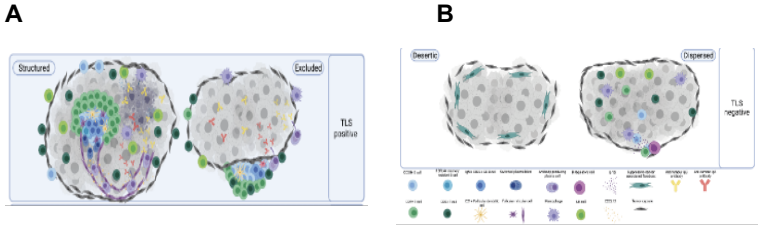


Figure 1. Four types of TME (Meylan, 2022). **A**. TLS-positive TME. **B**. TLS-negative TME.

Hypothesis: Based on these premises, we consider that the cellular and molecular composition of the TME may explain the absence of immune cells in the TME, which represents 50-60% of NSCLC cases (Lehtio, 2021). We hypothesize that the immune effector infiltration and TLS organization depend on the nature of tumor cells, CAFs subsets, blood and lymphatic vessels activation and patterning, and on ECM composition and structure.

Objectives: We aim to determine the cellular mechanisms and functional pathways leading to immune infiltration/organization in lung cancer and to identify strategies to warm up "immune cold" tumors with an organized immune microenvironment. Upon completion this project will provide novel therapeutic targets for improvement of immunotherapy response in these poor prognosis patients. We offer **3 inter-related work packages**:

WP1) Cellular and molecular classification of NSCLC patients and definition of TME signature (TME-Sign) associated with immune cell infiltration. To this end, Team 1 will perform a spatial transcriptomic analysis of the TME after a classification of NSCLC patients based on the type of adaptive immune infiltrates in their tumor. Patients will be classified as immune high (Immune^{Hi}) organized or not, and immune Low/Neg (Immune$^{Low/Neg}$). Transcriptomes of the various cell types in the TME of each group of

[1] La bibliographie peut être ajoutée en plus des 3 pages de description / *References can be added beyond the 3 pages of description*

patients will be compared to highlight the specific gene signatures and identify key candidate molecules linked to immune infiltration or exclusion (Team 4).

WP2) Functional assessment of immune cell migration and organization (Team 1 and 2), using a 3D microfluidic culture device set-up by Team 3 (Hautefeuille), and using patient derived tumor fragments (PDTFs).

WP3) Deciphering the molecular mechanisms involved in immune exclusion by focusing on the key target candidates identified in WP1. Team 1 will perform CRISPR invalidation of candidate molecules in different cell types of the TME to identify which of the signature genes control the shift of the TME from immune exclusion to immune infiltration. Functional assays will be performed using a heterotypic 3D microfluidic model of lung tumors set-up by Team 3 that mimics the structural organization of primary human tumors. We will identify "warming up" proteins, which could be further explored as pharmacological targets to improve response to immunotherapy.

Expected results and impact:

The IMMUNEX immuno-oncology project aims to bridge spatial analyses of tumor microenvironment by using transcriptomics and multiplex imaging combined with functional assays using 3D novel culture systems and PDTFs. This transdisciplinary project will lead to fundamental results:

- The characterization of tumor microenvironment that allows T and B cells to infiltrate the TME of lung tumors, leading to immune desert, exclusion or immune enriched (organized or not in TLS) TME.
- The development of a new 3D culture system for NSCLC, appropriate for subsequent functional analyses and a suitable model to test therapeutic agents in future experiments.
- The identification of molecules that can be targeted in the TME to "warm up" tumors, enabling T/B cell migration.

Spin-offs are also expected in onco-immunology and immunotherapy:

We expect this project to have significant impact in the field of cancerology and public health at several levels: i/ it will contribute to the identification of new molecules in the TME that could be targeted in lung cancer combined or not with currently used immunotherapy, to improve local antitumor immune response; ii/ the spatial transcriptomic data will constitute a precious resource for the scientific community; iii/ the 3D microfluidic culture model using NSCLC tumors from patients, with the ambitious objective to preserve the maximum of TME cell subtypes would be a useful tool to perform drug screening on the whole TME leading to personalized treatments.

Relevance and originality of the project regarding the state of the art:

Spectacular response rates to immunotherapy targeting PD-1/PD-L1 axis and CTLA-4 were observed only in approximately 30% of NSCLC patients (Pabani,2018), whereas other patients relapse. The integration of recent high throughput technologies with large-scale bioinformatic analysis has considerably improved our knowledge of the lung TME composition (Altorki, 2019; Lambrechts, 2018) (**Fig. 2A**), and allowed the identification of the major role of immune cell infiltrates in the rate of the response to immunotherapy.

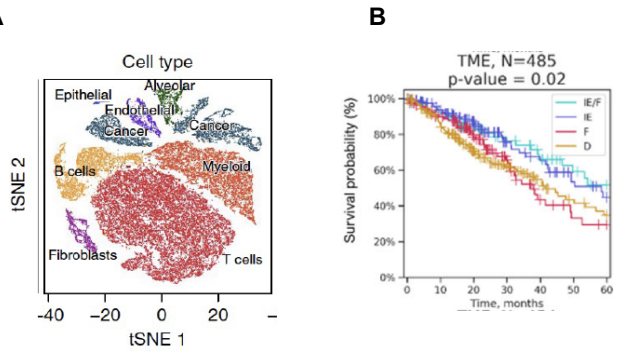


Figure 2. Heterogeneity of lung TME impacts clinical outcome. (A). Tumor heterogeneity revealed by scRNAseq (Lambrechts, 2018) **(B).** Four TME subtypes correlate with OS in lung adenocarcinoma (IE/F: Immune Enrich Fibrotic, E: Immune Enrich, F: Fibrotic, D: desert) (Bagaev, 2021).

Using multiparametric immunohistochemistry (IHC) and transcriptomic analyses, immune cell activation was observed in the TME, associated with complete or partial response to anti-PD-1 treatment and to increased overall survival (Damotte, 2019; Leader, 2021). We demonstrated that these immune cells are organized in TLS, composed of a T cell-rich zone with mDCs juxtaposing a B cell follicle with a germinal center surrounded by antibody

secreting plasma cells (Dieu-Nosjean, 2008; de Chaisemartin, 2011; Goc, 2014; Meylan, 2022). The presence of Tfh is revealed by CXCR5 and PD-1 co-labeling. High density of TLS, as well as high endothelial venules (HEVs), the major sites for lymphocyte entry in the TME, are associated with good clinical outcome, and correlates with disease free survival in patients treated with immunotherapy (Asrir, 2022; Sautes-Fridman, 2019; Petitprez, 2019; Schumacher & Thommen, 2022). On the contrary, immune desert or excluded patients (Kratz, 2021; Bagaev, 2021) poorly respond to treatment (**Fig. 2B**) (Bagaev, 2021).

By analyzing the spatial transcriptome of TLS **(Fig. 3A)**, we demonstrated the existence of different maturation stages of B cells and immunoglobulin production by plasma cells inside TLS, which supports the generation of an *in situ* antitumoral B cell response. Furthermore, we found dissemination of IgG and IgA positive plasma cells along CXCL12-positive fibroblastic tracks through the tumor bed. In conclusion, we evidenced that anti-tumor plasma cells are generated inside TLS which constitutes a new effector role of TLS in cancer, and that some CAFs subsets may help this process (**Fig. 3B,C**) (Meylan, 2022).

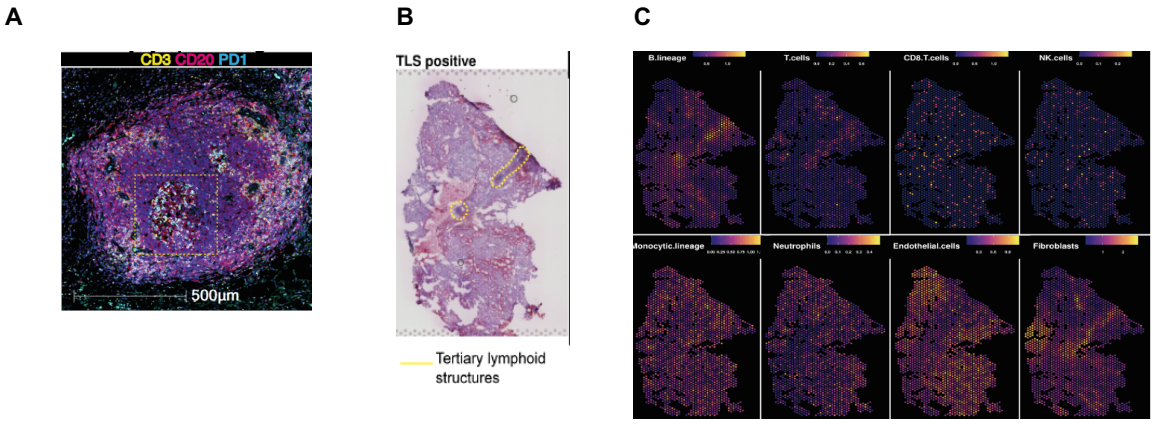


Figure 3: Spatial transcriptomics of immune and stromal cell population in the TME. (A) TLS organizatio the TME: Multiplex IHC showing CD3, CD20 and PD1 labeling. (**B)** Pathologist annotations of TLS on H&E slic **(C)** Abundance estimation of 8 immune and stromal cell populations by MCP-counter (Meylan, 2022).

In addition, several studies showed the involvement of tumor cells, CAFs or endothelial cells in immune cell infiltration: i/ Tumor mutations (*STK11*, *EGFR* and *TP53)* influence the immune landscape of TME (Spranger, 2018, Biton, 2018) and a high tumor mutational burden is positively correlated with TLS scoring (Rooney, 2015; Lin, 2020); Complement components expressed by tumor cells are associated with poor clinical outcome and less immune activation (Roumenina, 2019; Daugan, 2021); ii/ CAFs subsets expressing different collagen sets modulate immune cell homing (Salmon, 2012; Lambrechts, 2018; Grout, 2022; Hu, 2021); and iii/ vascular endothelial cell Notch pathway modulates the formation of TLS (Fleig, 2022).

However, despite the increasing understanding of TME structure and composition, we still do not understand why in some NSCLC patients the TME is devoid of immune cells, or devoid of a structured immune response, reflected by TLS organization. Indeed, the cellular and molecular mechanisms governing immune cells infiltration are not fully elucidated. The originality of this project is based on a combinatorial approach, using cutting edge technologies including spatial transcriptomic, multiplex IHC and 3D heterotypic microfluidic spheroids to perform functional assays.

Project feasibility (Max. 3 pages[1], Arial 11)

Brief description of implemented methodology and techniques (techniques that are already available or that are being validated):

WP1. Cellular and molecular classification of NSCLC patients and definition of the TME signature (TME-Sign) (Teams 1 & 4)

Task 1. Patient classification based on immune cell infiltrates

We will perform multiplex IHC labeling (Leica Bond RX automat on CHIC platform at CRC), on a retrospective cohort of 229 NSCLC patients, using the following markers: CD45 (pan immune cells), CD3 (T cells), CD20 (B cells), panCK (tumor cells), alphaSMA (fibroblasts), CD31 (EC: endothelial cells), Meca-79 (HEV), and LYVE1 (lymphatic vessels). After automated quantification of each marker using Halo AI software (Indica labs, available and regularly used in Team 1), we will classify the patients into 3 groups (we expect to obtain half of the patients in groups A and B):

- group A: no/low T/B cell infiltration ("immune desert")
- group B: presence of immune infiltrates
    - group B1: immune infiltrated not organized (absence of TLS)
    - group B2: infiltrated with organized response (presence of TLS)

Task 2. Spatial transcriptomic profile of the TME

The spatial transcriptomic profile will be performed (expertise of Prs WH and C Fridman in Team 1). We will select 10 patients from each group (A, B1, B2) and will perform a spatial transcriptomic profiling using Visium V2 technology (10X Genomics) on FFPE tissues. Each capture area (11 x 11 mm) contains 5,000 barcoded spots that are 55 µm in diameter (100 µm center to center between spots) providing an average resolution of 1 to 10 cells.

Task 3. Data processing and analysis (Visium data)

Bioinformatic analysis will be performed by bioinformaticians of the Team 4 (ARTbio platform at IBPS, Sorbonne Universite). Visium data will be processed as described in Meylan *et al*. (2022). Spatial spots belonging to TLS, tumor cells, fibroblasts, and endothelial/lymphatic cells will be defined by CD3/CD20, panCK, alphaSMA, and CD31/LYVE1/MECA-79 staining respectively, on the consecutive FFPE slide. The spatial immune and stromal infiltrates of each tumor will be estimated with MCP-counter (Becht, 2016) which computes abundances scores of 8 immune and stromal populations. Specific subset signatures for each cellular component of the TME will be obtained by comparing the groups of patients A vs B1 and B1 vs B2. From these signatures, we plan to select 60-80 genes, forming the TME-Sign (based on gene enrichment analysis), involved in specific functions of different cell types and differentially expressed between the groups of patients. We anticipate identifying new genes, in addition to confirming the involvement of candidate ones, described in **table 1** (identified among published and our preliminary data). **Table 1**:

Cell type	Candidate genes (probable impact on immune cell infiltration/organizatio
Tumor cells	CTNNB1 (WNT/β-catenin), FH, C1S (Luke, 2019; Daugan, 2021)
Fibroblasts	P-SMAD, C1s, C7, HGF, FGF7 (Hu, 2021; Grout, 2022)
EC	Notch1, RBP-J, HEY1, JAG1 (Fleig, 2022)

Task 3. Validation of gene expression signature TME-Sign on the whole cohort

The TME-Sign will be validated on the whole cohort (groups A and B1/B2 patients) using the Quantigene technology (thermoFisher, already used in Team 1).

⇒ Expected results: Defining a TME-Sign for each group of patients

WP2. Functional assessment of immune cell infiltration (Teams 2 & 3)

Task 1. Set-up a new perfusable microfluidic device to obtain 3D organization

A co-culture of all the different cell types of the TME will be performed in a physiologically-relevant microenvironment *in vitro*. This design will be inspired by merging two existing multicellular organ-on-chip models (OoC) mimicking pivotal cues of native tissues such as interstitial flow and extracellular matrix (ECM) composition and mechanics, to preserve the cell phenotypes. The device (**Fig. 4A**) preserves tissue explant structure and functions after dissociation, for several weeks thanks to the beneficial effect on endothelial cells of a controlled tissue-specific shear stress engineered inside the chip (Jun, 2019). An OoC device similar to those fabricated routinely by Team 3 (Vazquez 2019) was recently used to exploit the flow for a spontaneous assembly and organization of primary human blood B- and T-lymphocytes into ectopic lymphoid follicles (Goyal 2022), but migration processes

were not quantified. This idea will be used in the project and several flow conditions will be tested.

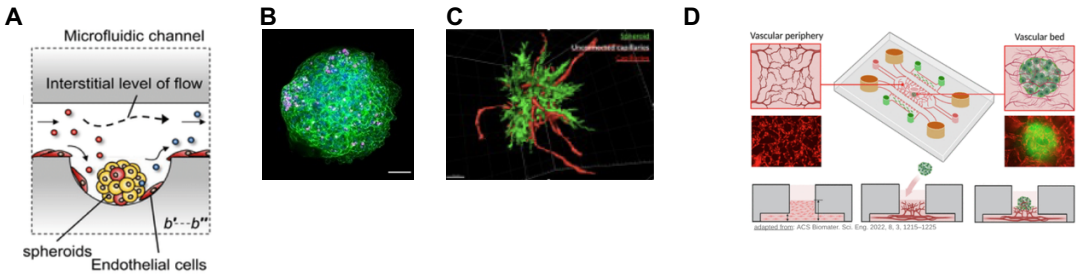


Figure 4: 3D spheroid culture. (A) Simple microfluidic channel will be designed with multiple microwells trapping the different TME cells and interstitial flow promoting the preservation of the primary cells phenotype (Jun, 2019). **(B)** A549 tumor spheroids. **(C)** Co-cultures of tumor spheroids of A549 with HUVECs in fibroblast derived conditioned medium + VEGF. Imaris reconstruction. (Brassard-Jollive, 2020) **(D)**. Set-up of a more elaborated chip will be fabricated (WP3) to vascularize 3D heterotypical spheroids of modified cell lines, for molecular studies. An ECM hydrogel perfused with growth factors will guide a capillary bed on which the spheroid is adhered.

Task 2. Functional screening of T and B cell migration

Tumor pieces from surgery (n=20-30 patients) will be divided in two pieces: one will be dedicated to 3D spheroid culture (see Task 1), one for PDTFs. T and B cell distribution and migration will be quantified by 3D confocal imaging in both models. For 3D culture, autologous T and B cells labeled with distinct fluorescent dyes will be added on spheroids. For PDTFs, we will track resident T and B after CD3 and CD20 labeling with directly-coupled fluorescent antibodies as described by Team 2 (Peranzoni, 2017).

Task 3. Data analyses and integration

Patients will be classified into groups A, B1, B2 by multiparametric IHC as in WP1T, and their TME-Sign will be defined as in WP1T3. The results of functional assays, ie. migration into the TME, will be analyzed for each group of patients, and will be linked to its TME-signature.

⇒ Expected results: Demonstrating different T and B cell migration, depending on the nature of the TME, characterized by some candidate genes of the CAFs and endothelial cells particularly.

WP3. Deciphering the molecular mechanisms involved in immune exclusion (Teams 1 & 3). This WP will be dedicated to validate the involvement of candidate genes already identified (Table 1) and in the WP1.

Task 1. Generation of cellular tools deficient for candidate genes

We will obtain tumor cells (A549), CAFs (already successfully cultured by Team 1 from primary lung tumors) and endothelial cells (HUVEC, human umbilical vein endothelial cells) deficient for genes specific of each cell type (CRISPR/Cas 9 approach) and most differentially expressed in the TME of patients from groups A, B1 and B2 (WP1T3).

Task 2. Functional screening assays using KO heterotypic spheroids

In addition to the OoC model presented in WP2, the use of cell lines may enable the formation of more complex 3D spheroids (simple A549 spheroids already obtained in team 1, **Fig 4B**) where tumor, stromal, immune and endothelial cells can be decoupled to evaluate more precisely the mechanisms of interest. Recently, vascularized micro-tumors (VMT) on-chip models successfully recapitulated TME *ex vivo* (Chen 2017, Hachey 2021, Campisi 2022). Inspired by this approach, the combined expertise of Team 1 in hydrogel vasculogenesis (**Fig. 4C**) and of Team 3 in OoC microfluidics will converge to build a perfused microvasculature made of HUVECs endothelial cells in hydrogel tissue to vascularize and perfuse the complex environment with the desired immune cells and study cell ingression mechanisms (**Fig. 4D**). Microdevices will be fabricated with the modified cell lines (as described in **Table 2**) and used to determine if the chosen target genes are involved in the migration of immune cells. The VMTs are transparent microfluidic chips allowing standard microscopy imaging and cell analyses, while permitting exogenous perfusion of cells and soluble molecules via the vessels to administer them inside the tissue in a more physiological way (Chen 2017).

Table 2: cocultures with tumor cells, CAFs and HUVEC

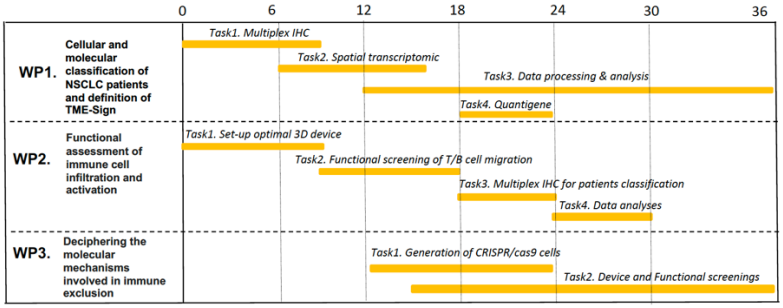
- WT tumor cells + WT CAFs + WT EC	- KO tumor cells + WT CAFs + WT EC
- WT tumor cells + WT CAFs + KO EC	- KO tumor cells + WT CAFs + KO EC
- WT tumor cells + KO CAFs + WT EC	- KO tumor cells + KO CAFs + WT EC
- WT tumor cells + KO CAFs + KO EC	- KO tumor cells + KO CAFs + KO EC

⇒ Expected results: Identify key molecules of tumor cells, fibroblasts or endothelial cells that limit T and B cell migration in the TME

Necessary biological resources:

- The cohort of NSCLC patients (n=229, operated in 2018, without neoadjuvant treatment) is already characterized for histology (n=161 adenocarcinoma, n=39 squamous cell carcinoma, n= 29 others) (Prs M Alifano, D Damotte, Cochin hospital, Paris). FFPE tumor specimens are already available, as well as clinical data.
- Prospective cohort of NSCLC patients will also be obtained from Prs. Alifano and Damotte. We plan to recruit 2 tumor samples per week.

Planned schedule and key steps:



Associated teams and added value:

Team 1: Inflammation, Complement and Cancer team of the Centre de Recherche des Cordeliers (I Cremer). The team has a long and strong expertise in the field of tumor immunology and highly contributed to decipher relationships between tumors and tumor infiltrating immune cells through intensive analysis of the complex tumor immune microenvironment. By using high-throughput bioinformatics combined with spatial transcriptomic analyses, cellular approaches and *in situ* analyses the team dissects how tumor cells shape the anti-tumor immune response. I Cremer (oncoimmunologist) will be responsible for managing the project. Expert researchers of the team will also be involved: C Fridman, WH Fridman (expertise in tumor immunology, TLS and spatial transcriptomics), L Roumenina (expertise in complement system in biology), D Damotte, M Alifano and A. Lupo (clinicians from Cochin hospital) and C Monnot (expertise in 3D cultures, vessels and extracellular matrix biology).

Team 2: Team Cancer and immune response of Cochin institute (E Donnadieu)

The team has a track record in analyzing T-cell activities on different levels of integration, ranging from *in vitro* to *in vivo* conditions, approaching translational research. Notably, this group has developed a powerful organotypic approach that, combined with dynamic imaging technology, permits the monitoring of T cells in fresh human tumors. Such innovative monitoring technology has been instrumental in the identification of obstacles contained within the tumor microenvironment. One engineer will be involved in this project.

Team 3: Team Dynamic and Multiscale processes of Auto-Organization in Tissue Morphogenesis of IBPS (M Hautefeuille)

The team has a wide expertise in the microfabrication of biomaterials and microfluidic systems for organ-on-chip models for fundamental and applied research (3 inventions patented). He recently incorporated IBPS to develop new tools for the development of microvascularized tissues on chips. Postdoctoral Researcher W. Xiao also brings her expertise in tissue culture in 3D microfluidic environments and will participate in this initiative.

Team 4: Team ARTbio of IBPS (C Antoniewski)

The ARTbio bioinformatics facility is led by Christophe Antoniewski and aims at providing biologists with a smooth access to information technologies and at accompanying them in high quality computational analyses of their data. Its expertise extends from the analysis of transcriptomics data to the calling and annotation of genomic variants in WGS and WES, including the implementation of regression, classification and inference statistical methods and the use of machine learning and deep learning. Analysis of single-cell RNAseq and the development of dimensional reduction and classification methods suited to biological data has been a priority axis of the team for several years (10.1093/gigascience/giaa102, 10.1186/s40478-019-0819-y, 10.1038/s41419-022-05358-8) and L. Bellenger, one of its member, is dedicated exclusively to this area.

Bibliography

Altorki NK, Markowitz GJ, Gao D, Port JL, Saxena A, Stiles B, McGraw T, Mittal V. (2019) The lung microenvironment: an important regulator of tumour growth and metastasis. Nat Rev Cancer. 19(1):9-31. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/30532012/

Asrir A, Tardiveau C, Coudert J, Laffont R, Blanchard L, Bellard E, Veerman K, Bettini S, Lafouresse F, Vina E, Tarroux D, Roy S, Girault I, Molinaro I, Martins F, Scoazec JY, Ortega N, Robert C, Girard JP. (2022) Tumor-associated high endothelial venules mediate lymphocyte entry into tumors and predict response to PD-1 plus CTLA-4 combination immunotherapy. Cancer Cell. S1535-6108(22)00004-6. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/35120598/

Bagaev A, Kotlov N, Nomie K, Svekolkin V, Gafurov A, Isaeva O, Osokin N, Kozlov I, Frenkel F, Gancharova O, Almog N, Tsiper M, Ataullakhanov R, Fowler N. (2021) Conserved pan-cancer microenvironment subtypes predict response to immunotherapy. Cancer Cell. 39(6):845-865.e7. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/34019806/

Becht E, Giraldo NA, Lacroix L, Buttard B, Elarouci N, Petitprez F, Selves J, Laurent-Puig P, Sautès-Fridman C, Fridman WH, de Reyniès A. (2016) Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. Genome Biol. 17(1):218. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/27765066/

Biton J, Mansuet-Lupo A, Pécuchet N, Alifano M, Ouakrim H, Arrondeau J, Boudou-Rouquette P, Goldwasser F, Leroy K, Goc J, Wislez M, Germain C, Laurent-Puig P, Dieu-Nosjean MC, Cremer I, Herbst R, Blons H, Damotte D. (2018) *TP53, STK11*, and *EGFR* Mutations Predict Tumor Immune Profile and the Response to Anti-PD-1 in Lung Adenocarcinoma. Clin Cancer Res. 24(22):5710-5723. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/29764856/

Brassard-Jollive N, Monnot C, Muller L, Germain S. (2020) *In vitro* 3D Systems to Model Tumor Angiogenesis and Interactions With Stromal Cells. Front Cell Dev Biol. 8:594903. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/33224956/

Campisi M, Shelton SE, Chen M, Kamm RD, Barbie DA, Knelson EH. (2022). Engineered Microphysiological Systems for Testing Effectiveness of Cell-Based Cancer Immunotherapies. Cancers, 14(15), 3561. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/35892819/

Chen MB, Whisler JA, Fröse J, Yu C, Shin Y, Kamm RD. (2017). On-chip human microvasculature assay for visualization and quantification of tumor cell extravasation dynamics. Nature protocols, 12(5), 865-880. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/28358393/

Damotte D, Warren S, Arrondeau J, Boudou-Rouquette P, Mansuet-Lupo A, Biton J, Ouakrim H, Alifano M, Gervais C, Bellesoeur A, Kramkimel N, Tlemsani C, Burroni B, Duche A, Letourneur F, Si H, Halpin R, Creasy T, Herbst R, Ren X, Morel P, Cesano A, Goldwasser F, Leroy K. (2019) The tumor inflammation signature (TIS) is associated with anti-PD-1 treatment benefit in the CERTIM pan-cancer cohort. J Transl Med. 17(1):357. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/31684954/

Daugan MV, Revel M, Thouenon R, Dragon-Durey MA, Robe-Rybkine T, Torset C, Merle NS, Noé R, Verkarre V, Oudard SM, Mejean A, Validire P, Cathelineau X, Sanchez-Salas R, Pickering MC, Cremer I, Mansuet-Lupo A, Alifano M, Sautès-Fridman C, Damotte D, Fridman WH, Roumenina LT. (2021) Intracellular Factor H Drives Tumor Progression Independently of the Complement Cascade. Cancer Immunol Res. 9(8):909-925. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/34039652/

de Chaisemartin L, Goc J, Damotte D, Validire P, Magdeleinat P, Alifano M, Cremer I, Fridman WH, Sautès-Fridman C, Dieu-Nosjean MC.Cancer Res. (2011) Characterization of chemokines and adhesion molecules associated with T cell presence in tertiary lymphoid

structures in human lung cancer. 71(20):6391-9. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/21900403/

Dieu-Nosjean MC, Antoine M, Danel C, Heudes D, Wislez M, Poulot V, Rabbe N, Laurans L, Tartour E, de Chaisemartin L, Lebecque S, Fridman WH, Cadranel J. (2008) Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. J Clin Oncol. 26(27):4410-7. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/18802153/

Fleig S, Kapanadze T, Bernier-Latmani J, Lill JK, Wyss T, Gamrekelashvili J, Kijas D, Liu B, Hüsing AM, Bovay E, Jirmo AC, Halle S, Ricke-Hoch M, Adams RH, Engel DR, von Vietinghoff S, Förster R, Hilfiker-Kleiner D, Haller H, Petrova TV, Limbourg FP. (2022) Loss of vascular endothelial notch signaling promotes spontaneous formation of tertiary lymphoid structures. Nat Commun. 13(1):2022. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/35440634/

Fucikova J, Becht E, Iribarren K, Goc J, Remark R, Damotte D, Alifano M, Devi P, Biton J, Germain C, Lupo A, Fridman WH, Dieu-Nosjean MC, Kroemer G, Sautès-Fridman C, Cremer I. (2016) Calreticulin Expression in Human Non-Small Cell Lung Cancers Correlates with Inreased Accumulation of Antitumor Immune Cells and Favorable Prognosis. Cancer Res. 76(7):1746-56. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/26842877/

Germain C, Gnjatic S, Tamzalit F, Knockaert S, Remark R, Goc J, Lepelley A, Becht E, Katsahian S, Bizouard G, Validire P, Damotte D, Alifano M, Magdeleinat P, Cremer I, Teillaud JL, Fridman WH, Sautès-Fridman C, Dieu-Nosjean MC. (2014) Presence of B cells in tertiary lymphoid structures is associated with a protective immunity in patients with lung cancer. Am J Respir Crit Care Med. 189(7):832-44. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/24484236/

Goc J, Germain C, Vo-Bourgais TK, Lupo A, Klein C, Knockaert S, de Chaisemartin L, Ouakrim H, Becht E, Alifano M, Validire P, Remark R, Hammond SA, Cremer I, Damotte D, Fridman WH, Sautès-Fridman C, Dieu-Nosjean MC. (2014) Dendritic cells in tumor-associated tertiary lymphoid structures signal a Th1 cytotoxic immune contexture and license the positive prognostic value of infiltrating CD8+ T cells. Cancer Res. 74(3):705-15. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/24366885/

Goveia J, …, Carmeliet P. (2020). An Integrated Gene Expression Landscape Profiling Approach to Identify Lung Tumor Endothelial Cell Heterogeneity and Angiogenic Candidates. Cancer Cell. 37(1):21-36.e13. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/31935371/

Grout JA, Sirven P, Leader AM, Maskey S, Hector E, Puisieux I, Steffan F, Cheng E, Tung N, Maurin M, Vaineau R, Karpf L, Plaud M, Casanova-Acebes M, Tabachnikova A, Keerthivasan S, Lansky A, LeBérichel J, Walker L, Rahman AH, Gnjatic S, Adam J, Martin JC, Wolf A, Flores R, Beasley MB, Pradhan R, Müller S, Marron TU, Turley SJ, Merad M, Kenigsberg E, Salmon H. (2022) Spatial positioning and matrix programs of cancer-associated fibroblasts promote T cell exclusion in human lung tumors. Cancer Discov. Aug 26:CD-21-1714. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/36027053/

Goyal, G., Prabhala, P., Mahajan, G., Bausk, B., Gilboa, T., Xie, L., ... & Ingber, D. E. (2022). Ectopic Lymphoid Follicle Formation and Human Seasonal Influenza Vaccination Responses Recapitulated in an Organ-on-a-Chip. Advanced Science, 2103241. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/35289122/

Jamal-Hanjani M, …, Swanton C, TRACERx Consortium. (2017) Tracking the evolution of Non-Small Cell Lung Cancer. The New England Journal of Medicine. 376(22):2109-2121. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/28445112/

Jun Y, Lee J, Choi S, Yang JH, Sander M, Chung S, Lee SH. (2019). In vivo–mimicking microfluidic perfusion culture of pancreatic islet spheroids. Science advances, 5(11), eaax4520. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/31807701/

Hachey SJ, Movsesyan S, Nguyen QH, Burton-Sojo G, Tankazyan A, Wu J., ...Hughes CC. (2021). An in vitro vascularized micro-tumor model of human colorectal cancer

recapitulates in vivo responses to standard-of-care therapy. Lab Chip, 21(7), 1333-1351. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/33605955/

Hu H, Piotrowska Z, Hare PJ, Chen H, Mulvey HE, Mayfield A, Noeen S, Kattermann K, Greenberg M, Williams A, Riley AK, Wilson JJ, Mao YQ, Huang RP, Banwait MK, Ho J, Crowther GS, Hariri LP, Heist RS, Kodack DP, Pinello L, Shaw AT, Mino-Kenudson M, Hata AN, Sequist LV, Benes CH, Niederst MJ, Engelman JA. (2021) Three subtypes of lung cancer fibroblasts define distinct therapeutic paradigms. Cancer Cell. 39(11):1531-1547.e10. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/34624218/

Kratz JR, Li JZ, Tsui J, Lee JC, Ding VW, Rao AA, Mann MJ, Chan V, Combes AJ, Krummel MF, Jablons DM. (2021) Genetic and immunologic features of recurrent stage I lung adenocarcinoma. Sci Rep. 11(1):23690. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/34880292/

Lambrechts D, Wauters E, Boeckx B, Aibar S, Nittner D, Burton O, Bassez A, Decaluwé H, Pircher A, Van den Eynde K, Weynand B, Verbeken E, De Leyn P, Liston A, Vansteenkiste J, Carmeliet P, Aerts S, Thienpont B. (2018) Phenotype molding of stromal cells in the lung tumor microenvironment. Nat Med. 24(8):1277-1289. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/29988129/

Leader AM, Grout JA, Maier BB, Nabet BY, Park MD, Tabachnikova A, Chang C, Walker L, Lansky A, Le Berichel J, Troncoso L, Malissen N, Davila M, Martin JC, Magri G, Tuballes K, Zhao Z, Petralia F, Samstein R, D'Amore NR, Thurston G, Kamphorst AO, Wolf A, Flores R, Wang P, Müller S, Mellman I, Beasley MB, Salmon H, Rahman AH, Marron TU, Kenigsberg E, Merad M. (2021) Single-cell analysis of human non-small cell lung cancer lesions refines tumor classification and patient stratification. Cancer Cell. 39(12):1594-1609.e12. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/34767762/

Lehtiö J, Arslan T, Siavelis I, Pan Y, Socciarelli F, Berkovska O, Umer HM, Mermelekas G, Pirmoradian M, Jönsson M, Brunnström H, Brustugun OT, Purohit KP, Cunningham R, Asl HF, Isaksson S, Arbajian E, Aine M, Karlsson A, Kotevska M, Hansen CG, Haakensen VD, Helland Å, Tamborero D, Johansson HJ, Branca RM, Planck M, Staaf J, Orre LM. (2021) Proteogenomics of non-small cell lung cancer reveals molecular subtypes associated with specific therapeutic targets and immune evasion mechanisms. Nat Cancer. 2(11):1224-1242. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/34870237/

Lin Z, Huang L, Li S, Gu J, Cui X, Zhou Y. (2020). Pan-cancer analysis of genomic properties and clinical outcome associated with tumor tertiary lymphoid structure. Sci Rep. 10(1):21530. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/33299035/

Luke JJ, Bao R, Sweis RF, Spranger S, Gajewski TF. (2019) WNT/β-catenin Pathway Activation Correlates with Immune Exclusion across Human Cancers. Clin Cancer Res. 25(10):3074-3083. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/30635339/

Meylan M, Petitprez F, Becht E, Bougoüin A, Pupier G, Calvez A, Giglioli I, Verkarre V, Lacroix G, Verneau J, Sun CM, Laurent-Puig P, Vano YA, Elaïdi R, Méjean A, Sanchez-Salas R, Barret E, Cathelineau X, Oudard S, Reynaud CA, de Reyniès A, Sautès-Fridman C, Fridman WH. (2022) Tertiary lymphoid structures generate and propagate anti-tumor antibody-producing plasma cells in renal cell cancer. Immunity. 55(3):527-541.e5. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/35231421/

Pabani A, Butts CA (2018) Current Landscape of Immunotherapy for the Treatment of Metastatic Non-Small-Cell Lung Cancer. Current Oncology, *25*(s1), 94-102. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/29910652/

Peranzoni E, Lemoine J, Vimeux L, Feuillet V, Barrin S, Kantari-Mimoun C, Bercovici N, Guérin M, Biton J, Ouakrim H, Régnier F, Lupo A, Alifano M, Damotte D, Donnadieu E. (2018) Macrophages impede CD8 T cells from reaching tumor cells and limit the efficacy of anti-PD-1 treatment. Proc Natl Acad Sci U S A. 115(17):E4041-E4050. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/29632196/

Petitprez F, de Reyniès A, Keung EZ, Chen TW, Sun CM, Calderaro J, Jeng YM, Hsiao LP, Lacroix L, Bougoüin A, Moreira M, Lacroix G, Natario I, Adam J, Lucchesi C, Laizet YH, Toulmonde M, Burgess MA, Bolejack V, Reinke D, Wani KM, Wang WL, Lazar AJ,

Roland CL, Wargo JA, Italiano A, Sautès-Fridman C, Tawbi HA, Fridman WH. (2020) B cells are associated with survival and immunotherapy response in sarcoma. Nature. 577(7791):556-560. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/31942077/

Rooney MS, Shukla SA, Wu CJ, Getz G, Hacohen N. (2015) Molecular and genetic properties of tumors associated with local immune cytolytic activity. Cell. 160(1-2):48-61. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/25594174/

Roumenina LT, Daugan MV, Noé R, Petitprez F, Vano YA, Sanchez-Salas R, Becht E, Meilleroux J, Clec'h BL, Giraldo NA, Merle NS, Sun CM, Verkarre V, Validire P, Selves J, Lacroix L, Delfour O, Vandenberghe I, Thuilliez C, Keddani S, Sakhi IB, Barret E, Ferré P, Corvaïa N, Passioukov A, Chetaille E, Botto M, de Reynies A, Oudard SM, Mejean A, Cathelineau X, Sautès-Fridman C, Fridman WH. (2019) Tumor Cells Hijack Macrophage-Produced Complement C1q to Promote Tumor Growth. Cancer Immunol Res. 7(7):1091-1105. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/31164356/

Roumenina LT, Daugan MV, Petitprez F, Sautès-Fridman C, Fridman WH. (2019) Context-dependent roles of complement in cancer. Nat Rev Cancer. 19(12):698-715. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/31666715/

Salmon H, Franciszkiewicz K, Damotte D, Dieu-Nosjean MC, Validire P, Trautmann A, Mami-Chouaib F, Donnadieu E. (2012) Matrix architecture defines the preferential localization and migration of T cells into the stroma of human lung tumors. J Clin Invest. 122(3):899-910. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/22293174/

Sautès-Fridman C, Petitprez F, Calderaro J, Fridman WH. (2019) Tertiary lymphoid structures in the era of cancer immunotherapy. Nat Rev Cancer. 19(6):307-325. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/31092904/

Schumacher TN, Thommen DS. (2022) Tertiary lymphoid structures in cancer. Science. 375(6576):eabf9419. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/34990248/

Spranger S, Gajewski TF. (2018) Impact of oncogenic pathways on evasion of antitumour immune responses. Nat Rev Cancer. 18(3):139-147. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/29326431/

Vazquez-Victorio G, Peto-Gutierrez C, Diaz-Bello B, Cano-Jorge M, Perez-Calixto D, Jimenez-Escobar A, …, Hautefeuille M. (2019) Building a microfluidic cell culture platform with stiffness control using Loctite 3525 glue. Lab Chip. 19(20), 3512-3525. https://pubmed-ncbi-nlm-nih-gov.proxy.insermbiblio.inist.fr/31544189/

Coordonnateur et équipes participantes / Coordinator and participating teams

Liste des équipes participantes / *List of participating teams* *Ajouter autant de lignes que nécessaire / Add as many lines as necessary*				
N° de L'équipe Team number	Titre, nom et prénom du responsable scientifique dans le cadre du projet *Title, name of the scientific manager in the frame of the project* [2]	Nom de l'équipe/du laboratoire/du service hospitalier *Name of the team/laboratory or hospital department*	Institution de rattachement et ville *Affiliated institution and city*	Engagement à participer au projet (insérer la signature scannée du responsable de l'équipe) *Commitment to the participation in the project*
1	Pr. Cremer Isabelle	Team Inflammation, Complement and Cancer	INSERM UMRS1138 - Centre de Recherche des Cordeliers, Paris	
2	Dr. Donnadieu Emmanuel	Tumor and Immune Response	INSERM U1016 Institut Cochin Paris	
3	Pr. Hautefeuille Mathieu	Team Dynamic processes in Morphogenesis	Laboratoire de Biologie du Développement (UMR7622), IBPS, Sorbonne Universite, Paris	
4	Dr. Antoniewski Christophe	ARTbio platform	IBPS, Sorbonne Universite, Paris	

[2] Peut être différent du responsable hiérarchique de l'équipe/ *Can be different of the hierarchical head of the team*

CV court du coordonnateur (max 2 pages sans publication) / *Short CV of the coordinator (max 2 pages without publications list)*

Isabelle Cremer
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Nationality: French
Date of Birth: 29/02/1968
Place of Birth: Versailles
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Email ID: isabelle.cremer@sorbonne-universite.fr
Researcher ID/ORCID: 0000-0002-0963-1031

EDUCATION

• **1998**: PhD, Microbiology (fundamental virology), University Pierre et Marie Curie, Paris, France
• **2010**: French research supervisor accreditation, HDR, University Pierre et Marie Curie

PROFESSIONAL CAREER

PROFESSIONAL EXPERIENCE

• **1994-1998**: PhD, Gene therapy of HIV infection by constitutive expression of IFN-beta, CMRS U177, Institut Curie, Orsay, France
• **1999-2001**: Post-doctoral researcher, INSERM U255, Institut Curie, Paris, France
• **2001-2012**: Assistant-professor in immunology, Universite Pierre et Marie Curie, Paris, France ; INSERM UMRS1138, Team Immune microenvironment and tumors. Centre de Recherche des Cordeliers.
• **2012-Now**: Professor in immunology (classe exceptionnelle), Sorbonne Universite. INSERM UMRS1138, Team Inflammation, Complement and Cancer. Centre de Recherche des Cordeliers.

Isabelle CREMER (IC) is full time professor at Sorbonne Universite where she obtained her HDR (French research supervisor accreditation) in 2010. IC is the director of the team Inflammation, Complement and Cancer in the Centre de Recherche des Cordeliers (CRC) since 2014, a team composed of 12 full tenure researchers and clinicians. IC was the director of the doctoral school Physiology, Physiopathology and Therapeutics from 2012 to 2018. IC is a member of the Steering Committee of labex Immuno-Oncology, member of the SIRIC CARPEM. IC heads her research at CRC (UMRS 1138 INSERM, Sorbonne Universite, Université de Paris), on immuno-oncology. Her projects consist in deciphering the cellular and molecular heterogeneity of the tumor microenvironment and the interplay between infectious agents and the tumor immune microenvironment, as well as to design novel therapeutics issues in lung tumors targeting immune cells. She uses in vivo spontaneous and orthotopic mice models, and tumors from operated patients from Cochin Hospital. In 2022, IC has published 99 articles in peer-review journals, and has approx. 100 contributions in conferences. H-index 43 (WOS).

RESEARCH ACTIVITY & EXPERTISE

• Immunology, Immuno-Oncology, chronic inflammation and cancer, tumor immune microenvironment, impact of viruses on tumor progression and on anti-tumoral immune cells, Natural Killer cell biology, immunotherapy, 3D culture, mice models of lung cancer, multiplex and multiparametric imaging, transcriptomics.
• Scientific directions (past & present): Master students (17), PhD students (7), Post doc (4).
President or jury members for about 40 thesis defenses.
• Co-president of the scientific council, UFR of life science, faculty of sciences and engineering, Sorbonne Universite.
• Members: SIRIC CARPEM Universite de Paris; Onco-Immunology labex Steering Committee, Universite de Paris; Scientific and Administrative council of UFR927, Sorbonne Universite; member

of national committee of Ligue contre le Cancer; expertise for AIRC (Association italienne de recherche contre le cancer), Cancer research UK, canceropole; member of HCERES evaluation committee for doctoral school (2016).

ACADEMIC CLASSES

• Lectures in the faculty of Sorbonne Universite: UFR927 (Life Sciences)
• Non-exhaustive list: fundamental immunology, antitumoral immune responses, translational immunology, immunotherapy.

PATENTS AND OTHER SCIENTIFIC PRODUCTION

• Cherfils-Vicini J, Fridman H and Cremer I. Methods for predicting the response to anti-cancer treatment with an agonist of TLR7 or an agonist of TLR8. US 2011/0195923 A1. EP09060555. INSERM
• Cremer I. Methods for predicting the response to treatment and for treating cancer. EP14305317.1. INSERM. Mars 2014.

HONORS AND AWARDS

• 2010-2013: Grant awarded as coordinator for ARC subvention (3 collaborative teams): TLR7 and TLR8 biomarkers to predict response to chemotherapy in Non-Small Cell Lung Carcinoma
• 2010-2012: Grant awarded as coordinator for Fondation de France subvention: infection and cancer: role of TLR7 and TLR8 in lung tumor progression and resistance to chemotherapy
• 2011-2014: Grant awarded as partner for INCa PL-BIO (Institut National du Cancer) (3 collaborative teams): Phenotypic and functional characterization of Natural Killer infiltrating human solid tumors
• 2016-2019: Grant awarded as coordinator for INCa PL-BIO (4 collaborative teams): Impact of respiratory viral infections dependent on TLR7 signaling pathway on tumor progression and resistance to chemo- and immunotherapy in lung cancers
• 2017-2021: Grant awarded as coordinator for INCa PL-BIO (2 collaborative teams): Identification of new NK checkpoint inhibitors in solid tumors
• 2012-2016: Award of a grant for investment in research (Sorbonne Universite)
• 2017-2021: Award of a grant for investment in research (Sorbonne Universite)
• 2019-2021: Grant awarded as partner for ITMO PCSI. Deciphering the Immunogenic cancer Cell deAth mechanisms triggered by COld PLASma treatments - Application to lung cancer
• 2021-2024: Grant awarded as coordinator for Fondation ARC (2 collaborative teams): Protumoral impact of influenza infection in lung cancer (labellisation).

Principaux articles publiés par le coordonnateur du projet attestant de son expertise dans le domaine concerné au cours des cinq dernières années / *Mains published articles of the project coordinator justifying his/her expertise in the project field during the last five years*[3]

Roumenina LT, **Cremer I.** (2022) COMPLEMENTing immunotherapy. Nat Cancer. 3:1144-1146. doi: 10.1038/s43018-022-00442-6.PMID: 36271171

Leonardi L, Siberil S, Alifano M, **Cremer I**, Joubert PE. (2022) Autophagy-Related Gene Signature Highlights Metabolic and Immunogenic Status of Malignant Cells in Non-Small Cell Lung Cancer Adenocarcinoma. Cancers (Basel). 14:3462. doi: 10.3390/cancers14143462.PMID: 35884522

Velut Y, Decroix E, Blons H, Alifano M, Leroy K, Petitprez F, Boni A, Garinet S, Biton J, **Cremer I**, Wislez M, Boudou-Rouquette P, Arrondeau J, Goldwasser F, Fournel L, Damotte D, Mansuet-Lupo A. (2022) SMARCA4-deficient lung carcinoma is an aggressive tumor highly infiltrated by FOXP3+ cells and neutrophils. Lung Cancer. 169:13-21. doi: 10.1016/j.lungcan.2022.05.001. PMID: 35597058

Leonardi L, Sibéril S, Alifano M, **Cremer I**, Joubert PE. (2021) Autophagy Modulatio by Viral Infections Influences Tumor Development. Front Oncol. 11:743780. doi: 10.3389/fonc.2021.743780. eCollection 2021.PMID: 34745965. Review

Russick J, Foy PE, Josseaume N, Meylan M, Hamouda NB, Kirilovsky A, Sissy CE, Tartour E, Smadja DM, Karras A, Hulot JS, Livrozet M, Fayol A, Arlet JB, Diehl JL, Dragon-Durey MA, Pagès F, **Cremer I.** (2021) Immune Signature Linked to COVID-19 Severity: A SARS-Score for Personalized Medicine. Front Immunol. 12:701273. doi: 10.3389/fimmu.2021.701273. eCollection 2021.PMID: 34322128

Joseph A, Juncheng P, Mondini M, Labaied N, Loi M, Adam J, Lafarge A, Astesana V, Obrist F, Klein C, Bloy N, Stoll G, Signolle N, Genestie C, Damotte D, Alifano M, Leary A, Pautier P, Morice P, Gouy S, Deutsch E, Chargari C, Dieu-Nosjean MC, **Cremer I**, Michels J, Kroemer G, Castedo M. (2021) Metabolic features of cancer cells impact immunosurveillance. J Immunother Cancer. 9(6):e002362. doi: 10.1136/jitc-2021-002362.PMID: 34162714

Daugan MV, Revel M, Russick J, Dragon-Durey MA, Gaboriaud C, Robe-Rybkine T, Poillerat V, Grunenwald A, Lacroix G, Bougouin A, Meylan M, Verkarre V, Oudard SM, Mejean A, Vano YA, Perkins G, Validire P, Cathelineau X, Sanchez-Salas R, Damotte D, Fremeaux-Bacchi V, **Cremer I**, Sautès-Fridman C, Fridman WH, Roumenina LT. (2021) Complement C1s and C4d as Prognostic Biomarkers in Renal Cancer: Emergence of Noncanonical Functions of C1s. Cancer Immunol Res. 9:891-908. doi: 10.1158/2326-6066.CIR-20-0532. PMID: 34039653

Daugan MV, Revel M, Thouenon R, Dragon-Durey MA, Robe-Rybkine T, Torset C, Merle NS, Noé R, Verkarre V, Oudard SM, Mejean A, Validire P, Cathelineau X, Sanchez-Salas R, Pickering MC, **Cremer I**, Mansuet-Lupo A, Alifano M, Sautès-Fridman C, Damotte D, Fridman WH, Roumenina LT. (2021) Intracellular Factor H Drives Tumor Progression Independently of the Complement Cascade. Cancer Immunol Res. 9:909-925. doi: 10.1158/2326-6066.CIR-20-0787. PMID: 34039652

Angrini M, Varthaman A, Garcia-Verdugo I, Sallenave JM, Alifano M, **Cremer I.** (2021) To Vaccinate or not: Influenza Virus and Lung Cancer Progression. Trends Cancer. 7:573-576. doi: 10.1016/j.trecan.2021.02.006. PMID: 33712391

Dubuisson A, Fahrner JE, Goubet AG, Terrisse S, Voisin N, Bayard C, Lofek S, Drubay D, Bredel D, Mouraud S, Susini S, Cogdill A, Rebuffet L, Ballot E, Jacquelot N, Thomas de

[3] En cas d'acceptation de publication (relative au projet) au cours de l'évaluation, une mise à jour peut être envoyée à l'INCa/ *In case of acceptance of publication (relative to the project) during the course of the evaluation, an update can be sent to INCa*

Montpreville V, Casiraghi O, Radulescu C, Ferlicot S, Figueroa DJ, Yadavilli S, Waight JD, Ballas M, Hoos A, Condamine T, Parier B, Gaudillat C, Routy B, Ghiringhelli F, Derosa L, Breuskin I, Rouanne M, André F, Lebacle C, Baumert H, Wislez M, Fadel E, **Cremer I**, Albiges L, Geoerger B, Scoazec JY, Loriot Y, Kroemer G, Marabelle A, Bonvalet M, Zitvogel L. (2021) Immunodynamics of explanted human tumors for immuno-oncology. EMBO Mol Med. 13:e12850. doi: 10.15252/emmm.202012850. PMID: 33372722

Russick J, Joubert PE, Gillard-Bocquet M, Torset C, Meylan M, Petitprez F, Dragon-Durey MA, Marmier S, Varthaman A, Josseaume N, Germain C, Goc J, Dieu-Nosjean MC, Validire P, Fournel L, Zitvogel L, Bindea G, Lupo A, Damotte D, Alifano M, **Cremer I.** (2020) Natural killer cells in the human lung tumor microenvironment display immune inhibitory functions. J Immunother Cancer. 2020 8:e001054. doi: 10.1136/jitc-2020-001054.PMID: 33067317

Russick J, Torset C, Hemery E, **Cremer I.** (2020) NK cells in the tumor microenvironment: Prognostic and theranostic impact. Recent advances and trends. Semin Immunol. 48:101407. doi: 10.1016/j.smim.2020.101407. PMID: 32900565. Review

Boulle G, Velut Y, Mansuet-Lupo A, Gibault L, Blons H, Fournel L, Boni A, **Cremer I**, Wislez M, Duchatelle V, Trédaniel J, Hammond SA, Herbst R, Alifano M, Giraud P, Damotte D. (2020) Chemoradiotherapy efficacy is predicted by intra-tumour CD8+/FoxP3+ double positive T cell density in locally advanced N2 non-small-cell lung carcinoma. Eur J Cancer. 135:221-229. doi: 10.1016/j.ejca.2020.04.040. PMID: 32610210

Angrini M, Varthaman A, **Cremer I. (**2020) Toll-Like Receptors (TLRs) in the Tumor Microenvironment (TME): A Dragon-Like Weapon in a Non-fantasy Game of Thrones. Adv Exp Med Biol.;1263:145-173. doi: 10.1007/978-3-030-44518-8_9. PMID: 32588327. Review.

Kasikova L, Truxova I, **Cremer I**, Sautes-Fridman C, Kepp O, Kroemer G, Spisek R, Fucikova J. (2020) Side-by-side comparison of flow cytometry and immunohistochemistry for detection of calreticulin exposure in the course of immunogenic cell death. Methods Enzymol.;632:15-25. doi: 10.1016/bs.mie.2019.05.025. PMID: 32000894

Hervier B, Russick J, **Cremer I**, Vieillard V. (2019) NK Cells in the Human Lungs. Front Immunol. 10:1263. doi: 10.3389/fimmu.2019.01263. eCollection 2019.PMID: 31275301. Review.

Dajon M, Iribarren K, Petitprez F, Marmier S, Lupo A, Gillard M, Ouakrim H, Victor N, Vincenzo DB, Joubert PE, Kepp O, Kroemer G, Alifano M, Damotte D, **Cremer I.** (2018) Toll like receptor 7 expressed by malignant cells promotes tumor progression and metastasis through the recruitment of myeloid derived suppressor cells. Oncoimmunology. 8:e1505174. doi: 10.1080/2162402X.2018.1505174. eCollection 2019.PMID: 30546943

Biton J, Ouakrim H, Dechartres A, Alifano M, Mansuet-Lupo A, Si H, Halpin R, Creasy T, Bantsimba-Malanda C, Arrondeau J, Goldwasser F, Boudou-Rouquette P, Fournel L, Roche N, Burgel PR, Goc J, Devi-Marulkar P, Germain C, Dieu-Nosjean MC, Cremer I, Herbst R, Damotte D. (2018). Impaired Tumor-Infiltrating T Cells in Patients with Chronic Obstructive Pulmonary Disease Impact Lung Cancer Response to PD-1 Blockade. Am J Respir Crit Care Med. 198:928-940. doi: 10.1164/rccm.201706-1110OC.PMID: 29518341

Biton J, Mansuet-Lupo A, Pécuchet N, Alifano M, Ouakrim H, Arrondeau J, Boudou-Rouquette P, Goldwasser F, Leroy K, Goc J, Wislez M, Germain C, Laurent-Puig P, Dieu-Nosjean MC, **Cremer I**, Herbst R, Blons HF, Damotte D. (2018) TP53, STK11 and EGFR mutations predict tumor immune profile and the response to anti-PD-1 in lung adenocarcinoma. Clin Cancer Res. pii: clincanres. 24:5710-5723. doi: 10.1158/1078-0432.CCR-18-0163. PMID: 29764856

Braud VM, Biton J, Becht E, Knockaert S, Mansuet-Lupo A, Cosson E, Damotte D, Alifano M, Validire P, Anjuère F, **Cremer I**, Girard N, Gossot D, Seguin-Givelet A, Dieu-Nosjean MC, Germain C. (2018) Expression of LLT1 and its receptor CD161 in lung cancer is associated with better clinical outcome. Oncoimmunology. 7:e1423184. doi: 10.1080/2162402X.2017.1423184. eCollection 2018.

Dajon M*, Iribarren K*, Petitprez F, Marmier S, Lupo A, Gillard M, Ouakrim H, Navas V, Di Bartolo V, Joubert PE, Kepp O, Kroemer G, Alifano A, Damotte D, **Cremer I**. (2018) Toll

like receptor 7 expressed by malignant cells promotes tumor progression and metastasis through the recruitment of myeloid derived suppressor cells. OncoImmunology. Oct 11;8(1):e1505174. *equal contribution. Oncoimmunology. doi: 10.1080/2162402X.2018.1505174. eCollection 2019.

Mansuet-Lupo A, Barritault M, Alifano M, Janet-Vendroux A, Zarmaev M, Biton J, Velut Y, Le Hay C, **Cremer I**, Régnard JF, Fournel L, Rance B, Wislez M, Laurent-Puig P, Herbst R, Damotte D, Blons H. (2019) Proposal for a combined histo-molecular algorithm to distinguish multiple primary adenocarcinomas from intrapulmonary metastasis in patients with multiple lung tumors. J Thorac Oncol. 14:844-856. doi: 10.1016/j.jtho.2019.01.017. PMID: 30721797

Holicek P, Truxova I, Kasikova L, Vosahlikova S, Salek C, Rakova J, Holubova M, Lysak D, **Cremer I**, Spisek R, Fucikova J. (2020) Assessment of NK cell-mediated cytotoxicity by flow cytometry after rapid, high-yield isolation from peripheral blood. Methods Enzymol. 631:277-287. doi: 10.1016/bs.mie.2019.05.034. PMID: 31948552

Kasikova L, Truxova I, **Cremer I**, Sautes-Fridman C, Kepp O, Kroemer G, Spisek R, Fucikova J. (2020) Side-by-side comparison of flow cytometry and immunohistochemistry for detection of calreticulin exposure in the course of immunogenic cell death. Methods Enzymol. 632:15-25. doi: 10.1016/bs.mie.2019.05.025. PMID: 32000894

Kasikova L, Hensler M, Truxova I, Skapa P, Laco J, Belicova L, Praznovec I, Vosahlikova S, Halaska MJ, Brtnicky T, Rob L, Presl J, Kostun J, **Cremer I**, Ryska A, Kroemer G, Galluzzi L, Spisek R, Fucikova J. (2019) Calreticulin exposure correlates with robust adaptive antitumor immunity and favorable prognosis in ovarian carcinoma patients. J Immunother Cancer. 7:312. doi: 10.1186/s40425-019-0781-z.PMID: 31747968

Plan financier / Financial plan

Détailler uniquement le financement demandé (en €) / Please describe the requested budget only (in €)

Equipe / *Team*	**Type de dépense / *Type of costs***	**Budget**
1	Personnel / *Staff (36 months post-doc)*	154 500
	Consommable / *Consumables*	211 000
	Equipement / *Equipment*	15 000
	Frais de gestion / *Overheads*	30 440
2	Personnel / *Staff*	0
	Consommable / *Consumables*	33 000
	Equipement / *Equipment*	0
	Frais de gestion / *Overheads*	2 640
3	Personnel / *Staff (12 months experienced-engineer*	44 900
	Consommable / *Consumables*	27 500
	Equipement / *Equipment*	0
	Frais de gestion / *Overheads*	5 792
4	Personnel / *Staff 24-months bioinformatics post-doc*	103 000
	Consommable / *Consumables*	0
	Equipement / *Equipment*	12000
	Frais de gestion / *Overheads*	9 200
Total		**648 972**

Ajouter autant d'équipes que nécessaire / Add as many teams as necessary