Immune Responses in Cancer and Infection
2nd International Symposium

IRCI-2022

PROGRAM
&
ABSTRACT BOOK

June 15th-17th, 2022
Amphithéâtre Charles Mérieux | Lyon, France

www.irci2022.insight-outside.fr

Centre International de Recherche en Infectiologie

CRCL CANCER RESEARCH CENTER OF LYON

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Preface

The International Center for Infectiology Research (CIRI) and the Cancer Research Center of Lyon (CRCL) are jointly organizing this 2nd International Symposium on Immune Responses in Cancer and Infection and are pleased to welcome you at the Charles Mérieux Amphitheater, Lyon.

The symposium will bring together clinicians and researchers interested in the immunology of cancers and infections to present and discuss the latest scientific advances. How the current understanding of immune mechanisms might enable the development of next-generation vaccines or curative therapies for patients with cancer or infections will be an over-arching theme of this international symposium.

We are most pleased to welcome a panel of 20 prestigious invited speakers from 8 countries, who will discuss their latest research. To complete this high-quality program, the Organizing Committee has selected 18 researchers to give short-talks and over 130 posters will be presented. We feel that this symposium represents an excellent opportunity to share your research and form new and meaningful collaborations.

This symposium could not have taken place without the strong and generous support of our numerous sponsors and partners. We would like to take this opportunity to express our thanks to them and all those that participated in the scientific programming and local organization.

We wish you all a very pleasant and exciting conference and a nice stay in Lyon, a city with a considerable historical heritage in addition to being known as the gastronomic capital of France.

Drs Uzma Hasan & Julien C. Marie, heads of the Organizing and Scientific Committee

Organizing and Scientific Committee

Uzma Hasan, CIRI
Julien Marie, CRCL
Pierre Chaumont, CRCL
Marlène Dreux, CIRI
Yenkel Grinberg-Bleyer, CRCL
Marie-Cécile Michallet, CRCL
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CIRI

The CIRI (Centre International de Recherche en Infectiologie) is a joint research unit of the University of Lyon 1, Inserm, CNRS and ENS de Lyon, Jean Monnet University and Hospices Civils de Lyon in partnership with VetAgro Sup and the Institut Pasteur. The CIRI brings together scientific and medical research communities from complementary disciplinary backgrounds, grouped into 3 specialties namely bacteriology, immunology and virology, as well as epidemiology and eco-infectiology. They all work together with the main objective of understanding the interactions between microbes and their hosts in order to better fight against infectious and immune diseases. In close collaboration with hospitals and private companies, CIRI conducts research projects ranging from fundamental to translational, leading to scientific publications, patent applications, public-private collaborations and start-ups creations.

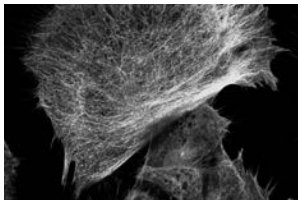
The CIRI is composed of more than 400 people, researchers, faculty members, graduate students, engineers and technicians divided in 24 teams and 2 emerging CIRI groups mainly based in Lyon Gerland. The Center is directed by Dr. François-Loïc Cosset, his deputy director Professor François Vandenesch assisted by six delegate directors: Thierry Walzer (Immunology), Thomas Henry (Bacteriology), Andrea Cimarelli (Virology), Nathalie Alazard-Dany, Jacqueline Marvel and Patricia Doublet.

The research programs of CIRI cover major research areas of infectious diseases, immunology and epidemiology :

- Lessons from the diversity of clinical and environmental strains
- Cell biology of infections and innate immunity
- Physiopathogenesis of emerging and highly pathogenic viruses in humans
- Inflammatory diseases
- Immunotherapy and Vaccination

With his partners, the CIRI plays an active part in local and national initiatives concerning infectious diseases, such as, for example, the PIExmico levels 3 platforms, the Ecofect LabEx and the InfectioTron EquipEx+ projects, that aim at developing in Lyon the “One Health” concept in the field of emerging infectious diseases or several National Reference Centers on infectious diseases directed by clinicians or researchers from the CIRI.

CIRI Website: http://ciri.ens-lyon.fr

CRCL

The Cancer Research Center of Lyon is a research structure affiliated with the University Claude Bernard Lyon 1, the National Health and research bodies (Inserm and CNRS), the Léon Bérard Comprehensive Cancer Centre (CLB) and with the Lyon University Hospitals (HCL) as clinical partners. The CRCL was officially created in January 2011. With 24 research teams, and around 500 members, the CRCL is regarded as one of the biggest research centers dedicated to cancer in France. The CRCL is directed by Dr. Patrick Mehlen, assisted by Pr. Charles Dumontet and Dr. Véronique Maguer-Satta as Deputies.

The CRCL is organized around two scientific departments focusing on the following research topics:

- Tumor escape, resistance and immunity – TERI
- Cancer initiation and tumor cell identity – CITI

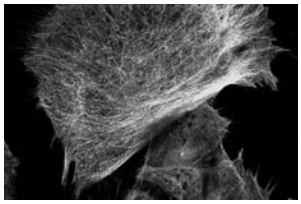
The scientists at the CRCL can rely on state-of-the-art equipment allowing analysis in single cell, spectral flow cytometry, spectral cell sorter, bi-photonic microscopy…

One of the main goals of the CRCL is to support the development of strong translational research to enable patients to rapidly benefit from breakthroughs in basic research. This bridge from bench to bedside was rendered possible due to the strong collaboration between scientific teams and clinicians/pathologists of the CLB and HCL, creating a continuity between basic research and clinical applications.

Beyond its own research programs, the CRCL has been a stepping-stone for the development of new research structures in Lyon: the DEVweCAN LabEx (Dir. Dr Patrick Mehlen) and the LYRICAN (Lyon Integrated Research Institute in Cancer, Dir. Prof Jean-Yves Blay). The creation of these two structures, linking both hospitals and the CRCL, was a key element towards the implantation of a comprehensive cancer center in Lyon.

Finally, the CRCL actively participated to the creation with the Lyon 1 University to a Master's program dedicated to oncology, in which immuno-oncology and viro-oncology are actively taught.

CRCL website: www.crcl.fr

Program

Wednesday 15th of June, 2022 | *Amphithéâtre Charles Mérieux*

8:30 AM to 10 AM — Registration and welcome coffee

10:00 AM — **Opening**

10:15 AM — Keynote Lecture: **Ruslan Medzhitov**, Yale University School of Medicine, Howard Hughes Medical Institute, USA
Tissue Homeostasis and Inflammation

SESSION 1

11:00 AM — **Innate Immunity - *Endorsed by Innasco***
Moderators: *Benedicte Py & Giorgio Trinchieri*

- Sandra S. Diebold, National Institute for Biological Standards and Control, UK
  Targeted delivery of endosomal toll-like receptor agonists for cancer therapy
- Clara Taffoni, Institut de Génétique Humaine, France
  The cooperation between the cgas-sting and DNA-PK pathways shapes cancer-related inflammation
- Geert Van Loo, VIB-UGent Center for Inflammation Research, Belgium
  Otulin in inflammation, cell death, and disease

12:00 PM — **Lunch and Posters Topics 1 & 2**

2:00 PM — Moderators: *Benedicte Py & Giorgio Trinchieri*

- Nelson O. Gekara, Laboratory for Molecular Infection Medicine Sweden
  The role of the Immune DNA sensors cGAS and ALRs in genome stability and host-microbiota interactions
- Nathalie Bendriss-Vermare, Centre de Recherche en Cancérologie de Lyon, France
  Interleukin-33 drives polyfunctionality and antitumor activity of a unique ST2+ NK cell population
- Jonathan C. Kagan, Harvard Medical School and Boston Children's Hospital, USA
  Hyperactive dendritic cells and the control of anti-tumor immunity

3:30 PM — **Coffee Break**

Wednesday 15th of June, 2022 | *Amphithéâtre Charles Mérieux*

SESSION 2

4:00 PM **Immuno-metabolism**

Moderators: Thierry Walzer & Nadine Laguette

- Ping-Chih Ho, Department of Fundamental Oncology, University of Lausanne, Switzerland
  Targeting mitochondrial dynamics for tailoring T cell exhaustion program and anti-tumor response
- Leonid Pobezinsky, Universtity of Massachussetts, USA
  Let-7 miRNAs define CD8 T cell fate
- Antoine Marçais, Centre International de Recherche en Infectiologie, France
  IL-15 and IL-18 mobilize both classic and non-canonical pathways to activate mTORC1 in primary NK cells
- Hélène Poinot, Institute of Pharmaceutical Sciences of Western Switzerland
  Effect of endogenous glucocorticoids on the antitumor immune response in renal cancer
- Federica M. Marelli-Berg, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, UK
  Glucose Transporter 2 regulates CD8+ T cell metabolism and function via environment sensing

6:00 PM **Poster teasers – Topics 1&2**

FROM 6:30 PM **Welcome Cocktail and posters - Topics 1&2**

Thursday 16th of June, 2022 | *Amphithéâtre Charles Mérieux*

SESSION 3

9:00 AM **Immunotherapy and clinic** - ***Endorsed by Fondation Arc***

Moderators: *Christophe Caux & Thomas Gajewski*

- John Wherry, Perelman School of Medicine, University of Pennsylvania, USA
- Lucas Blanchard, Institut de Pharmacologie et Biologie Structurale, France
  Tumor-associated high endothelial venules mediate lymphocyte entry into tumors and predict response to PD-1 plus CTLA-4 combination immunotherapy
- Stéphane Depil, Centre de Recherche en Cancérologie de Lyon, France
  Human Endogenous Retroviruses represent a source of shared tumor epitopes inducing high-avidity cytotoxic T cells for cancer immunotherapy

10:15 AM **Coffee Break**

SESSION 3

10:45 AM

Moderators: Christophe Caux & Thomas Gajewski

- Julie Déchanet-Merville, ImmunoConcEpT, CNRS, Bordeaux University, France
  Harnessing Gamma-Delta T cell functions against cancer and infectious diseases
- Mariana Diniz**,** Division of Infection and Immunity, University College London, UK
  NK Cells Limit CD8+T-cell Immunity in a PDL1-dependent Manner
- Mansun Law, The Scripps Research Institute, USA
  The genetically conserved VH1-69 neutralizing antibody response in hepatitis C infection and vaccination
- Ido Amit, Weizmann Institute of Science, Israel
  The power of ONE: Immunology in the age of single cell genomics

12:30 PM to 2:30 PM **Lunch and posters - Topics 3&4**

1:30 PM to 2:15 PM **Meet the editors**

SESSION 4

2:30 PM **Microbiota** - ***Endorsed by Maat Pharma***

Moderators: Yasmine Belkaid & Julien Marie

- Thomas F. Gajewski, Duchossois Center for Advanced Medicine (DCAM), USA
  Microbiome and germline variants can control ani-tumor immunity by regulating myeloid cells within the tumor microenvironment
- Meriem Messaoudene, University of Montreal Research Center, Canada
  Polyphenol castalagin exerts antitumor activity and potentiates anti-PD-1 immune efficacy through a beneficial shift in microbiome composition
- Jonathan D. Schertzer, Farncombe Family Digestive Health Research Institute, Canada
  Blood glucose interacts with host-microbe symbiosis

3:45 PM **Poster teasers – Topics 3&4**

4:00 PM **Coffee Break**

Thursday 16th of June, 2022 | *Amphithéâtre Charles Mérieux*

SESSION 4

4:30 PM

Moderators: Yasmine Belkaid & Julien Marie

- Giorgio Trinchieri, Laboratory of Integrative Cancer Immunology, USA
  Targeting the microbiota for cancer therapy
- Lars Vereecke, VIB Center for Inflammation Research, Belgium
  Host-microbiota interactions during colorectal cancer development

5:15 PM Keynote Lecture: **Yasmine Belkaid,** National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA
Multi-kingdom control of tissue physiology and repair

7:30 PM **Gala dinner at "Le Selcius" Restaurant** (see p.13)

Friday 17th of June, 2022 | *Amphithéâtre Charles Mérieux*

SESSION 5

9:30 AM **Genetics and epigenetics**

Moderators: Lynnette Fernandez-Cuesta & Yenkel Grinberg-Bleyer

- Esteban Ballestar, Josep Carreras Institute, Spain
  Atlasing Human Primary Immunodeficiency Patients and Inspecting their Responses to Infections
- Laurent Genestier, Centre International de Recherche en Infectiologie, France
  JARID2 deletion drives NKT cell lymphomagenesis

10:15 AM **Coffee Break**

10:45 AM

Moderators: Lynnette Fernandez-Cuesta & Yenkel Grinberg-Bleyer

- Julien C. Marie, Centre de Recherche en Cancérologie de Lyon, France
  TGF-ß signaling Ensures the Stability of the Epigenetic program of Already Differentiated TH17 cells preventing from inflammation-induced cancer
- Lucie Etienne, Centre International de Recherche en Infectiologie, France
  Distinct evolutionary trajectories of sars-cov-2 interacting proteins in bats and primates identify host determinants of covid-19
- Anne Puel, Laboratory of Human Genetics of Infectious Diseases, France
  Human genetic and immunological host factors in critical COVID-19 pneumonia

12:00 PM **Poster teasers - Topics 5&6**

12:15 PM **Lunch and posters - Topics 5&6**

SESSION 6

1:45 PM **Infections and immune responses**

Moderators: Marie-Cécile Michallet & John Wherry

- Mala K. Maini, Division of Infection and Immunity, University College London, UK
  Pre-existing polymerase-specific T cells expand in abortive seronegative SARS-CoV-2 infection
- Adrian Hayday, The Francis Crick Institute, UK
  "Titre"
- Lion Uhl, Universtity of Oxford, UK
  The role of Interferon-gamma-mediated T cell collectivity on CD8+ T cell responses

3:00 PM **Coffee break**

1:45 PM **Infections and immune responses**

Moderators: Marie-Cécile Michallet & John Wherry

- Sophie Trouillet-Assant, Centre International de Recherche en Infectiologie, France
  Deciphering the Type I interferon response during viral infection
- Carlson Tsui, The Peter Doherty Institute of Infection and Immunology, Australia
  The molecular regulation of CD8 T cell exhaustion in chronic infection

4:15 PM Keynote Lecture: **Antonio Bertoletti,** Duke-NUS Graduate Medical School, Singapore
Engineering virus-specific T lymphocytes for treatment of virus-related cancer and viral infection

5:00 PM **Poster prizes and Closing Ceremony**

Conference venue access

• How to get to the Amphitéâtre Charles Mérieux Ecole Normale Supérieure de Lyon, 46 allée d'Italie, 69007 Lyon.

From the Airport to Downtown Lyon

The RhônExpress Tramway links the airport to the city center in 30 minutes and runs every 15 minutes. The RhônExpress ticket desk is located on the 1st floor of the airport's main hall, open from 5am to 11 pm. Tickets can also be reserved in advance on-line (https://www.rhonexpress.fr/en). The terminus is at the Part-Dieu railway station in central Lyon.
Price: one way: from 16.30€, return: from 28.30€

Taxi numbers: *(around 50€)*

– -Taxi Airport: +33(0)6 58 76 78 64
- Taxi Lyonnais: +33(0)4 78 26 81 81

From Part-Dieu Station and RhônExpress terminus

The conference center at the ENS can be reached from the Part-Dieu station using the B metro line (direction "Gare d'Oullins") getting off at "Debourg" metro station (the journey should take about 10 minutes and there are 5 stops).

From Perrache Station

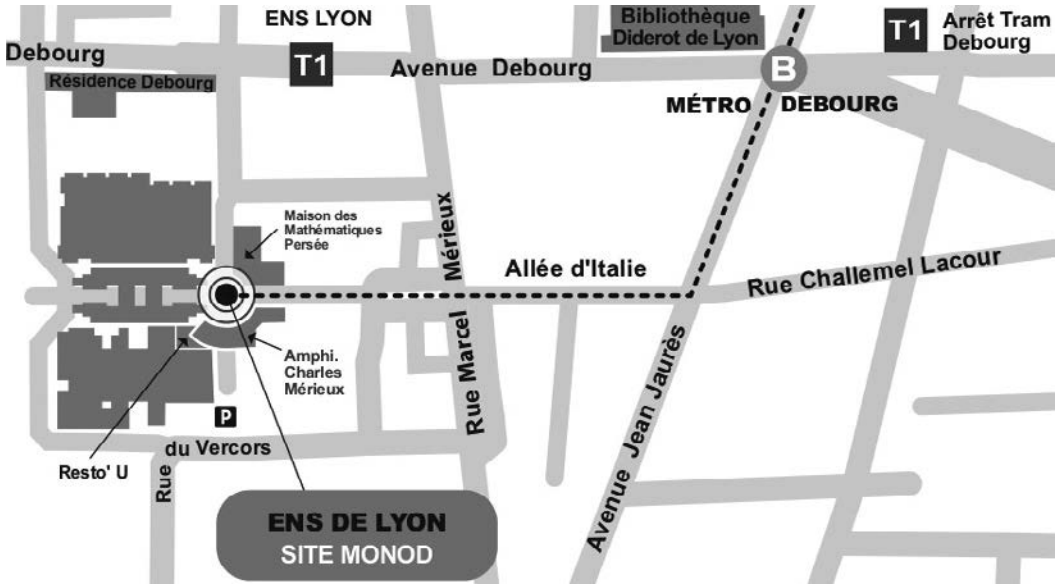
Take the Tram line 1 direction "Debourg" and get off at "Debourg" (terminus).
Alternatively take the C22 bus direction "Grange Blanche" and get off at "Debourg".

Local Public Transport (see map in your bag)

Lyon has a comprehensive public transport system including buses, trams and underground lines. Tickets can be purchased at metro stations or tram stops.
Price: Single tickets (valid for 1 hour): 1.90€

ATTENTION: there is no public transport between 12 pm and 5 am!

ENS access map:



Practical information

• Useful numbers and addresses

- European emergency calls : 112
- Emergency ambulance service : 15
- SOS doctors: +33(0)4 78 83 51 51
- Police : 17

HOSPITAL

- Saint Joseph / Saint Luc: 20 quai Claude Bernard, 69007 Lyon – Phone : +33(0)8 26 28 81 81

CHEMISTS NEAR THE CONFERENCE VENUE

- *Pharmacie centrale de Gerland :*
  285 Avenue Jean Jaurès 69007 Lyon
  Phone: +33(0)4 78 72 62 41

LATE NIGHT CHEMISTS

- *Grande Pharmacie Lyonnaise :*
  22 rue de la République, 69002 Lyon
  Phone: +33(0)4 72 56 44 00 – open from 8 pm to 8 am

• General information

WELCOME / REGISTRATION DESK
The welcome desk is located in the Auditorium's entrance hall.
Upon your arrival, take your e-badge and go directly to the welcome desk to get your congress bag and lanyard.
Registration will be open from 8.30 am on the 15th of June, (8.30 am on the 16th and 17th) to 6 pm.

BADGE
Please wear your badge at all times in order to gain access into the meeting rooms.

LANGUAGE
The official meeting language is English. There will be no simultaneous translation.

INSURANCE
Organizers do not assume any liability for personal injuries sustained or loss of, or damage to, property belonging to congress participants (or their accompanying persons), either during or as result of the congress. Participants are requested to make their own arrangements with respect to health and travel insurance.

CERTIFICATE OF ATTENDANCE
Registered participants will receive a certificate of attendance by email, after the event.

CLOAKROOM
A cloakroom located next to the registration desk will be open throughout the conference to store luggage and coats

WIFI
Free WIFI connection will be available in the entrance hall and exhibition area.

CATERING FACILITIES
Coffee breaks and lunches are included in the registration fee for delegates.

TRADE AND POSTER EXHIBITION
The trade and poster exhibition are located within the Auditorium facilities.

DRESS CODE
The dress code for the Gala dinner is smart casual.

ACCOMPANYING PERSON
Accompanying persons may attend the welcome cocktail. Tickets for the gala dinner should be collected from the registration desk

CURRENCY AND BANKING
The official currency is the €uro. Money can be exchanged in banks and exchange currency offices located in the city center and at the airport.

TELEPHONE
The international code for France is +33 (dial 0033).

ELECTRICITY
Electricity in France is 220V, 50Hz. Plugs are European 2-pin.

• Tourist information center

Pavillon du Tourisme, Place Bellecour, Lyon 2
Phone : +33(0)4 72 77 69 69
www.lyon-france.com

Social Program

WELCOME COCKTAIL

Wednesday, the 15th of June, 2022
Amphithéâtre C. Mérieux, ENS Lyon

Wednesday evening, at the end of the first day of conferences, please join us for a welcome cocktail.

GALA RECEPTION

Thursday, the 16th of June, 2022
"Le Selcius" Restaurant
43, Quai Rambaud, 69002 Lyon

Historically the Selcius building was a salt warehouse where salt was stored during its transfer by ship from the south to the north of France. Located in the Confluence district and recognizable by its atypical architecture, its characteristic arches were preserved during the restoration of the river Saône's quays.

Please note that the gala dinner is an extra-option and is not included in the registration fees.

POSTER PRIZE AWARDS

Friday, the 17th of June, 2022
Auditorium Charles Mérieux, ENS Lyon

The poster award ceremony will take place on Wednesday 15th February at 5pm.

Institutional partners

Centre Léon Bérard
CNRS
Ecole Normale Supérieure de Lyon
Hospices Civils de Lyon
Inserm
Programme d'investissements d'avenir
Université Claude Bernard Lyon 1
Université de Lyon

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Cancers - Open Access Journal by MDPI
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Miltenyi Biotec
NEOMED Scientific
Ozyme
PeproTech
ProteiGene
Thermo Fisher Scientific

Keynote

TISSUE HOMEOSTASIS AND INFLAMMATION

Ruslan Medzhitov
Yale University School of Medicine, Howard Hughes Medical Institute

Mammalian tissues consist of a multiple of cell types. These cells communicate through exchange of growth factors and other paracrine signals, forming circuit interactions to ensure tissue homeostasis. However, the underlying principles of tissue organization remain poorly understood. Here I will discuss some of the properties of cell interactions as they relate to tissue homeostasis and inflammation. I will discuss key features of inflammation and its relation to tissue homeostasis. Finally, I will present a new perspective on the control logic of growth factor production, cellular composition, and regulation of tissue compartment size.

IRCI-2022

Immune Responses in Cancer and Infection
2nd International Symposium

Oral Session 1

Innate Immunity

18	O 01 - 01	TARGETED DELIVERY OF ENDOSOMAL TOLL-LIKE RECEPTOR AGONISTS FOR CANCER THERAPY Sandra S. Diebold, National Institute for Biological Standards and Control, UK
19	O 01 - 02	THE COOPERATION BETWEEN THE CGAS-STING AND DNA-PK PATHWAYS SHAPES CANCER-RELATED INFLAMMATION Clara Taffoni, Institut de Génétique Humaine, France
20	O 01 - 03	OTULIN IN INFLAMMATION, CELL DEATH, AND DISEASE Geert Van Loo, VIB-UGent Center for Inflammation Research, Belgium
21	O 01 - 04	THE ROLE OF THE IMMUNE DNA SENSORS CGAS AND ALRS IN GENOME STABILITY AND HOST-MICROBIOTA INTERACTIONS Nelson O. Gekara, The Wenner-Gren Institute, Sweden
22	O 01 - 05	INTERLEUKIN-33 DRIVES POLYFUNCTIONALITY AND ANTITUMOR ACTIVITY OF A UNIQUE ST2+ NK CELL POPULATION Nathalie Bendriss-Vermare, Centre de Recherche en Cancérologie de Lyon, France
23	O 01 - 06	HYPERACTIVE DENDRITIC CELLS AND THE CONTROL OF ANTI-TUMOR IMMUNITY Jonathan C. Kagan, Harvard Medical School and Boston Children's Hospital, USA

O **01** - 01

TARGETED DELIVERY OF ENDOSOMAL TOLL-LIKE RECEPTOR AGONISTS FOR CANCER THERAPY

Diana Corogeanu[1,2], Andrew Beavil[3], James N Arnold[4], Sandra S Diebold[1]

[1] Cellular Immunology Section, Biotherapeutics, NIBSC, Potters Bar, UK

[2] Newcastle upon Tyne Hospitals NHS Foundation Trust, Freeman Hospital, Newcastle upon Tyne, UK

[3] King's College London, School of Basic & Medical Biosciences, Randall Centre for Cell and Molecular Biophysics, London, UK

[4] King's College London, Faculty of Life Sciences and Medicine, School of Cancer and Pharmaceutical Sciences, London, UK

Endosomal toll-like receptors (TLRs) are pattern recognition receptors that upon detection of molecular patterns associated with intracellular pathogens activate innate immune cells and instruct adaptive immune responses towards a Th1 phenotype with strong cytotoxic T cell effector functions. Agonists of endosomal TLRs (TLR3, TLR7/8 or TLR9) were shown to promote anti-tumour immune responses when administered to the tumour site and the TLR7 agonist imiquimod is approved for topical administration in basal cell carcinoma and stage zero melanoma. However, when administered systemically, endosomal TLR agonists induce broad immune activation that leads to adverse reactions, which prevent their use for non-topical administration. Novel strategies for targeted delivery of TLR agonists to the tumour tissue may allow to overcome these limitations.

We have explored different approaches of linking endosomal TLR agonists to Trastuzumab, a clinically used HER2-specific antibody, to achieve targeted delivery to HER2-expressing tumour tissue. We have investigated the feasibility of the approach and its efficiency in promoting anti-tumour immune responses in a proof of principle study. Physicochemical and in vitro assays were established to characterise the composition of the generated compounds and their bioactivity on cells. In addition, selected compounds were evaluated in a mouse tumour model expressing transgenic human HER2. The data have highlighted advantages, but also limitations of individual approaches.

Notes:

O **01** - 02

THE COOPERATION BETWEEN THE CGAS-STING AND DNA-PK PATHWAYS SHAPES CANCER-RELATED INFLAMMATION

Clara Taffoni[1], Johanna Marines[1], Mathilde Saccas[1], Amel Bouzid[1], Hanane Chamma[1], Isabelle Vila[1], Fabien Blanchet[2], Nadine Laguette[1]

[1] Institut de Génétique Humaine, CNRS, Université de Montpellier, Molecular Basis of Inflammation Laboratory, Montpellier, France

[2] Institut de Recherche en Infectiologie de Montpellier, CNRS, Université de Montpellier, Biologie Quantitative du Trafic Membranaire et Pathogénèse, Montpellier, France

Keywords: cGAS-STING pathway, DNA-PK pathway, anti-tumoral responses

Genetic instability and DNA replication stress can trigger cytosolic release of self-DNA, which can be recognized by pattern recognition receptors (PRR) to promote pro-inflammatory cytokines and Interferon (IFN) production. The cGAS PRR, and its downstream adaptor protein STING, have emerged as key for the induction of type I IFN production in the presence of cytosolic dsDNA. Additionally, cGAS-STING activation promotes anti-tumor responses in vivo, prompting the development of therapeutic strategies activating this pathway to boost the immune system and promote tumor regression (1). However, numerous tumors repress cGAS and/or STING expression (2) and are consequently unresponsive to cGAS/STING-targeting strategies. This calls for the identification of alternative means to target inflammatory pathways in these contexts. Recently, the DNA-PK complex, canonically involved in DNA repair, has been identified as involved in innate-immune sensing (3). Yet, the impact of DNA-PK associated inflammatory responses in tumorigenesis is unexplored.
We used glioblastoma cell lines and patient-derived glioma cell lines, that downregulate cGAS expression to investigate the role of DNA-PK in promoting inflammatory responses in absence of cGAS. We thereby show that the DNA-PK complex can be activated in absence of cGAS, promoting STING-independent, IRF3-dependent type I IFN responses, upon challenge with exogenous dsDNA and upon genotoxic stress. Interestingly, we show that re-expression of cGAS in glioblastoma cells restores cGAS-dependent IFN responses, but also unveil a cooperation between DNA-PK and cGAS for optimal type I IFN responses. Indeed, unexpectedly, we show that DNA-PK potentiates cGAS-dependent cGAMP production. Finally, we show that this cooperation between cGAS and DNA-PK operates at early time points following challenge with dsDNA. However, it is lost at later time points, where DNA-PK negatively regulates cGAS activity. Consequently, combining cGAS-STING activation and DNA-PK inhibition promotes monocytes polarization towards anti-tumoral M1 macrophages, providing a rational for which adequate use of DNA-PKcs inhibitor alone or in combination with STING agonists could be used to boost anti-tumoral responses.

(1) M. Motwani, S. Pesiridis, and K.A. Fitzgerald, Nat Rev Genet (2019).
(2) T. Xia, H. Konno, J. Ahn, and G.N. Barber, Cell Rep (2016).
(3) C. Taffoni, A. Steer, J. Marines, H. Chamma, I.K. Vila, and N. Laguette, Front Immunol (2021).

Notes: ----------

O **01** - 03

OTULIN IN INFLAMMATION, CELL DEATH, AND DISEASE

Lien Verboom[1], Kim Lecomte[1], Esther Hoste[1], Geert van Loo[1]

[1] VIB-UGent Center for Inflammation Research, Gent, Belgium

Keywords: inflammation, innate immunity, ubiquitin signaling, deubiquitinase, ORAS

Tight control of inflammatory signaling pathways is an absolute requirement to avoid chronic inflammation and disease. One of the proteins responsible for such control is OTU deubiquitinase with linear linkage specificity (OTULIN), the only mammalian deubiquitinating enzyme (DUB) described thus far that exclusively hydrolyzes linear ubiquitin chains from proteins modified by the linear ubiquitin chain assembly complex (LUBAC). Loss-of-function mutations in OTULIN underlie a severe early-onset human autoinflammatory disease termed ORAS (OTULIN-related auto-inflammatory syndrome).

Through experimental studies with OTULIN knockout and mutant knockin mice we demonstrate the critical importance of OTULIN in protecting cells from death, thereby preventing the development of chronic tissue inflammation and eventually cancer.

Notes:

O **01** - 04

THE ROLE OF THE IMMUNE DNA SENSORS CGAS AND ALRS IN GENOME STABILITY AND HOST-MICROBIOTA INTERACTIONS.

Saskia F. Erttmann[1], Patrycja Swacha[2], Kyaw Min Aung[2], Björn Brindefalk[3], Hui Jiang[2], Anetta Härtlova[4], Bernt Eric Uhlin[1], Sun N. Wai[1], Nelson O. Gekara[1,2]

[1] The Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå Centre for Microbial Research (UCMR), Department of Molecular Biology, Umeå University, SE-90187 Umeå, Sweden

[2] Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, 106 91 Stockholm, Sweden

[3] CBRN Def ence and Security, Swedish Defence Research Agency, Umeå, Sweden.

[4] The Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg, Gothenburg, Sweden

Cyclic GMP-AMP synthase (cGAS) and the AIM2-like receptors (ALRs) are DNA binding proteins best known for their role in alerting the immune systems alerts the innate immune system to the presence of foreign or self-DNA in the cytoplasm and have thus been shown to influence the outcome of infections, inflammatory diseases, senescence, and cancer. Recently we have found that these DNA sensors are also nuclear-localized, wherein they function as regulators of chromatin structure and repair. During this seminar, I will discuss our latest findings on the role of cGAS and ALRs in immune priming, genome stability, microbiota-host interactions, and their potential as pharmacological targets for managing immune and genome instability-driven disorders.

Notes:

O **01** - 05

INTERLEUKIN-33 DRIVES POLYFUNCTIONALITY AND ANTITUMOR ACTIVITY OF A UNIQUE ST2+ NK CELL POPULATION

Anaïs Eberhardt[1], Valentin Picant1, Elena Blanc[1], Vincent Alcazer[1], Yamila Rocca[1], Maude Ardin[1], Aurélien Voissière[1], Fanny Onodi[1], Céline Rodriguez[1], Laurie Tonon[1], Benjamin Estavoyer[1], Lyvia Moudombi[1], Emilie Charrier[4], Xi Wang[5-6], Ana Stojanovic[7], Tilman Rau[3], Olivier Tredan[2], Isabelle Treilleux[2], Marie-Cécile Michallet[1], Jenny Valladeau-Guilemond[1], Antoine Marçais[4], Thierry Walzer[4], Philippe Krebs[3], Adelheid Cerwenka[7-8], Margaux Hubert[1], Christophe Caux[1]*, Nathalie Bendriss-Vermare[1]*

[1] Centre de Recherche en Cancérologie de Lyon, INSERM U1052, CNRS UMR5286, Université de Lyon, Université Lyon 1, Centre Léon Bérard, F-69000 Lyon, France.

[2] Department of Medical Oncology, Centre Léon Bérard, F-69000 Lyon, France.

[3] Institute of Pathology, University of Bern, Bern, Switzerland.

[4] CIRI, Centre International de Recherche en Infectiologie Lyon, France; INSERM, U1111, Lyon, France; École Normale Supérieure de Lyon, Lyon, France; Université Lyon 1, Lyon, France; and CNRS, UMR 5308, Lyon, France.

[5] State Key Laboratory of Reproductive Medicine, Nanjing Medical University, Nanjing 211166, China

[6] Division of Cellular Immunology, German Cancer Research Center, 69120 Heidelberg, Germany

[7] Department of Immunobiochemistry, Mannheim Institute for Innate Immunoscience (MI3), Heidelberg University, 68167 Mannheim, Germany

[8] European Center for Angioscience (ECAS), Medical Faculty Mannheim, University of Heidelberg, 68167 Mannheim, Germany

Aim/Objective: Natural Killer (NK) cells are key players in cancer immunosurveillance but they become dysfunctional in tumors. Harnessing NK cell potential through cytokines for cancer immunotherapy is a promising approach. The alarmin Interleukin-33 (IL-33), via its ST2 receptor, controls infections via NK cell activation. However, its role in tumors is still controversial and depends on tumor types and immune microenvironments.

Design: We explored IL-33 ability to activate NK cells through functional, transcriptomic, *in situ*, and *in silico* analyses on biological tissues from healthy donors (HD) and cancer patients as well as *in vivo* experiments in murine tumor models.

Results: We showed that IL-12 signaling drives ST2 expression on a subset of CD56dim NK cells that became fully activated by IL-33. TLR-activated dendritic cells induced ST2 expression in NK cells through their IL-12 production. ST2+ NK cells exhibited a distinct differentiation state between canonical CD56bright and CD56dim subsets, featuring high proliferative properties and polyfunctionality (cytokines/chemokines production, cytotoxicity). A subpopulation of tumor-infiltrating (ti)-NK cells from breast cancer (BC) lesions expressed ST2, in accordance with the identification of Ti-NK cells enriched for a ST2 gene signature in scRNAseq datasets. Furthermore, around 20% of blood and ti-NK cells from BC patients produced IFN-γ in response to IL-12 and IL-33. Accordingly, IL-33/IL-12 co-injection resulted in a NK-dependent IFN-γ secretion, decreased tumor formation, and improved survival in a mouse model of mammary tumor. Finally, an *IL33*hi-*NK*hi score correlated with increased progression-free survival for BC patients.

Conclusion: Our findings identify an unprecedently characterized subset of ST2+ polyfunctional NK cells, with potent antitumor activity and warrant further investigation for the use of IL-33 for cancer immunotherapy.

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O **01** - 06

HYPERACTIVE DENDRITIC CELLS AND THE CONTROL OF ANTI-TUMOR IMMUNITY

Jonathan C. Kagan

Harvard Medical School and Boston Children's Hospitaly=

The central goal of my research is to understand the molecular basis of inflammation. We aim to create a comprehensive map of the subcellular sites of innate immune signal transduction, and determine how manipulations of early signaling events influence protective immunity in the context of infection and cancer. Particular focus is placed on understanding how microbial or self-derived molecules engage pattern recognition receptors, and the functional consequences of this engagement. In this seminar, I will discuss our recent investigations of innate immune signal transduction, with an emphasis on defining how regulators of signal transduction interact with one another dynamically and functionally to execute effective host defenses.

Notes:

IRCI-2022

Immune Responses in Cancer and Infection
2nd International Symposium

Oral Session 2

Immuno-metabolism

25	O 02 - 01	TARGETING MITOCHONDRIAL DYNAMICS FOR TAILORING T CELL EXHAUSTION PROGRAM AND ANTI-TUMOR RESPONSES Ping-Chih Ho, Department of Fundamental Oncology, University of Lausanne, Switzerland
26	O 02 - 02	LET-7 MIRNAS DEFINE CD8 T CELL FATE Leonid Pobezinsky, Universtity of Massachussetts, USA
27	O 02 - 03	IL-15 AND IL-18 MOBILIZE BOTH CLASSIC AND NON-CANONICAL PATHWAYS TO ACTIVATE MTORC1 IN PRIMARY NK CELLS Antoine Marçais, Centre International de Recherche en Infectiologie, France
28	O 02 - 04	EFFECT OF ENDOGENOUS GLUCOCORTICOIDS ON THE ANTITUMOR IMMUNE RESPONSE IN RENAL CANCER Hélène Poinot, Institute of Pharmaceutical Sciences of Western Switzerland
29	O 02 - 05	GLUCOSE TRANSPORTER 2 REGULATES CD8+ T CELL METABOLISM AND FUNCTION VIA ENVIRONMENT SENSING Federica M. Marelli-Berg, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, UK

O **02** - 01

TARGETING MITOCHONDRIAL DYNAMICS FOR TAILORING T CELL EXHAUSTION PROGRAM AND ANTI-TUMOR RESPONSES

Yi-Ru Yu[1,2], Yingxi Xu[1,2], Ping-Chih Ho[1,2]

[1] Department of Fundamental Oncology, University of Lausanne, Switzerland

[2] Ludwig Institute for Cancer Research, University of Lausanne, Switzerland

The metabolic challenges present in tumors attenuate the metabolic fitness and anti-tumor activity of tumor-infiltrating T lymphocytes (TILs). However, it remains unclear whether persistent metabolic insufficiency can imprint permanent T cell dysfunction. We found that TILs accumulated depolarized mitochondria as a result of decreased mitophagy activity and displayed functional, transcriptomic and epigenetic characteristics of terminally exhausted T cells. Mechanistically, reduced mitochondrial fitness in TILs was induced by the coordination of T cell receptor stimulation, microenvironmental stressors and PD-1 signaling. Enforced accumulation of depolarized mitochondria with pharmacological inhibitors induced epigenetic reprogramming toward terminal exhaustion, indicating that mitochondrial deregulation was causal of T cell exhaustion. Furthermore, supplementation with nicotinamide riboside enhanced T cell mitochondrial fitness and improved responsiveness to anti-PD-1 treatment. Together, our results reveal new insights on how mitochondrial dynamics and quality orchestrate T cell anti-tumor responses and commitment to the exhaustion program.

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O **02** - 02

LET-7 MIRNAS DEFINE CD8 T CELL FATE

Leonid Pobezinsky[1]

[1] *University of Massachusetts, Amherst, United States*

Keywords: memory, exhaustion, microRNA, mTOR, ROS

Effector and memory CD8 T cells are critical for an efficient immune response, but in chronic infection or cancer prolonged exposure to antigen leads to the stage of exhaustion, where T cells lose effector function and eventually die. Therefore, controlling differentiation of T cells into effector, memory, or exhausted cells has much therapeutic potential, but the mechanism behind these processes remains poorly understood. Using transgenic mouse models, we found that the inhibition of let-7 microRNA biogenesis in CD8 T cells promotes terminal differentiation including exhaustion, while increased expression of let-7 leads to differentiation into memory T cells. Comparative transcriptome analysis of let-7 transgenic and deficient CD8 T cells revealed dysregulation of early signaling events in T cells.Specifically, we found that let-7 inhibits mTOR-pathway that controls Notch activity and ROS production that are both essential for differentiation of terminal effector T cells. Furthermore, we observed that such let-7-mediated regulation of CD8 T cell fate has a direct impact on anti-tumor immune responses. Therefore, we identified a novel molecular pathway that is involved in terminal differentiation of CD8 T cells and can be antagonized by let-7 which promotes memory formation.

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O **02** - 03

IL-15 AND IL-18 MOBILIZE BOTH CLASSIC AND NON-CANONICAL PATHWAYS TO ACTIVATE MTORC1 IN PRIMARY NK CELLS

Lucie Fallone[1,2,3,4,5], Anne Gonzalez de Peredo[6], Romain Roncagalli[7], Thierry Walzer[1,2,3,4,5,*], Antoine Marçais[1,2,3,4,5,*]

[1] CIRI, Centre International de Recherche en Infectiologie - International Center for Infectiology Research, Lyon, 69007 France.

[2] Inserm, U1111, Lyon, France.

[3] Ecole Normale Supérieure de Lyon, Lyon, France.

[4] Université Lyon 1, Lyon, France.

[5] CNRS, UMR5308, Lyon, France

[6] IPBS Toulouse, France

[7] CIML, Marseille, France

** equal contributors*

Aim/objective: Natural killer (NK) cells are cytotoxic innate lymphocytes with antitumoral functions. These functions are strongly potentiated by *in vitro* treatment with IL-15 and IL-18 acting in synergy; a property used to generate activated NK cells for immunotherapy. Despite its physiological relevance, the molecular basis of IL-15/18 action on NK cells remains elusive.

Design: We recently observed that both cytokines activate mTORC1, a complex controlling NK cell effector capacities. To decipher the respective pathways leading to mTORC1 activation following IL-15 and IL-18 stimulation, we undertook two complementary approaches: 1) a hypothesis driven strategy using pharmacological inhibitors and CRISPR/Cas9 gene KO and 2) an unbiased approach relying on the characterization of the mTOR interactome by quantitative mass-spectrometry.

Results: We confirm the synergistic impact of IL-15/18 on NK cell biology. Indeed, this activation leads to robust proliferation correlating with a boost in effector functions, metabolic activity and protein translation; cellular processes regulated by mTORC1. Pharmacological inhibitors reveal that IL-15 activates mTORC1 through the canonical PI3K/Akt/TSC pathway while IL-18 stimulation is relayed to mTORC1 via an unconventional MyD88/TRAF6/p38 MAPK pathway. These results were validated by CRISPR/Cas9 gene KO in primary NK cells. In parallel, analysis of mass-spectrometry experiments suggests that the composition of the mTOR interactome differs downstream IL-15 and IL-18 stimulations. We are now testing in a mouse tumor model the relevance of the different molecular players identified.

Conclusion: We uncover the fact that IL-15 and IL-18 converge on mTORC1 through two complementary pathways, a property that could inform future immunotherapies.

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O **02** - 04

EFFECT OF ENDOGENOUS GLUCOCORTICOIDS ON THE ANTITUMOR IMMUNE RESPONSE IN RENAL CANCER

Hélène Poinot[1,2], Eloïse Dupuychaffray[1,2], Montserrat Alvarez[1,2], Jérémie Tachet[1,2], Guillaume Disner[1,2], Eulalia Olesti[1,2], Ounss Ezzar[1], Victor Gonzalez Ruiz[1,2], Serge Rudaz[1,2], Carole Bourquin[1,2,3], Aurélien Pommier[1,2]

[1] Institute of Pharmaceutical Sciences of Western Switzerland (ISPOS), University of Geneva, Geneva, Switzerland

[2] School of Pharmaceutical Sciences, University of Geneva, Geneva, Switzerland

[3] Department of Anaesthetics, Pharmacology, Intensive Care and Emergencies, Faculty of Medicine, Geneva, Switzerland

Keywords: Renal cancer, Immunotherapy, Glucocorticoids

Novel treatments for cancer are required to improve the outcome of patients not responsive to current immunotherapies that have made a breakthrough in the last decade with the development of immune checkpoint inhibitors. Endogenous steroids have strong immunomodulatory functions but their role in resistance to immunotherapy in cancer is poorly understood. Using TCGA data, we found the glucocorticoid regeneration pathway strongly associated with clinical outcome of renal cancer patients. The expression of 11beta-hydroxysteroid dehydrogenase type 1 (hsd11b1), which regenerates inactive glucocorticoids into active glucocorticoids, was associated with a poor clinical outcome. Interestingly, HSD11B1 expression was correlated with immune repression signatures in these patients (Th2, immune checkpoints). We confirmed HSD11B1 expression at protein level by immunohistochemistry and localized HSD11B1 mostly expressed in immune cells in human renal cancer samples. In mice, we found that the level of HSD11B1 substrate was much higher in the kidney than in plasma. Therefore, we explored the potential of inhibiting the endogenous glucocorticoid pathway to improve the antitumor immune response and the efficacy of immune checkpoint blockade. In a murine coculture assay with dendritic cells, T cells and renal cancer cells we demonstrated that HSD11B1 inhibition increases the T cell dependent killing of tumor cells by stimulating the antigen-mediated T cell activation. In a human antigen recall assay, we also showed that antigen-specific T cell activation was decreased in the presence of HSD11B1 substrate. Surprisingly, treatment with HSD11B1 inhibitor synergized with anti-PD-1 to improve the efficacy of immunotherapy in vitro. These results were also observed in primary tumor samples from a renal cancer patient and support the hypothesis that combination of HSD11B1 pharmacological inhibitor with immune checkpoint blockade could be beneficial in renal cancer patients. We are currently investigating the role of HSD11B1 in vivo in several mouse renal cancer models

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O **02** - 05

GLUCOSE TRANSPORTER 2 REGULATES CD8+ T CELL METABOLISM AND FUNCTION VIA ENVIRONMENT SENSING

Hongmei Fu[1], Juho Vuononvirta[1], Guosu Wang[1], Fabrizia Bonacina[2], Davide Lucchesi[1], Rachel Coleby[1], David Tarussio[3], Bernard Thorens[3], M. Paula Longhi[1], Michele Bombardieri[1], Dave Smith, Giuseppe Danilo Norata[2], Federica M. Marelli-Berg[1]

[1] William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ, UK;

[2] Department of Pharmacological and Biomolecular Sciences (DisFeB), Università Degli Studi di Milano, Milan, 20133, Italy.

[3] Falculty of Biology and Medicine, Center for Integrative Genomics, Génopode Building - UNIL Sorge, CH-1015, University of Lausanne, Switzerland

T cell activation is associated with a profound and rapid metabolic remodelling which serves to meet increased energy demands for cell division, differentiation, and development of effector function. Glucose uptake and engagement of the glycolytic pathway are major checkpoints underpinning this event. Glucose transporter 1 (Glut1) is the primary glucose transporter to fuel glycolysis upon CD4+ T cell activation. However, CD8+ T cell responses appear to be less dependent on Glut1 function. In this study, we show that Glut2 plays a pivotal role in the development of CD8+ T cell responses. Further, Glut2 expression by $CD8^+$ T cells is modulated by environmental factors such as glucose and oxygen availability, and acidification during T cell recirculation in different tissues. Mechanistically, we show that Glut 2 expression is regulated by a combination of molecular interactions involving HIF1a, Galectin 9 and Stomatin. Finally, we present evidence that human T cells also rely on this glucose transporter, thus providing a potential target for therapeutic modulation of the immune response.

Notes:

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Oral Session 3

Immunotherapy and clinic

31	O 03 - 01	John Wherry, Institute for Immunology, USA
32	O 03 - 02	TUMOR-ASSOCIATED HIGH ENDOTHELIAL VENULES MEDIATE LYMPHOCYTE ENTRY INTO TUMORS AND PREDICT RESPONSE TO PD-1 PLUS CTLA-4 COMBINATION IMMUNOTHERAPY Lucas Blanchard, Institut de Pharmacologie et Biologie Structurale, France
33	O 03 - 03	HUMAN ENDOGENOUS RETROVIRUSES REPRESENT A SOURCE OF SHARED TUMOR EPITOPES INDUCING HIGH-AVIDITY CYTOTOXIC T CELLS FOR CANCER IMMUNOTHERAPY Stéphane Depil, Centre de Recherche en Cancérologie de Lyon, France
34	O 03 - 04	HARNESSING GAMMA-DELTA T CELL FUNCTIONS AGAINST CANCER AND INFECTIOUS DISEASES Julie Déchanet-Merville, ImmunoConcEpT, CNRS, France
35	O 03 - 05	NK CELLS LIMIT CD8+T-CELL IMMUNITY IN A PDL1-DEPENDENT MANNER Mariana Diniz, Division of Infection and Immunity, University College London, UK
36	O 03 - 06	THE GENETICALLY CONSERVED VH1-69 NEUTRALIZING ANTIBODY RESPONSE IN HEPATITIS C INFECTION AND VACCINATION Mansun Law, The Scripps Research Institute, USA
37	O 03 - 07	THE GENETICALLY CONSERVED VH1-69 NEUTRALIZING ANTIBODY RESPONSE IN HEPATITIS C INFECTION AND VACCINATION Ido Amit, Weizmann Institute of Science, Israel

O **03** - 01

John Wherry

Perelman School of Medicine, University of Pennsylvania, USA

Notes:

O **03** - 02

TUMOR-ASSOCIATED HIGH ENDOTHELIAL VENULES MEDIATE LYMPHOCYTE ENTRY INTO TUMORS AND PREDICT RESPONSE TO PD-1 PLUS CTLA-4 COMBINATION IMMUNOTHERAPY

Lucas Blanchard[1], Assia Asrir[1], Claire Tardiveau[1], Juliette Coudert[1], Robin Laffont[1], Elisabeth Bellard[1], Krystle Veerman[1], Sarah Bettini[1], Fanny Lafouresse[1], Estefania Vina[1], Dorian Tarroux[1], Severine Roy[2], Isabelle Girault[2], Irma Molinaro[2], Frédéric Martins[3], Jean-Yves Scoazec[2], Nathalie Ortega[1], Caroline Robert[2], Jean-Philippe Girard[1]

[1] Institut de Pharmacologie et de Biologie Structurale, IPBS, Université de Toulouse, CNRS, UPS, Toulouse, France

[2] Gustave Roussy, Villejuif, France

[3] Plateforme Genome et Transcriptome, GeT, Genopole , Toulouse, France

Keywords: cancer immunotherapy, lymphocyte trafficking, tumor blood vessels, high endothelial venule, tumor-infiltrating lymphocytes

Recruitment of lymphocytes into tumors is critical for anti-tumor immunity and efficacious cancer immunotherapy. However, the mechanisms governing lymphocyte recruitment into tumor are incompletely characterized. We previously reported that MECA-79+ tumor blood vessels reminiscent of lymph node high endothelial venules (HEVs)1,2 are frequently observed in human solid tumors3,4. These tumor-associated HEVs (TA-HEVs) correlate with the density of tumor-infiltrating lymphocytes and with favorable clinical parameters in primary breast cancer and melanoma. In murine tumor models, we now demonstrate that TA-HEVs are major sites of lymphocyte entry into tumors both at baseline and during treatment with combined anti-PD-1/anti-CTLA-4 immune checkpoint blockade (ICB)5. TA-HEV endothelial cells (TA-HECs) derive from post-capillary venules, co-express MECA-79+ HEV sialomucins and inflammatory E/P-selectins, and are associated with homing and infiltration into tumors of various T cell subsets. Intravital microscopy further shows that TA-HEVs are the main sites of lymphocyte arrest and extravasation into ICB-treated tumors. Increasing TA-HEC frequency and maturation increases the proportion of tumor-infiltrating stem-like CD8+ T cells, and ameliorates ICB efficacy. Finally, analysis of tumor biopsies from 93 patients with metastatic melanoma reveals that TA-HEVs are predictive of better response and survival upon treatment with anti-PD-1/anti-CTLA-4 combination. These studies provide important insights into the mechanisms governing lymphocyte trafficking in cancer immunity and immunotherapy.

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Notes: ----------

O **03** - 03

HUMAN ENDOGENOUS RETROVIRUSES REPRESENT A SOURCE OF SHARED TUMOR EPITOPES INDUCING HIGH-AVIDITY CYTOTOXIC T CELLS FOR CANCER IMMUNOTHERAPY

Paola Bonaventura[1,2]†, Vincent Alcazer[1]†, Virginie Mutez[3], Laurie Tonon[4], Juliette Martin[5], Nicolas Chuvin[3], Emilie Michel[3], Rasha E. Boulos[3], Yann Estornes[3], Jenny Valladeau-Guilemond[1], Alain Viari[4], Qing Wang[6], Christophe Caux[1,2], Stéphane Depil[1,2,3,7]

[1] Centre de Recherche en Cancérologie de Lyon (CRCL), UMR INSERM U1052 CNRS 5286, Lyon, France.

[2] Centre Léon Bérard, Lyon, France.

[3] ErVaccine Technologies, Lyon, France.

[4] Synergie Lyon Cancer, Plateforme de bioinformatique « Gilles Thomas », Lyon, France.

[5] CNRS- Institut de Biologie et Chimie des Protéines UMR 5086, Lyon, France

[6] Complete Omics, Baltimore, USA

[7] Université Claude Bernard Lyon 1, Lyon, France.

† These authors contributed equally to this work

Background. Human endogenous retroviruses (HERVs) represent 8% of the human genome. HERVs are silenced by epigenetic mechanisms in normal cells but are aberrantly expressed by tumor cells. Given their viral origin, HERV products may represent tumor antigens relevant for cancer immunotherapy.

Results. We developed a systematic bioinformatics-based approach to identify shared $CD8^+$ T cell epitopes derived from cancer-associated HERVs in solid tumors. Six HLA-A2 epitopes among the most shared epitope candidates with evidence of translation were selected for further immunological evaluation. *In vitro* priming assays showed the induction of specific $CD8^+$ T cells leading to polyfunctional T cell responses. The functionality of the sorted T cell clones was confirmed by Elispot ($GrzB^+$ IFN-γ^+) before TCR sequencing. Interestingly, these TCRs were predicted to interact with a high affinity with their respective MHC-peptide complexes in 3D models. This was confirmed by measurement of the functional avidity, which was in the same order as CMV-specific T cell clones. HERV-specific $CD8^+$ T cells induced specific cell death of HLA-$A2^+$ cancer cell lines presenting HERV epitopes on HLA molecules, as demonstrated by mass spectrometry. Furthermore, HERV-specific $CD8^+$ T cells were identified by dextramer-staining among tumor infiltrating lymphocytes (TILs) from HLA-$A2^+$ breast and ovarian cancer patients. Finally, we showed that HERV-specific T cells can lyse patient-derived organoids (Bonaventura et al. Sci Adv 2022). Synthetic long peptides containing these HERV epitopes have been validated for the development of a cancer vaccine. TCR engineered T cells specific to these HERV epitopes have been generated and their functionality and specificity have been confirmed.

In parallel, we also evaluated HERV expression in Acute Myeloid Leukemia (AML). We used a complete database of 14,968 HERVs functional units to provide a thorough analysis of HERVs in normal and AML bone marrow cells. We found that HERV retrotranscriptome accurately characterizes normal and leukemic cell subpopulations, including leukemia stem cells, in line with different epigenetic profiles. We then showed that HERV expression separates distinct AML subtypes of different prognosis. We selected $CD8^+$ T cell epitopes derived from AML-specific HERVs and we showed that patients' marrow infiltrating lymphocytes at diagnosis also contain naturally occurring $CD8^+$ T cells against HERV epitopes. Furthermore, we demonstrated that HERV-specific $CD8^+$ T cells specifically recognize AML cells (Alcazer et al. In revision).

Conclusion. Our bioinformatic approach allowed us to identify shared HERV-derived $CD8^+$ T cell epitopes specifically expressed by tumor cells and inducing high-avidity T cell clones able to kill tumor cells in a class I-restricted manner. The detection of TILs recognizing HERV peptides suggests natural presentation of these epitopes in the tumors. These HERV-derived epitopes may thus represent relevant targets for the development of new immunotherapeutic approaches, especially in tumors with a low or moderate mutational burden. We are currently developing a therapeutic vaccine as well as TCR engineered T cells specific to these HERV epitopes.

O **03** - 04

HARNESSING GAMMA-DELTA T CELL FUNCTIONS AGAINST CANCER AND INFECTIOUS DISEASES

Julie Déchanet-Merville

ImmunoConcEpT, CNRS, Bordeaux University, Bordeaux, France

Gamma-delta T lymphocytes hold great promise for the development of new immunotherapies in cancer and in infectious diseases and concrete strides have been recently made down that road. However, a better understanding of how they recognize and kill cancer or infected cells is required to harness full clinical potential of these cells. I will present the strategy we developed to identify the molecules construed by gamma-delta T cells as stress signals on the surface of modified cells. These signals, representing new types of pathogen-induced or tumor antigens, have interesting potential as biomarkers of disease evolution or as targets for immunotherapy. Cancer cell- or infected cell-specific gamma-delta T cells can themselves be harnessed as cell therapy and monitoring their phenotype and functions can be used to improve patient care. I will also present our most recent developments toward this end.

Notes:

O **03** - 05

NK CELLS LIMIT CD8+T-CELL IMMUNITY IN A PDL1-DEPENDENT MANNER

Mariana Diniz[1], Anna Schurich[1], Senthil Chinnakannan[2], Marion Duriez[1], Kerstin Stegmann[1], Jessica Davies[1], Stephanie Kucykowicz[1], Kornelija Suveizdyte[1], Frances Alcock[1], Tamsin Cargill[2], Eleanor Barnes[2], Mala Maini[1]

[1] Division of Infection and Immunity, University College London, London, United Kingdom

[2] Nuffield Dept of Medicine, University of Oxford, Oxford, United Kingdom

Keywords: NK cell, T cell, PD-1, HBV, liver

Successful therapeutic vaccines for chronic infection and cancer need to overcome immunoregulatory mechanisms to boost antigen-specific T cells functionality. Following up on studies from our group showing the capacity of NK cells to kill virus-specific CD8 T cells in patients with chronic hepatitis B (CHB), we investigated whether NK cells can limit T cell responses to therapeutic vaccination in vivo, using a mouse model of persistent Hepatitis B virus (HBV) infection. We found that NK cell depletion enhanced the frequency and functionality of antigen-specific T cell responses to chimp adenoviral vector vaccination (ChAdOx-HBV, Vaccitech, Oxford). HBV infection drove upregulation of PD-L1 on liver-resident NK cells and, concomitantly, PD-1 on intrahepatic T cells. In vivo PD-1 blockade increased vaccine immunogenicity to the same extent as NK cell depletion, pointing to a dominant role for NK cell regulation through the PD-L1/PD-1 axis. Cytokine-stimulation to increase NK cell effector function, as well as their capacity to boost T cell responses, has been explored in the development of NK cell-based therapies against cancer. However, we found a pronounced upregulation of PD-L1 on cytokine-stimulated NK cells, which limited their T cell helper function. Pre-treating NK cells with PD-L1 blocking antibody abrogated their capacity to constrain HBV-specific T cells responses in vivo. This mechanism proved to be translationally relevant as PDL-1 blocked cytokine-activated NK cells promoted expansion of HBV-specific T cells from donors with CHB. Our findings delineate a novel NK cell regulatory mechanism via PD-L1 and provide a rationale for including PD-L1 blockade or knockdown, in order to preserve the beneficial potential of NK cells, while avoiding their detrimental inhibition of PD-1hiCD8+T cells.

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O **03** - 06

THE GENETICALLY CONSERVED VH1-69 NEUTRALIZING ANTIBODY RESPONSE IN HEPATITIS C INFECTION AND VACCINATION

Mansun Law

Department of Immunology and Microbiology, The Scripps Research Institute, La Jolla, California, USA

Keywords: HCV vaccine, bnAb, VH1-69, multi-donor class antibody, public immunity

A vaccine designed to target highly conserved functional immune epitopes, known as an epitope-focused vaccine, is an important strategy against viruses that can readily evade host immunity, such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV). These viruses have developed diverse viral features, including rapid mutation, epitope shielding, and/or encoding viral gene products, that circumvent host immune responses. Among the host defense mechanisms, broadly neutralizing antibodies (bnAbs) target conserved vulnerable sites on the viruses for broad protection. A vaccine eliciting high levels of bnAbs can be a solution against viral diseases that are not preventable by traditional vaccines.

The interest in eliciting bnAbs by vaccination underscores the importance of understanding how these antibodies are generated and their functions at the molecular and structural levels. In HCV, a conserved antigenic surface on the E2 envelope glycoprotein, the E2 neutralizing face (NF), has been identified to be a prime target for rational vaccine design. E2 NF is composed of the conserved antigenic site 412-423 (AS412), the E2 front layer (FL) and the CD81 binding loop (CD81bl) regions. E2 NF is frequently targeted by bnAbs utilizing the human antibody heavy chain variable gene *IGHV1-69* in HCV patients. Compared to HIV bnAbs that require high levels of somatic hypermutation (SHM) for maturation, the low to medium SHM rate for the HCV bnAbs should be more readily achievable through vaccination.

In the structural analysis of VH1-69 bnAbs isolated from different patients, the antibodies recognize E2 NF using different binding modes, and that E2 NF can adopt different conformations for neutralization. Intriguingly, macaques were found to use a gene orthologous to human VH1-69 to elicit E2 NF-targeting bnAbs in response to vaccination. This unusual multi-donor class bnAb response and the functional convergence of a germline-encoded bnAb response within primates provide an excellent opportunity for HCV rational vaccine design and testing.

Notes:

O **03** - 07

THE POWER OF ONE: IMMUNOLOGY IN THE AGE OF SINGLE CELL GENOMICS

Ido Amit

Weizmann Institute of Science

The immune system is a complex, dynamic and plastic network composed of various interacting cell types that are constantly sensing and responding to environmental cues. From very early on, the immunology field has invested great efforts to characterize the various immune cell types and elucidate their functions. However, accumulating evidence indicates that current technologies and classification schemes are limited in their ability to account for the functional heterogeneity of immune processes. Single cell genomics hold the potential to revolutionize the way we characterize complex immune cell assemblies and study their spatial organization, dynamics, clonal distribution, pathways, and crosstalk. This emerging field can greatly affect basic and translational research of the immune system. I will discuss how recent single cell genomic studies are changing our perspective of various immune related pathologies from cancer to autoimmune disease and neurodegeneration. Finally, I will consider recent and forthcoming technological and analytical advances in single cell genomics and their huge potential impact on the future of immunology research and immunotherapy.

Notes:

IRCI-2022

Immune Responses in Cancer and Infection
2nd International Symposium

Oral Session 4

Microbiota

O **04** - 01

MICROBIOME AND GERMLINE VARIANTS CAN CONTROL ANI-TUMOR IMMUNITY BY REGULATING MYELOID CELLS WITHIN THE TUMOR MICROENVIRONMENT

Thomas F. Gajewski, M.D., Ph.D.

Most cancers express tumor antigens that can be recognized by T cells of the host. The fact that cancers become clinically relevant and grow nonetheless implies that immune escape must occur to allow cancer outgrowth. We have observed two major phenotypes of human melanoma metastases based on gene expression profiling and confirmatory assays. One subgroup of patients has a T cell-inflamed phenotype that includes expression of chemokines, T cell markers, and a type I IFN signature. In contrast, the other major subset lacks this phenotype and appears to display immune "exclusion". Factors that influence the degree of spontaneous immune infiltration are being investigated, as sources of inter-patient heterogeneity. These include tumor cell-intrinsic oncogenic events, the composition of the gut microbiota , and polymorphisms in immune regulatory genes. We now know that each of these dimensions can be functionally important. The first tumor cell-intrinsic oncogenic pathway identified that mediates immune exclusion is the Wnt/β-catenin pathway. Tumors with active β-catenin fail to recruit Batf3-lineage dendritic cells into the tumor site. Regarding the commensal microbiota, mouse models identified commensal Bifidobacterium as one key component that augments spontaneous anti-tumor immunity and increases efficacy of anti-PD-L1 therapy in vivo. Similar analyses in human cancer patients revealed bacteria sequences enriched in anti-PD-1 responders, and also bacteria sequences enriched in non-responders. Fecal transfer into germ-free mice has confirmed a causal role for the gut microbiota in regulating immunotherapy efficacy. Recent experiments have revealed that one major mechanism by which gut microbes impact on distant ani-tumor immunity is through modulation of immune-regulatory myeloid cells, ie the M1/M2 ratio and MDSCs. Regarding germline variants, our first identified SNP connected to immune cell infiltration is in the PKCδ gene. Loss of function variants are associated with greater immune cell infiltration. PKCδ knockout hosts show improved immune-mediated tumor control and anti-PD-L1 efficacy, but with comparable T cell priming. However, activated T cell accumulation in the tumor microenvironment increases overtime, which is associated with a shift from M2 to M1. Myeloid cell-specific PKCδKO mice using LysM-Cre Tg mice recapitulate the phenotype. Thus, tumor and host factors can impact on anti-tumor immunity by modulating myeloid cell participation and differentiation state.

Notes:

O **04** - 02

POLYPHENOL CASTALAGIN EXERTS ANTITUMOR ACTIVITY AND POTENTIATES ANTI-PD-1 IMMUNE EFFICACY THROUGH A BENEFICIAL SHIFT IN MICROBIOME COMPOSITION

Meriem Messaoudene[1], Reilly Pidgeon[2], Corentin Richard[1], Genevieve Pilon[3], Florian Plaza Oñate[4], Emmanuelle Le Chatelier[4], Guido Kroemer[5], André Marette[3], Bastien Castagner[2], Bertrand Routy[1]

[1] University of Montreal Research Center, Montreal, Canada

[2] McGill University, Montreal, Canada

[3] Laval University, Montreal, Canada

[4] University Paris-Saclay, INRAE, MGP, Jouy-en-Josas, France

[5] Metabolomics and Cell Biology Platforms, Gustave Roussy Cancer Campus, Villejuif, Canada

Keywords: cancer, microbiome, prebiotics, immunotherapy, polyphenol

Immune checkpoint inhibitors (ICI) have shown unparalleled clinical success for treating several cancers and currently represent the standard of care for numerous cancers, including metastatic melanoma and non-small cell lung cancer. Nonetheless, primary resistance remains frequent. The gut microbiome recently emerged as a key mediator of cancer-immune set points. Several approaches to manipulate the gut microbiome improving the activity of cancer ICI are currently under evaluation. Camu-camu (CC), also known as Myrciaria dubia, is an amazonian berry containing several phytochemicals and has been shown to exert protective prebiotic effects against obesity and related metabolic disorders in mice through increasing the abundance of A. muciniphila and Bifidobacterium in the gut. Here, we evaluated whether the prebiotic action of CC could also be harnessed to shift the gut microbiome and improve antitumor activity. By performing shotgun metagenomics and 16S sequencing, we showed that oral supplementation with CC in mice shifted gut microbial composition, which translated into antitumor activity and a stronger anti–PD-1 response. Using reversed-phase chromatography, we identified castalagin, an ellagitannin, as the active compound in CC. We then characterized the immune-potentiating effect of castalagin using several approaches including flow cytometry, immunofluorescence, RNA sequencing, and in vivo CD8+ T-cell killing assay. We showed that oral administration of castalagin enriched bacteria associated with efficient immunotherapeutic responses (Ruminococcaceae and Alistipes) and improved the CD8+/FOXP3+CD4+ ratio within the tumor microenvironment. We also identified the CD8+ T cell–dependent mechanism of castalagin. Moreover, we investigated the impact of castalagin on metabolite production by performing a combination of chromatographic and mass spectrometric methods. The results showed that castalagin induced metabolic changes, resulting in an increase in taurine-conjugated bile acids. Oral supplementation of castalagin following fecal microbiota transplantation from ICI-refractory patients into mice supported anti–PD-1 activity. Finally, using a fluorescein-castalagin conjugate, we found that castalagin binds to Ruminococcus bromii and promoted an anticancer response. Altogether, our results identify castalagin as a polyphenol that acts as a prebiotic to circumvent anti–PD-1 resistance.

MICROBIOTA

Notes: ----------

O **04** - 03

BLOOD GLUCOSE INTERACTS WITH HOST-MICROBE SYMBIOSIS

Jonathan D. Schertzer[1]

[1] Department of Biochemistry and Biomedical Sciences, Farncombe Family Digestive Health Research Institute, Centre for Metabolism, Obesity and Diabetes Research, McMaster University, Hamilton, Canada

The source of compartmentalized inflammation in metabolic disease is poorly understood. The gut microbiota may provide inflammatory cues to the host, but it important to move beyond characterizing microbial taxonomy and identify bacterial-derived molecules that influence metabolic inflammation, such as postbiotics that include microbial metabolites and components.

Lipopolysaccharide (LPS) is a postbiotic that engages host immunity via toll-like receptor 4 (TLR4) during metabolic endotoxemia, a low-level increase of LPS in the blood during metabolic disease. It is not clear which aspect of metabolic disease initiates metabolic endotoxemia or if certain metabolic disease factors alter the type of metabolic endotoxemia. We discovered that metabolic endotoxemia may be beneficial or detrimental depending on the type of LPS, where the acylation status of lipid A in LPS, derived from different strains of bacteria, dictated changes in blood glucose control in obese mice. We now show that certain metabolic disease characteristics alter the type and compartmentalization of metabolic endotoxemia. We used leptin-deficient *ob/ob* mice of different ages, and hyperglycemic Akita mice with and without insulin delivery and quantification of the TLR4 activation properties of LPS using a cell-reporter assay. Our results show that obesity and hyperglycemia can alter the type and compartmentalization of metabolic endotoxemia. Hyperglycemia of sufficient duration increased the TLR4 activity of the feces and in the blood of mice. We also found that under-acylated LPS acts as a weak agonist for TLR4 activation and competes with the potent activation of TLR4 by hexa-acylated LPS, which sets up the potential for different types of LPS to be used as a postbiotic to mitigate metabolic inflammation. These results show that elevated blood glucose influences the commensal microbe symbiosis with the host. We then tested the implications of obesity and elevated blood glucose for outcomes from enteric pathogens.

Obesity and type 2 diabetes can increase the risk and severity of bacterial infection, but it is not clear how obesity versus elevated blood glucose modify the risk and severity of enteric infection. We show that hyperglycemia prior to infection increased incidence and severity of diarrhea during a community outbreak of *Escherichia coli* and *Campylobacter* in humans. Elevated blood glucose, but not obesity, increased mortality during infection with the diarrhea-causing pathogen *Citrobacter rodentium* in mice. Obese (*ob/ob*) mice and lean Akita mice had increased mortality during *Citrobacter rodentium* infection, but only when mice were hyperglycemic. Implanting continuous insulin pellets lowered blood glucose and improved survival during infection in all mice. Mechanistically, hyperglycemia was not associated with overt bacteremia, but rather promoted increased activation of Wnt/β-catenin in the distal gut and treatment with Wnt pathway inhibitor improved survival during enteric infection in diabetic mice.

Overall, these results show that elevated blood glucose for a sufficient duration promotes a permissive and inflammatory environment linked to a change in commensal microbes and higher TLR4 activation in the gut lumen and blood. Elevated blood glucose also promotes the severity of outcomes from enteric pathogens. Therefore, blood glucose modifies both commensal and pathogen symbiosis with the host.

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O **04** - 04

TARGETING THE MICROBIOTA FOR CANCER THERAPY

Giorgio Trinchieri

Laboratory of Integrative Cancer Immunology, Center of Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

Growing evidence suggests that the gut microbiota modulates the efficacy and toxicity of cancer therapy, most notably immunotherapy and its immune-related adverse effects. The impaired response to immunotherapy in patients treated with antibiotics supports this role of the microbiota. Until recently, results pertaining to the identification of the microbial species responsible for these effects were incongruent, and relatively few studies analyzed the underlying mechanisms. Now, a better understanding of the taxonomy of the species involved and of the mechanisms of action has been achieved. Defined bacterial species have been shown to promote a response to immune checkpoint inhibitors through the production of different products or metabolites, although a suppressive effect of Gram(-) bacteria may be dominant in some unresponsive patients. Machine learning approaches trained on patients' microbiota composition can predict the ability of patients to respond to immunotherapy with some accuracy. Thus, the interest in modulating the microbiota composition to improve patients' responsiveness to therapy has been mounting. Clinical proof of concept studies demonstrated that fecal microbiota transplant or diet alteration may be utilized clinically to improve cancer immunotherapy's success rate.

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O **04** - 05

HOST-MICROBIOTA INTERACTIONS DURING COLORECTAL CANCER DEVELOPMENT

Maude Jans[1,2,3], Magdalena Kolata[5,6], Ioanna Petta[1,2,3], Gillian Blancke[1,2,3], Marie Thorp[1,2,3], Alexandra Thiran[1,2,3], Vanessa Andries[1,2,3], Korneel Barbry[1,2,3], Geert Berx[3,4], Geert Van Loo[1,3,4], Han Remaut[5,6], Lars Vereecke[1,2,3]

[1] VIB Center for Inflammation Research, Ghent, Belgium

[2] Department of Internal Medicine and Pediatrics, Ghent University, Ghent, Belgium

[3] Cancer Research Institute Ghent (CRIG), Ghent , Belgium

[4] Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium

[5] Structural & Molecular Microbiology, VIB-VUB Centre for Structural Biology, Brussels, Belgium

[6] Structural Biology Brussels, Vrije Universiteit Brussel, Brussles, Belgium

Keywords: Microbiota, Colorectal cancer, Inflammation, Germ-free and gnotobiotic Mouse technology, Trnsgenic mouse CRC models

Colorectal cancer (CRC) is highly prevalent in Western society, with increasing evidence indicating a role for the intestinal microbiota in CRC initiation, progression and metastasis. The notion that CRC development is mediated by bacterial-host interactions is supported by the identification of several CRC-promoting or 'onco-bacteria', including Fusobacterium nucleatum and pathogenic E. coli strains. We recently developed a new mouse model of CRC by expressing the EMT-regulator Zeb2 specifically in intestinal epithelial cells (IECs). Zeb2IEC-Tg mice have a destabilized intestinal epithelial barrier and develop severe colon inflammation, leading to the development of invasive CRC. Zeb2IEC-Tg mice display distinct shifts in microbiota composition, and germfree Zeb2IEC-Tg mice are completely protected from CRC development, indicating that microbiota-derived signals are essential in this model. Using this mouse model, we are characterizing bacterial strains with suggested CRC promoting functions. We show that the adherent invasive E. coli (AIEC) strain CCR20, a human CRC isolate, significantly promotes tumor development in Zeb2IEC-Tg mice by driving mucus epithelial invasion. Using CCR20 knockout strains, we show that epithelial adhesion and tissue invasion depends on the expression of type 1 pilus adhesins FimH and FmlH. Interestingly, in monocolonized Zeb2IEC-Tg mice, or in a minimal microbiota setting, CCR20 does not promote CRC progression. CCR20 thus requires a complex bacterial ecosystem and adhesin-mediated tissue binding to promote CRC progression. Targeting the epithelial adhesion of AIEC strains could thus be an effective strategy to attenuate cancer progression and opens perspectives for the development of microbiota-based therapies in CRC. In addition, we generated a goblet-cell specific Cre line, to study goblet cell biology in health and disease. Using diphtheria-toxin based depletion models, we can transiently or constitutively delete intestinal goblet cells and the mucus layer, and study epithelial-bacterial interactions in mucus deprived conditions. Goblet cell deficient mice develop spontaneous microbiota dependent CRC. Together, using elegant transgenic mouse CRC models and germ-free technology, we are unraveling complex host-microbiota interactions during CRC initiation and progression.

MICROBIOTA

Notes: ----------

Keynote

MULTI-KINGDOM CONTROL OF TISSUE PHYSIOLOGY AND REPAIR

Yasmine Belkaid
National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA

The immune system has coevolved with extensive microbial communities living on barrier sites that are collectively known as the microbiota. Microbial antigens and metabolites engage in a constant dialogue with the immune system, leading to microbiota-specific immune responses that occur in the absence of inflammation. A remarkable property of these responses is that, in contrast to those induced by infections, initiation of T and B cell responses to the microbiota and accumulation of these cells in tissues occur in the absence of inflammation, a process referred to as homeostatic immunity. Within the skin, immunity to the microbiota controls numerous aspects of tissue physiology including enhanced barrier immunity and epithelial repair. We will discuss the role of endogenous retrovirus in the promotion of immune responses to the microbiota and how the nutritional and metabolic status of the host can impact this multi-kingdom dialog.

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Oral Session 5

Genetics and epigenetics

O **05** - 01

ATLASING HUMAN PRIMARY IMMUNODEFICIENCY PATIENTS AND INSPECTING THEIR RESPONSES TO INFECTIONS

Javier Rodríguez-Ubreva[1], Anna Arutyunyan[2], Louis-François Handfield[2], Marc Jan Bonder[3], Lucía Del Pino-Molina[4], Stephen J. Clark[5], Carlos de la Calle-Fabregat[1], Luz Garcia-Alonso[2], Laura Ciudad[1], Eduardo Andrés-León[6], Felix Krueger[7], Francesc Català-Moll[1], Virginia C. Rodríguez-Cortez[1], Krzysztof Polanski[2], Lira Mamanova[2], Stijn van Dongen[2], Vladimir Yu. Kiselev[2], María T. Martínez-Saavedra[8], Holger Heyn[9], Javier Martín[6], Bärbel Keller[10], Klaus Warnatz[10], Eduardo López-Granados[4], Carlos Rodríguez-Gallego[8], Oliver Stegle[3], Gavin Kelsey[5], Roser Vento-Tormo[2], Esteban Ballestar[1]

[1] Epigenetics and Immune Disease Group, Josep Carreras Research Institute (IJC), Badalona, Barcelona, Spain

[2] Cellular Genetics Department, Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, UK

[3] European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Wellcome Genome Campus, Hinxton, Cambridgeshire, UK

[4] Clinical Immunology Department, La Paz University Hospital and Lymphocyte Pathophysiology in Immunodeficiencies Group, La Paz Institute for Health Research (IdiPAZ) and Center for Biomedical Network Research on Rare Diseases (CIBERER U767), Madrid, Spain

[5] Epigenetics Programme, Babraham Institute, Cambridge, UK

[6] Instituto de Parasitología y Biomedicina López-Neyra, Consejo Superior de Investigaciones Científicas (IPBLN-CSIC), Granada, Spain

[7] Bioinformatics Unit, Babraham Institute, Cambridge, UK

[8] Department of Immunology, Hospital Universitario de Gran Canaria Dr Negrín, Las Palmas de Gran Canaria, Spain.

[9] CNAG-CRG, Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain

[10] Department of Rheumatology and Clinical Immunology, Medical Center, University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany

Common variable immunodeficiency (CVID), the most frequent symptomatic primary immunodeficiency, is characterized by impaired terminal B-cell differentiation and defective antibody responses. CVID can only be partially explained by genetics, as only 20% of CVID cases can be accounted for by monogenic gene defects and there is an ample phenotypic expressivity. This suggests the participation of additional pathogenic mechanisms. Monozygotic (MZ) twins discordant for CVID are unique for studying the contribution of epigenetics to the disease. I will here present our most recent single-cell epigenomics and transcriptomics study, where we studied a CVID-discordant MZ twin pair and the analysis of a CVID cohort. In this study, we obtained a single-cell omics census of different B cell subpopulations (naïve and unswitched and switched memory B cells) in CVID. We identified DNA methylation, chromatin accessibility and transcriptional defects in memory B-cells that reflect defective cell-cell communication upon activation in CVID. Our findings provided a comprehensive multi-omics map of alterations in naïve-to-memory B-cell transition in CVID and reveal links between the epigenome and immune cell cross-talk. In this presentation, I will also present single cell omics data of the dynamics of viral infection (before, during and after) of CVID patients in parallel with healthy individuals, where a number of cell populations and pathways are shown to be altered. These studies pave the way for future diagnosis and treatments of CVID patients.

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O **05** - 02

JARID2 DELETION DRIVES NKT CELL LYMPHOMAGENESIS

Rémy Robinot[1], Emilie Bardel[1], Dimitri Chartoire[1], Sylvain Mareschal[1], Amel Chebel[1], Boris Lipinski[1], Sylvain Carras[1], Sammara Chaubard[1], Florian Pesce[1], Anthony Scotta[1], Alexandra Traverse-Glehen[1], Pierre Sujobert[1], Emmanuel Bachy[1], Laurent Genestier[1]

[1] CIRI, Lyon, France

Keywords: Epigenetic, T-cell , Lymphoma, Jarid2

Peripheral T cell lymphomas (PTCL) are a heterogeneous entity of neoplasm with poor prognosis and lack of effective therapies. We recently identified a novel entity of PTCL originating from CD1d-restricted T cells in mice and humans (Bachy et al. J Exp Med 2016) and demonstrated the driver role of chronic TCR stimulation, in particular by bacteria, in NKT cell lymphomagenesis (Robinot et al. Blood 2018). To further study molecular mechanisms associated with NKT lymphomagenesis, whole-exome sequencing was performed on 6 PTCL-NKT revealing recurrent large losses in chromosome 13 with a minimal deletion region including the epigenetic regulator gene Jarid2. Jarid2 is a member of the Jumonji family, but lacks the conserved residues essential for histone demethylase activity and hence is predicted to be catalytically inactive. However, Jarid2 has been reported as a component of the PRC2 complex that promotes H3K27 methylation but also G9a- and GLP-containing protein complex regulating H3K9 di- and tri-methylation. Within NKT cells, JARID2 is a direct binding partner of SETDB1 also promoting H3K9 di- and tri-methylation. It thus negatively regulates the expression of target genes, such as Zbtb16 gene (encoding PLZF), the master gene of NKT cell differentiation. Our data indicate that hemizygosity of Jarid2 within mouse PTCL-NKT results in loss of Jarid2 RNA and protein expression. In addition, the function of Jarid2 seems to be invalidated in PTCL-NKT since we have demonstrated a decrease in H3K9 trimethylation, associated with a very strong PLZF expression. To further study epigenetic regulation of NKT cells by Jarid2, ATAC-seq was performed on WT and Jarid2-deficient NKT cells, that revealed several significant open chromatin regions, such as in ARID5b gene, also regulating the H3K9 methylation, in Jarid2-deficient NKT cells. The role of Jarid2 in NKT cell lymphomagenesis was further demonstrated by the significant increase incidence of PTCL-NKT in p53-/-Jaridf/f CD4-cre compared to their control mice. Finally, Jarid2-deficient PTCL-NKT expressed high level of the survival receptor ICOS and in vivo blocking of ICOS/ICOS-L interaction significantly delayed PTCL-NKT development.

Our work thus represents the first demonstration of a direct role of Jarid2 inactivation in T-cell lymphomagenesis and as a molecular hallmark of PTCL-NKT.

Notes:

O **05** - 03

TGF-ß SIGNALING ENSURES THE STABILITY OF THE EPIGENETIC PROGRAM OF ALREADY DIFFERENTIATED TH17 CELLS PREVENTING FROM INFLAMMATION-INDUCED CANCER

Valentin Thevin*[1], Olivier Fesneau*[1], Hector Hernandez-Vargas*[1], Chloé Goldsmith[1], Valérie Pinet[2], Valérie Dardalhon[2], Tanguy Fenouil[3], Gitta Stockinger[4], Nicolas Benech[1], Julien C. Marie[1]

[1] CRCL, INSERM 1052 CNRS 5286, Centre Léon Bérard, University Lyon 1, Lyon France

[2] IGMM, CNRS UMR5535, Montpellier, France

[3] Hospices Civils de Lyon, France

[4] Francis Crick institute, London, United Kingdom

T_H17 cells are largely abundant in the gut, where they contribute to the intestinal homeostasis and bacterial protection. The Transforming Growth Factor beta 1 (TGF-ß1) is known to contribute to the differentiation of naïve CD4 T cells in TH17 cells. However, whether this cytokine plays a role on T_H17 cells once they are already differentiated remains totally unknown. Here, we reveal that TGF-ß signaling in already differentiated T_H17 cells is crucial to avoid intestinal-chronic inflammation which leads to intestinal adenocarcinoma. Using fate-mapping approaches, as well as gain and loss of TGF-ß signaling selectively in already differentiated T_H17 cells, we demonstrate that in the absence of TGF-ß signaling, differentiated T_H17 cells become T_H1 like cells and Cytotoxic T Lymphocytes (CTL) in the gut. Sc-RNA-seq and flow cytometry analysis reveal that T_H17 cells converted in T_H1 cells and CTL acquire pathogenic features, responsible for the inflammation-induced cancer. Sc-ATAC-seq and methylation profile analysis demonstrate that, in the gut, TGF-ß signaling controls at the epigenetic level the stability of the T_H17 cell program by sustaining the expression of the several transcription factors which in turn avoid T-bet expression, and thus from T_H1 and CTL differentiation. The intestinal source of TGF-β1 responsible for the T_H17 epigenetic stability is provided by the intestinal epithelial cells (IEC). Finally, the relevance of our findings in mice was confirmed in patients. Thus, this study reveals that IEC ensure the stability of the epigenetic program of T_H17 cells which is essential to prevent from inflammation-induced intestinal cancer.

Notes:

O **05** - 04

DISTINCT EVOLUTIONARY TRAJECTORIES OF SARS-COV-2 INTERACTING PROTEINS IN BATS AND PRIMATES IDENTIFY HOST DETERMINANTS OF COVID-19

Marie Cariou[1], Léa Picard[1], Laurent Guéguen[2], Stéphanie Jacquet[1,2], Andrea Cimarelli[1], Oliver Fregoso[3], Antoine Molaro[4], Vincent Navratil[5], Lucie Etienne[1]

[1] CIRI, Centre International de Recherche en Infectiologie, Lyon, France

[2] Laboratoire de Biométrie et Biologie Evolutive, Lyon, France

[3] UCLA, University of California Los Angeles, Department of Microbiology, Immunology, and Molecular Genetics, Los Angeles, CA, United States

[4] Genetics, Reproduction & Development Institute (GRED), Clermont-Ferrand, France

[5] PRABI, Rhône-Alpes Bioinformatics Center, Lyon, France

Keywords: SARS-CoV-2, COVID-19, comparative genetics, virus-host coevolution, bats and primates

The COVID-19 pandemic is caused by a novel coronavirus, SARS-CoV-2 that spilled from the bat reservoir. Despite scores of clinical trials and vaccines, the burden remains immense, and the host determinants of SARS-CoV-2 susceptibility and COVID-19 severity remain largely unknown. Comparative functional-genetic analyses of the modern genomes from primates –which include humans– and bats –which include the natural reservoir of SARS-CoVs– inform us on their past adaptation to pathogenic viruses and the virus-host interfaces. To identify key SARS-CoV adaptive loci and the functional genetic differences between bats and primates, we performed high-throughput evolutionary analyses of 334 SARS-CoV-2 interacting proteins. Using DGINN (Detection of Genetic INNovation), we identified 38 bat and 81 primate proteins with strong marks of positive selection. Seventeen genes, including the ACE2 receptor, represent a core set with adaptive marks in both mammalian orders, suggesting (i) past epidemics of pathogenic coronaviruses shaping bat and primate genomes, and (ii) common virus-host interfaces. Yet, we found important distinct adaptations in bats and primates. Notably, the residues for ubiquitination and phosphorylation of the inflammatory RIPK1 have been rapidly evolving in bats, suggesting different regulations of inflammation versus humans. Furthermore, we discovered specific protein sites with typical virus-host arms-race marks, as in the primate entry factor TMPRSS2, pointing to in vivo important interfaces that may be drug targets. Finally, by combining phylogenetics with genome-wide association studies (GWAS), we identified specific sites in the FYCO1 autophagy adaptor that may be crucial for SARS-CoV-2 pathogenesis and replication. Overall, we identified functional adaptations involved in SARS-CoV-2 restriction in bats and primates, which critically shed light on modern genetic determinants of virus susceptibility and severity.

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O **05** - 05

HUMAN GENETIC AND IMMUNOLOGICAL HOST FACTORS IN CRITICAL COVID-19 PNEUMONIA

Anne Puel, Qian Zhang, Paul Bastard, Aurélie Cobat, Emmanuelle Jouanguy, Jean-Laurent Casanova

St Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, The Rockefeller University, New York, NY, USA.
Laboratory of Human Genetics of Infectious Diseases, Necker Branch, INSERM, U1163, Necker Hospital for Sick Children, Paris, France.
University of Paris Cité, Imagine Institute, Paris, France.

Most individuals, following infection with SARS-CoV-2 will be asymptomatic or develop a benign infection. However, approximately 10% of the infected cases will develop hypoxemic COVID-19 pneumonia, leading to critical disease in around 3% of cases. The resultant risk of death (approximately 1% across age and gender) doubles every five years from childhood onwards and is around 1.5 times greater in men than in women. The molecular and immunological host factors of critical COVID-19 pneumonia will be discussed, in particular, inborn errors of type I interferons (IFNs), found 1-5% of patients with critical pneumonia and neutralizing auto-antibodies against IFN-α, IFN-β and/or IF-Nω, found in approximately 15-20% of patients with critical pneumonia.

Notes:

IRCI-2022

Immune Responses in Cancer and Infection
2nd International Symposium

Oral Session 6
Infections and immune responses

O **06** - 01

PRE-EXISTING POLYMERASE-SPECIFIC T CELLS EXPAND IN ABORTIVE SERONEGATIVE SARS-COV-2 INFECTION

Leo Swadling[1]*, Mariana O. Diniz[1]^, Nathalie M. Schmidt[1]^, Oliver E. Amin[1]^, Aneesh Chandran[1]^, Emily Shaw[1]^, Corinna Pade[2], Joseph M. Gibbons[2], Nina Le Bert[3], Anthony T. Tan[3], Anna Jeffery-Smith[1,2], Cedric Tan[4], Christine Y. L. Tham[3], Stephanie Kucykowicz[1], Gloryanne Aidoo-Micah[1], Joshua Rosenheim[1], Jessica Davies[1], Marina Johnson[5], Melanie P. Jensen[6,7], George Joy[6,8], Laura E McCoy[1], Ana M Valdes[9,10], Benjamin M Chain[1], David Goldblatt[5], Lucy van Dorp[4], Daniel M. Altmann[11], Rosemary J. Boyton[12,13], Charlotte Manisty[6,8], Thomas A. Treibel[6,8], James C. Moon[6,8], COVIDsortium investigators[$], Francois Balloux[4], Áine McKnight[2], Mahdad Noursadeghi[1v], Antonio Bertoletti[3,14v], Mala K. Maini[1*]

Individuals with potential exposure to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) do not necessarily develop PCR or antibody positivity, suggesting that some individuals may clear subclinical infection before seroconversion. T cells can contribute to the rapid clearance of SARS-CoV-2 and other coronavirus infections. We hypothesised that pre-existing memory T cell responses, with cross-protective potential against SARS-CoV-2, would expand in vivo to support rapid viral control, aborting infection. We measured SARS-CoV-2-reactive T cells, including those against the early transcribed replication–transcription complex (RTC) in intensively monitored healthcare workers (HCWs) who tested repeatedly negative according to PCR, antibody binding and neutralization assays (seronegative HCWs (SN-HCWs)). SN-HCWs had stronger, more multispecific memory T cells compared with a cohort of unexposed individuals from before the pandemic (prepandemic cohort), and these cells were more frequently directed against the RTC than the structural-protein-dominated responses observed after detectable infection (matched concurrent cohort). SN-HCWs with the strongest RTC-specific T cells had an increase in IFI27, a robust early innate signature of SARS-CoV-2, suggesting abortive infection. RNA polymerase within RTC was the largest region of high sequence conservation across human seasonal coronaviruses (HCoV) and SARS-CoV-2 clades. RNA polymerase was preferentially targeted (among the regions tested) by T cells from prepandemic cohorts and SN-HCWs. RTC-epitope-specific T cells that cross-recognized HCoV variants were identified in SN-HCWs. Enriched pre-existing RNA-polymerase-specific T cells expanded in vivo to preferentially accumulate in the memory response after abortive compared to overt SARS-CoV-2 infection. Our data therefore highlight RTC-specific T cells as targets for vaccines against endemic and emerging *Coronaviridae*.

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O **06** - 02

IMMUNE SURVEILLANCE OF CELL PATHOLOGY IN CANCER AND COVID-19: FROM THE BENCH TO THE CLINIC AND BACK

Adrian Hayday[1,2], Yin Wu[1], Duncan McKenzie[2], Florian Rubelt[3], Jan Berka[3], Magdalene Joseph[1]

[1] Peter Gorer Dept of Immunobiology, King's College London, UK

[2] Francis Crick Institute, London, UK

[3] Roche Diagnostics, 4300 Hacienda Drive, Pleasanton, CA., USA

αβ T cells and B cells can target cancer primarily via the capacity of their diverse antigen receptors to identify neo-epitopes resulting from genomic instability and mutation. γδ T cells also bear highly diverse antigen receptors, but they seemingly deploy them in a different way, focussed on the consequences of infection and cell transformation rather than on antigens specific to pathogenic processes and/or infectious agents. This poses the challenge of distinguishing dysregulated self from healthy self. This presentation will review our evidence for so-called "normality-sensing" wherein PDL1-like molecules expressed by healthy epithelial cells are detected by γδ TCR-dependent mechanisms, thereby suppressing the T cells' activation but maintaining their competence to respond to stress-antigens associated with cell pathology, as are typical of cancer and of virus infection. Evidence will be drawn from animal models of body surface immune surveillance; from studies of human breast and lung cancer; and from studies of COVID-19 patients, in all of which systems, γδ T cells display prominent potentially host-protective behaviours. We shall also consider the current application of this information in ongoing clinical trials of γδ T cell immuntherapeutics.

Notes:

O **06** - 03

THE ROLE OF INTERFERON-GAMMA-MEDIATED T CELL COLLECTIVITY ON CD8+ T CELL RESPONSES

Lion Uhl[1], Audrey Gerard[1]

[1] University of Oxford, Oxford, United Kingdom

Keywords: T cells, CD8, IFNg

CD8+ T cell immune responses are characterised by a remarkable degree of poly-clonality and poly-functionality. However, their overall population response is consistent and robust. Much research has focused on individual T cell activation, but how individual, heterogenous T cell responses are integrated at the population-level remains incompletely understood. Upon encountering their cognate antigen in secondary lymphoid organs, CD8+ T cells arrest for prolonged period of times around antigen-presenting cells forming dense cell clusters, which can function as communication hubs enabling T cells to directly communicate with each other and therefore directly modulate each other's behaviour. Here we investigated how endogenous CD8+ T cells regulate their response through direct T cell-T cell (T-T) communication, with a particular focus on the cytokine interferon-gamma (IFN-γ) and its effect on T cell responses. We found that specific deletion of the IFN-γ receptor on CD8+ T cells results in augmented expansion of antigen-specific effector T cells and in a skewing of the average functional avidity of the T cell repertoire, thus modulating multiple aspects of the response. In addition, we discovered that activating CD8+ T cells preferentially receive IFN-γ from other CD8+ T cells and furthermore that this IFN-γ-mediated communication between T cells is restricted to a specific time window during the activation phase of the response. This study highlights an underappreciated facet of the regulatory role of IFN-γ on endogenous CD8+ T cell responses against infection

Notes:

O **06** - 04

DECIPHERING THE TYPE I INTERFERON RESPONSE DURING VIRAL INFECTION

K. Saker, K. Brengel-Pesce, A. Pizzorno, JS Casalegno, R Pescarmona, M Perret, C Lombard, M Mezidi, M Rosa-Calatrava, A Belot, W Mouton, JC Richard, S Paul, J Lopez, D Goncalves, M Mommert, JL Casanova, T Walzer, P Bastard, S Trouillet-Assant

Aim/Objective _ Type I IFNs (IFN-I) produced by innate cells are critical antiviral molecules. Impairment of IFN-I immunity has been reported in critically ill COVID-19 patients. We aimed to decipher the causes of this impairment, investigate if other viral infections lead to the same phenomenon and develop new tools to rapidly identify subjects with a low IFN response.

Design _ Cohorts composed of more than 350 subjects with severe or moderate respiratory viral infections caused by the SARS-CoV-2 or by influenza viruses were constituted at the Hospices Civils de Lyon. The IFN-I response was monitored by analyzing the transcriptome response using Nanostring® and FilmArray® technologies from longitudinal blood and nasal swabs. The association between IFN-I impairment and the presence of anti-interferon autoantibodies detected by ELISA was also investigated.

Results_ We have shown that the IFN-I deficiency was responsible for 1/5 admissions into intensive care for severe covid. We demonstrated that this impairment can be detected from naso-paryngeal swabs at the time of diagnosis of viral infection within 45 minutes. This impairment is mostly associated with the presence of autoantibodies neutralizing interferon alpha and omega. We also identified the presence of these autoantibodies in 5% of subjects hospitalized for severe influenza. Finally, using reconstituted human airway epithelia, we reported that neutralizing auto-Abs also blocked the antiviral function of type I IFNs leading to strong viral replication.

Conclusion _ We have demonstrated that IFN-I impairment could be one of the main causes for severe viral infection. Furthermore, we have examined new tools by which individuals at highest risk of life-threatening viral infection can be identified. Here we offer propose a perspective strategy for personalized medicine based on therapeutic recombinant IFN-I administration.

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O **06** - 05

THE MOLECULAR REGULATION OF CD8 T CELL EXHAUSTION IN CHRONIC INFECTION

Carlson Tsui[1], Lorenz Kretschmer[2], Svenja Rapelius[2], Sarah Gabriel[1], David Chisanga[3], Konrad Knöpper[5], Stephen Nutt[4], Wolfgang Kastenmüller[5], Veit Buchholz[2], Axel Kallies[1]

[1] The Peter Doherty Institute of Infection and Immunology, University of Melbourne, Melbourne, Australia

[2] Institute for Medical Microbiology, Immunology and Hygiene, Technical University of Munich, Munich, Germany

[3] Olivia Newton-John Cancer Research Institute, Heidelberg, Australia

[4] The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

[5] Würzburg Institute of Systems Immunology, the Julius-Maximilians-Universität Würzburg, Würzburg, Germany

Keywords: CD8 T cells, Exhaustion, Chronic infection, Checkpoint inhibition

CD8 T cells chronically exposed to high amounts of antigen, for example during chronic viral infection or cancer, enter a state known as T cell exhaustion. Exhausted T cells are characterised by dampened effector function, sustained expression of inhibitory receptors and reduced proliferative capacity. Despite this, exhausted T cells persist long-term, exert some control over viral and tumour antigens, and can be boosted using therapeutic checkpoint inhibition. Recent studies have shown that TCF1-expressing exhausted T cells, termed precursor exhausted T (TPEX) cells, are critical in maintaining chronic T cell responses by undergoing self-renewal and generating effector progenies. Importantly, TPEX cells are key to the responsiveness of therapeutic PD-1 checkpoint blockade. However, the cellular and molecular mechanisms that governs TPEX self-renewal and differentiation are not fully understood. Here we reveal and map additional complexity of the exhausted T cell hierarchy during chronic infection using single-cell RNA sequencing, adoptive transfer experiments and different genetic models. We also identify the transcription factor c-Myb as a central regulator of the exhausted T cell function that underpins prolonged T cell activities and responsiveness to checkpoint inhibition. Our study thus links two fundamental aspects of T cell exhaustion to a single transcription factor and provides novel insights into the molecular regulation of exhausted T cell responses in chronic infection.

Notes:

Keynote

ENGINEERING VIRUS-SPECIFIC T LYMPHOCYTES FOR TREATMENT OF VIRUS-RELATED CANCER AND VIRAL INFECTION

Antonio Bertoletti, MD

Program in Emerging Infectious Diseases, Duke-NUS Graduate Medical School, Singapore

(antonio@duke-nus.edu.sg)

Immunotherapy based on adoptive transfer of T cells engineered to express HLA class I restricted T-cell receptor (TCR) or a chimeric antigen receptor (CAR) targeting tumor antigens has generated impressive results in treating some cancers. T cells are, however, also essential for the control of viral infections.

Libraries of virus-specific TCRs can be generated from T cells of individuals who, for example, control HBV or SARS-CoV-2 infection and virus-specific T lymphocytes can be engineered to express stably or transiently the selected TCRs.

The demonstration that HBV antigen originated from HBV-DNA integration in HBV-related hepatocellular carcinoma (HCC) can be used as a specific tumor antigen for HBV-specific CD8 T cell recognition lead us to an initial clinical trial of HBV-specific T cell immunotherapy in patients with primary or secondary HBV-related HCC.

Furthermore, I will also discuss how we co-transfect T cells with mRNA coding for virus-specific TCR and an altered form of Calcineurin B, which allows us to engineer Immunosuppressive Drugs Resistant Armoured (IDRA) virus-specific T cells. These engineered IDRA-TCR-T cells retain their functionality in the presence of therapeutic concentrations of immunosuppressive drugs (i.e. Tacrolimus) and thus can be used not only to target HBV-HCC relapses in liver transplanted patients but also the persistent SARS-CoV-2 infection occurring in kidney transplanted patients who poorly respond to COVID-19 vaccination.

IRCI-2022

Immune Responses in Cancer and Infection
2nd International Symposium

Posters sessions

Wednesday 15th of June, 2022

Session 1

Innate Immunity

Session 2

Immuno-metabolism

P **01** - 001

DECIPHERING THE ROLE OF NEUTROPHILS IN HUMAN COLORECTAL CARCINOGENESIS

Sarabi Matthieu[1,2,3], Manuela Pereira Abrantes[2,3], Lyvia Moudombi[2], Aurélien Dupre[3,4], Patrice Peyrat[4], Michel Rivoire[3,4], Justine Berthet[2,5], Sarah Barrin[2,5], Vincent Garbit[6], Damien Blehaut[6], Olivier Raspado[6], Gwenaelle Garin[7], Hervé Perrier[6], Caroline Renard[8], Thibault Andrieu[9], Christophe Caux[2,5], Marie-Cécile Michallet[2]

[1]Department of Medical Oncology, Centre Léon Bérard Cancer Center, Lyon, France; [2]CRCL - TERI (Tumor Escape, Resistance and Immunity) Department, Centre de Recherche en Cancérologie de Lyon, Centre Léon Bérard, Université de Lyon, Université Claude Bernard Lyon 1, INSERM 1052, CNRS 5286, Lyon, France; [3]BMIC - Université Claude Bernard Lyon 1, Lyon, France; [4]Department of Surgery, Centre Léon Bérard Cancer Center, Lyon, France; [5]CRCL - Laboratory for Immunotherapy of Cancer of Lyon (LICL), Lyon, France; [6]Infirmerie Protestante de Lyon , Caluire-et-cuire, France; [7]Centre Léon Bérard - Direction de la Recherche clinique et de l'Innovation, Lyon, France; [8]Centre Léon Bérard - Département de Biopathology, Lyon, France; [9]CRCL - Plateforme de cytométrie, Lyon, France

Keywords: Colorectal, Adenoma, neutrophils, carcinogenesis

Colorectal cancer (CRC) is a major health matter with nearly 45000 new cases and 17000 deaths each year. Screening program has been set up to detect and resect early CRC and preneoplastic lesions (Ad). Neutrophils (Nph) has been extensively associated with inflammatory hallmarks of carcinogenesis, tumor spread, metastasis and poor outcome in many cancers. However, little is known about the role of this heterogeneous and multifaceted cell in CRC, especially in Ad. Our hypothesis is that Nph may play different roles across tumorigenesis and potentially inside CRC progression.

We are studying Nph in tissues from patients undergoing surgery (ColonIM program NCT03841799), through sampling of CRC, matched with non-tumoral adjacent tissues (NTa), and with adjacent Ad on surgical explant. To decipher the immune landscape, we performed multiparametric flow cytometry analysis (FACS) and multiplexed immunofluorescence (mIF) stainings on 162 samples (67 NTa, 24 Ad and 71 CRC) and 30 FFPE specimens, respectively. To go deeper in the characterization of Nph, we did bulk RNAseq and scRNAseq on FACS-sorted neutrophils (NTa, Ad and CRC), and on whole cell samples (n = 25, 8 NTa, 10 Ad and 7 CRC).

FACS showed an increase of Nph in Ad and even more in CRC compared to NTa. Nph infiltrate was more pronounced from localized to metastatic setting. There was a tendency to increased EpCam+ Nph through NTa, the Ad and CRC. On correlograms of immune cells infiltrates, Nph were increase with IgA Plasma cells (Pc) in Ad, and with IgG Pc in CRC, respectively. IgG Pc were augmented in CRC with RAS mutation. Conversly there was a tendency to increased Nph infiltrate in RASwt tumors. Nph and NK cells were positively correlated in NTa colon.

mIF was performed on 30 cases of CRC with 13 RAS wild type tumors, 4 BRAF muted tumors, 4 cases of deficient mismatch repair tumors and 15 cases with RAS mutation. Cell density analysis reported presence of neutrophils in both in stroma and tumor cells trabeculae. Cell cluster and proximity analyses are ongoing.

Transcriptomic (Tc) analyses gives us an insight to functional differences among Nph from NTa, Ad and CRC. In one hand, Tc profiles of bulk cell suspensions from Ad and CRC have more in common than with NTa ; in the other hand, sorted Nph Tc profils, from Ad and NTa are closer than Tc profils from CRC.

In conclusion, our datas support the hypothesis of Nph quantitative/ qualitative modifications along carcinogenesis that may impact microenvironment.

P **01** - 002

DISTINCT IMMUNE PHENOTYPES IN ENDOMETRIOSIS SUBSETS REVEALED BY MULTIPLEX IMMUNOHISTOCHEMISTRY

Jonathan Moore[1,2], Dimitrios Kalaitzopoulos[3], Jonathan S. Moore[1], Stephan Ryser[1], Nicolas Liaudet[4], S. Intidhar Labidi-Galy[1,5], Pierre E. Samartzis[3]

[1]Department of Medicine and Center of Translational Research in Onco-Hematology, Faculty of Medicine, University of Geneva; Swiss Cancer Center Leman, Genève, Switzerland , Geneva, Switzerland; [2]Developmental Biology Laboratory, Department of Animal Biology, School of Biology, College of Science, University of Tehran, Iran, Tehran, Iran; [3]Department of gynecology, Zurich University Hospital, Zurich, Switzerland, Zurich, Switzerland; [4]Bioimaging Core Facility, Faculty of Medicine, University of Geneva, Genève, Switzerland, Geneva, Switzerland; [5]Department of Oncology, Hôpitaux Universitaires de Genève, Genève, Switzerland , Geneva, Switzerland

Keywords: Immune repertoire, NK cells, Macrophages, endometriosis, tissue microarray

Endometriosis is a frequent chronic neuroinflammatory disease in females of reproductive age, defined by the presence of endometrial-like tissue in the peritoneal cavity and is responsible for pain and infertility. The adaptation of immune cell populations within the ectopic endometrium tissues remains elusive. Here, we developed a sequential multiplexed immunohistochemistry method that could sustain 7 consecutive markers (CD45, CD68, CD163, CD20, CD8, FOXP3 and Cytokeratin) and NKp46. We applied it to a tissue microarray that included clinically annotated endometriosis (N=182) and eutopic endometrium samples as control (N=74). Most of immune cells were more abundant in rASRM stage III-IV, as compared to rASRM stage I-II endometriosis. There was a fluctuation of immune cell populations during the menstrual cycle. Specifically, endometriosis lesions showed an increase of B cells, NK cells and CD163+ M2 macrophages during the secretory phase, as compared to normal endometrium. Patients with endometriosis-associated infertility had significant increase of macrophages. Our results suggest that endometriosis lesions differ immunologically from normal endometrium and that immune cells recruited to endometriotic lesions are influenced by the phase of menstrual cycle, suggesting hormonal regulation. We anticipate that a tolerogenic immune environment enriched in M2 macrophage promotes the expansion of endometriosis lesions and favors infertility.

P **01** - 003

SEEKING ILC'S TRUE COLOURS - MULTIPARAMETRIC ANALYSIS OF HUMAN INNATE LYMPHOID CELLS USING SPECTRAL FLOW CYTOMETRY

Sarah Benezech[1], Alexandre Belot[1], Thierry Walzer[1]

[1]Centre International de Recherche en Infectiologie , Lyon, France

Keywords: Innate Lymphoid Cells, Natural Killer Cells, Cytometry

Innate lymphoid cells (ILCs) are major components of innate immune response against external pathogens and tumors. Shifts in phenotypes of human ILCs subsets among the 5 described to date (i.e. Natural Killer (NK) Cells, ILCs 1, 2, 3 and Lymphoid Tissue Inducer Cells) have been depicted in various tumoral, inflammatory and dysimmune conditions. However, studies are still limited due to the absence of clear phenotype for human ILCs 1 and overall plasticity of ILCs. Here we present a 39 parameter, 35-colour full spectrum flow cytometry panel dedicated to in-depth analysis of circulating ILCs subsets in cryopreserved human peripheral blood mononuclear cells (PBMCs). This panel moreover includes a selection of 3 markers related to type-I and type-II interferon (IFN) signaling being ISG15, CD169 and CD64, providing an integrated cytometric interferon signature. Design and optimization of this panel was performed on Cytek®Aurora-5L, on a series of cryopreserved PBMCs samples from healthy controls and pediatric patients presenting with dysregulated type I/II IFN signaling. In addition to an extensive characterization of NK cells maturation, activation, and exhaustion profile, classical supervised analysis allowed a precise and reliable discrimination of circulating ILCs 1, 2 and 3 in healthy controls as well as in pathological conditions. Cytometric IFN signature was reliably correlated with the IFN signature as assessed by gold standard technique with gene expression (NanoString nCounter). Unsupervised analysis was able to highlight major phenotypic trends of ILCs through

the course of diverse immune conditions. First implementation of this panel will be dedicated to explore the impact of chronic interferon signaling on ILCs phenotype and function, and conducted on a series of pediatric patients presenting with interferon-signaling dysregulation associated diseases. Further research projects, within cancer or infectious diseases fields, should benefit from this multi-parametric exploration of human circulating ILCs.

P **01** - 004

INFLUENCE OF IMMUNOSUPPRESSIVE MYELOID CELLS ON CANCER STEMNESS PROMOTION

Thomas Boyer[1], Celine Blaye[1,2], Justine Vaché[1], Clément Klein[1,5], Aurélia Le Dantec[1], Christine Varon[3,4], Nicolas Larmonier[1,4], Charlotte Domblides[1,5]

[1]CNRS UMR 5164, ImmunoConcEpT, Bordeaux, France; [2]Department of Medical Oncology, Institut Bergonié, Bordeaux, France; [3]INSERM U1035, Bordeaux Research in Translational Oncology, Bordeaux, France; [4]UF Biology, University of Bordeaux, Bordeaux, France; [5]CHU Bordeaux, Bordeaux, France

Keywords: Immunosuppressive myeloid cells, cancer stem cells

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 in we demonstrate that monocyte-derived suppressor cells are endowed with the capability to promote stemness features in different types of cancer cells in a contact-dependent manner. Moreover, these interactions confer to cancer cells chemotherapy resistance properties. Finally, our data provide insights into the ability of mouse-derived MDSC to increase tumorsphere formation.

P **01** - 005

CHARACTERIZATION OF MURINE INNATE LYMPHOID CELLS DEVELOPMENT USING HIGH DIMENSIONAL SPECTRAL CYTOMETRY

Noemi Rousseaux[1], Thierry Walzer[1]

[1]CIRI, Lyon, France

Keywords: Spectral Cytometry, ILCs, Mouse, Differentiation

Innate Lymphoid cells (ILCs) group several types of lymphoid cells with various functions such as cytotoxicity for Natural Killer (NK) cells or helper functions for ILC1, 2, 3 and Lymphoid tissue inducer (LTi) cells. These helper ILCs participate to tissue homeostasis and defense against infection. All ILCs originate from the common innate lymphoid progenitor in the bone marrow. During maturation, ILCs progressively lose their capacity to proliferate, and modify their trafficking machinery. In particular they egress from the bone marrow to circulate through the blood and gain several organs. Indeed, NK cells and ILCs show a very broad distribution among the organism (Bone marrow, spleen and lungs for NK cells, liver for ILC1, liver and lungs for ILC2, liver and intestine for ILC3 and LTi). All helper ILCs express the IL-7 receptor, (CD127), and they can further be classified according to the expression of both membrane and nuclear markers: T-bet and NK1.1/CD49a, Gata-3 and CD25, RorgT and NKp46/IL-18R respectively for ILC1, ILC2 and ILC3/LTi. Thanks to the use of spectral cytometry (Cytek Aurora ©), we could better characterize and understand the engagement into ILC lineage, the maturation and plasticity among these lineages as well as events of activation in a cancer or infectious context. To this order, we have designed a 30 colors panel to analyze samples from different strains of mice with spectral cytometry. Through immunostaining of membrane markers like activating or inhibiting receptors, cytokines receptors, markers of maturation and activation, we can monitor the cell journey into ILC differentiation. Thanks to nuclear staining of transcription factors such as Tcf7, Eomes, T-bet, RorgT and Gata-3 in a sufficient set of organs (Bone Marrow, Spleen, Liver, Lungs), we are able to distinguish the different subsets of ILCs and any variation of expression of these transcription factors. The use of mutant mouse for genes known to be involved in NK engagement and differentiation such as Eomes, T-bet and Zeb1 have helped us to validate this panel. We are currently sampling other strains of mice mutated for different molecules involved in ILC differentiation in order to better characterized NK cells differentiation and to better understand the parameters governing ILCs’ differentiation. The combined use of supervised and unsupervised analysis might also help us to identify unexpected subsets of ILCs.

P **01** - 006

DECIPHER THE ROLE OF DENDRITIC CELLS IN EARLY IMMUNE SURVEILLANCE DURING BREAST CANCER DEVELOPMENT

Aurélien Voissiere[1], Margaux Hubert[1], Léo Laoubi[1], Cyril Degletagne[1], Dominique Poujol[1], Alexia Gazeu[1,2], Christophe Caux[1], Jenny Valladeau[1], Nathalie Bendriss-Vermare[1]

[1]UMR Inserm 1052 CNRS 5286,Cancer Research Center of Lyon, Lyon, France; [2]Department of Pathology-Groupement Hospitalier Est, Hospices Civils de Lyon, Lyon, France

Keywords: Immune surveillance, Dendritic cells, Interferons, Breast Cancer

While tumor immune evasion mechanisms are now well characterized in mammary tumors, the very early events implicated in the immune sensing of preneoplastic cells remain poorly understood and hypothetical due to the lack of human biological samples and the difficulties to develop an appropriate preclinical mouse model. In order to characterize the molecular and cellular mechanisms of early immune surveillance in triple-negative breast cancer, we used a spontaneous mammary tumor model in mice (BLR-Cre, BRCA1f22-24/f22-24, p53+/-) which recapitulates the different stages of development (normal, adenosia, hyperplasia, and invasive carcinoma). Given the important role of dendritic cells (DC) and interferon (IFN) pathways in the initiation of anti-tumor immune response, we hypothesize they could play a central role in the anti-tumor immune surveillance. We have previously demonstrated that type 1 conventional DC (cDC1) infiltrate human breast tumors and are associated, with their production of type III IFN (IFN-III), to a good prognosis. First, we characterized the phenotype of DC infiltrate in the spontaneous tumor model at different stages of development by flow cytometry. All DC subsets are present from the hyperplasia stage, but, the proportion of cDC1 and cDC2 decreases during tumor progression while plasmacytoid DC (pDC) remain stable. We also demonstrated the functional abilities of infiltrating DCs (cytokine production, expression of activation markers) ex vivo in response to TLR agonists by spectral flow cytometry using a 23-color panel. Furthermore, we performed the first in-depth analyses of DC types sorted from mammary tissues at the different stages by single cell RNA sequencing (scRNAseq). Preliminary analysis revealed DC heterogeneity (e.g., presence of mature DCs) and different activation states (e.g., DC in cell cycle) that will be compared between the different stages of development. By exploiting a spontaneous mouse mammary tumor model and powerful technologies to study early TNBC immune surveillance, our project will help to understand the cellular and molecular mechanisms involved in the immune surveillance of preneoplastic cells and ultimately to identify new therapeutic targets promoting anti-tumoral functions of DC and IFNs, in advanced tumors resistant to conventional immunotherapies.

P **01** - 007

IFITM PROTEINS REGULATE IMMUNE-RELATED CELL SURFACE MOLECULES IN CERVICAL CARCINOMA

Nela Friedlova[1,2], Lenka Dosedelova[1], Filip Zavadil Kokas[1], Lenka Hernychova[1], Ted Hupp[1,3], Borivoj Vojtesek[1], Marta Nekulova[1]

[1]Research Centre for Applied Molecular Oncology, Masaryk Memorial Cancer Institute, Brno, Czechia; [2]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czechia; [3]Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom

Keywords: IFITM, interferon, cervical cancer, immunosurveillance, tumor progression

Interferon-induced transmembrane (IFITM) proteins form a family of plasma and endosome membrane proteins expressed by most cell types especially as a response to interferon stimulation. IFITMs are a part of innate immune response against a broad range of viruses. Besides, IFITMs also regulate adaptive immune system, especially immune cell activation and development. Deregulated expression of IFITMs has been described in most types of solid tumors as well as hematolymphoid malignancies. Using a human cervical cancer model, we have previously described that IFITMs are linked with tumor immunogenicity through regulation of major histocompatibility complex class I exposure level on tumor cell surface. Moreover, IFITM expression inversely correlates with lymph node positive cervical cancers. To further clarify the role of IFITMs in metastasis and immunity, we determined changes in surfaceome of IFITM1-deficient Siha cervical cancer cell line using differential proteomics approach. Revealed cell surface protein alterations could affect interactions between tumor and immune cells. We performed a validation analysis of preselected candidate proteins that confirmed a downregulation of CD166 and CD40 in IFITM1 and IFITM3 deficient SiHa derivatives established by CRISPR/Cas method. We further focus on functional analysis of CD166 protein (ALCAM, „activated leukocyte cell adhesion molecule“). Beyond cancer-stem cell marker function, CD166 serves as a CD6 ligand and thus intervenes in the T cell activation process. CD166 also regulates epithelial-mesenchymal transition, adhesion and metastatic spread of cancer cells. In our model, CD166 and IFITM proteins support cervicosphere formation and tumor cell migration. Interestingly, SiHa cell line sorted according to CD166 surface level exerts inversed IFITM1 expression and a transient CD166 silencing resulted in IFITM1 downregulation that confirms a reciprocal relationship between these proteins. Despite IFITM and CD166 overexpression and pro-carcinogenic role in different cancers, these proteins possibly help tumor immunosurveillance and thus prevent cervical cancer spread. The mutual regulation of IFITMs and CD166 described here might be important for better understanding of the relationship between the innate and adaptive immunity.

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P **01** - 008

DETECTION AND CORRELATION ANALYSIS OF SERUM CYTOKINE LEVELS IN CHRONIC HEPATITIS C VIRUS INFECTION

Ismail Che Noh[1,2], Asma Abdullah Nurul[3], Imran Ahmad[4], Abu Bakar Ruzilawati[1]

[1]Department of Pharmacology, School of Medical Sciences, Universiti Sains Malaysia, Kota Bharu, Malaysia; [2]Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Malaysia; [3]Biomedicine Programme, School of Health Sciences, Universiti Sains Malaysia, Kota Bharu, Malaysia; [4]Department of Family Medicine, School of Medical Sciences, Universiti Sains Malaysia, Kota Bharu, Malaysia

Keywords: Hepatitis C, Cytokines, Serum level, Elisa, Luminex assay

Background: Chronic hepatitis C virus (HCV) infection is one of the major causes of liver cirrhosis and carcinoma. Intravenous drug usage remains one of the major risk factors for HCV transmission, and the issue continues to burden healthcare and social systems. Studies have indicated that an imbalance of cytokine activities could contribute to the pathogenesis of chronic HCV infection. Objective: The aim of this study was to assess and correlate serum cytokine levels with chronic HCV infection in Malay male subjects. Method: Thirty-nine subjects were enrolled from various health clinics in Kota Bharu, Kelantan, Malaysia, and divided into two groups: patients with chronic HCV infection (HP) and healthy control (HS). The serum cytokines IL-10, IL-6, TNF-α were simultaneously measured using Luminex assay, and serum TGF-β1 was measured by ELISA. Result: There were statistically significant differences in the mean serum levels of IL-10, IL-6, and TGF-β1 in HP compared to HS ($p = 0.0096$, $p = 0.0180$, and $p=0.0005$, respectively). There was no significant difference in the mean serum level of TNF-α in HP compared to HS group. Conclusion: Serum levels of IL-10 and IL-6 is associated with chronic HCV infection. However, serum level of TGF-β1 was negatively associated with chronic HCV infection and there was no significant association observed for TNF-α.

P **01** - 009

INNATE IMMUNE MEMORY – TRANSLATION TO VETERINARY SPECIES

Simon Paris[1], Ludivine Chapat[1], Manon Lambiel[1], Pierre-Yves Durand[1], Lauriane Piney[1], Pierre Bergamo[2], Jeanne-Marie Bonnet[3], Ludovic Freyburger[3], Karelle De Luca[1]

[1]Boehringer Ingelheim Animal Health France_Global innovation, ST PRIEST, France; [2]Boehringer Ingelheim Animal Health France_Animal facilities, ST VULBAS, France; [3]Agressions Pulmonaires et Circulatoires dans le Sepsis (APCSe), VetAgro Sup Campus Vétérinaire, MARCY L'ETOILE, France

Keywords: trained immunity, beta-glucans, adjuvants, dog, rabies vaccine

Host defense against infections relies on physical, chemical barriers as well as immunity, composed of two arms: the innate and the adaptive immunity. Adaptive immunity requires specific T and B lymphocytes, able to recognize with a high specificity a huge variety of pathogens. It is responsible for the immune memory and is the target of vaccination strategies. However, the last years have seen many publications describing features of the memory of the innate immunity, enabling a heightened response to secondary exposure to homologous as well as heterologous pathogens. It was demonstrated that the training of monocytes with vaccines (BCG) or prototypical agonists like b-glucans induce epigenetic and metabolic modifications leading to an enhanced inflammatory cytokine secretion when the host encounters pathogens. This memory was shown to last several months thanks to the modulation of hematopoietic progenitors and myelopoiesis. The objective of our research program is to investigate this innate immune memory in dogs. We developed an in vitro model of trained innate immunity in dogs as described by Netea and coll. Canine blood enriched monocytes stimulated with b-glucans showed an increased production of inflammatory cytokines (IL-6, TNF-a) in response to LPS or Pam3CKS4 stimulation. The use of inhibitors of histone methylation, autophagy and metabolic pathways during the training of the cells completely abolished the effect on cytokine secretion. These in vitro results confirm that the trained innate immunity follows similar mechanisms in dogs, with cellular mechanisms close to those described in mice and humans. Furthermore, in a vivo study, we assessed the potential immune training effects of β-glucans as well as their capacity to enhance the adaptive immune response of an inactivated rabies vaccine (Rabisin®). Injection of β-glucan from Euglena gracilis was performed one month before vaccination with Rabisin® supplemented or not with the same β-glucan used as adjuvant. Our results support that adjuvantation of Rabisin® vaccine with β-glucan elicit a higher B-lymphocyte immune response, the prevailing factor of protection against rabies. β-glucan also tend to stimulate the T cell response as shown by the cytokine secretion profile of PBMCs ex vivo re-stimulated. Our data are providing new insights on the impact of trained immunity on the adaptive immune in dogs.

P **01** - 010

INSIGHTS ON THE ANTIVIRAL MECHANISMS OF ACTION OF THE TLR1/2 AGONIST PAM3CSK4 IN HEPATITIS B VIRUS (HBV)-INFECTED HEPATOCYTES

Manon Desmares[2], Marion Delphin[2], Brieux Chardes[2], Caroline Pons[1,2], Juliette Riedinger[1], Maud Michelet[2], Michel Rivoire[3], Bernard Verrier[4], Anna Salvetti[1,2], Julie Lucifora[1,2], David Durantel[1,2]

[1]CIRI - INSERM U1111, University of Lyon (UCBL1), CNRS UMR_5308, ENS de Lyon, Lyon, France; [2]CRCL - INSERM U1052, University of Lyon (UCBL1), CNRS UMR_5286, Centre Léon Bérard, Lyon, France; [3]INSERM U1032, Lyon, France; [4]LBTI, CNRS UMR_5305, University of Lyon (UCBL1), Lyon, France

Keywords: HBV, PRR, TLR2, NFkB, Agonist

Background and Aims: Pegylated interferon-alpha (peg-IFN) is yet used in clinics, some time in first line, to treat chronically HBV-infected patients, despite its poor safety profile and relative weak efficacy at inducing HBsAg loss. If an immune-stimulator is argued to be an instrumental component of future combination therapies aiming at curing HBV infections, it would be interesting to find alternatives to peg-IFN. We have previously identified the TLR2 agonist Pam3CSK3, as one of the best agonist to reduce HBV replication, including cccDNA level and activity, in infected hepatocytes (Lucifora et al., Scientific Rep. 2018). Yet the detailed mode of action (MoA) of this potential anti-HBV asset remained elusive. The aim of this study was to precisely decipher the MoA of Pam3CSK4 in vitro, by determining the kinetic of antiviral events, identifying the upstream and most relevant step of blockade of HBV replication, working-out its specificity of action, discovering host-effectors involved in the long-lasting antivirals phenotypes.

Methods: We used experimentally-HBV-infected differentiated HepaRG cells and primary human hepatocytes (PHH), as well as rather standard molecular and cellular virologic methods to decipher the MoA of Pam3CSK4. This included RNA-seq analyses for the identification of antiviral host-effectors and loss-of-functions studies to validate them.

Results: Pam3SCK4 strongly and durably (no rebound within 5-weeks off drug) inhibited HBV replication by engaging the TLR2 innate receptor and canonical NF-kB pathway. The overall long-lasting antiviral phenotype is the double consequence of an immediate effect on HBV RNA biogenesis, by both reduction of RNA transcription and acceleration of RNA decay, as well as a reduction of cccDNA level; the latter phenotype, which kinetically occurs second, reinforce the first, as cccDNA is the main template of HBV RNA synthesis. RNA-seq analyses and loss-of-function approaches allowed the investigation of two host-effectors, respectively MCPIP1 and FEN-1, as involved in the reduction of HBV RNA accumulation and cccDNA level.

Conclusion: Using relevant cell culture models, we have uncovered the MoA of Pam3CSK4, a TLR2 ligand, which is currently under preclinical evaluation in a liver-deliverable and nanoparticular form for the treatment of chronic HBV infections.

P **01** - 011

THE TYROSINE KINASE INHIBITOR PEXIDARTINIB NEGATIVELY IMPACT DENDRITIC CELL SUBSETS THROUGH INHIBITION OF FLT3 SIGNALING

Celine Rodriguez[1,2], Aurélien Voissière[2], Carlos Gomez-Roca[5], Axelle N'kodia[1], Justine Berthet[1,2], Gwenaelle Garin[3], Isabelle Treilleux[4], Anne-Sophie Bidaux[3], David Perol[3], Christophe Caux[1,2], Bertrand Dubois[1,2], Christine Menetrier-Caux[1,2], Nathalie Bendriss-Vermare[1,2], Philippe Cassier[6]

[1]Laboratory of Cancer Immunotherapy of LYON (LICL), Centre Léon Bérard, 69008 Lyon, France , LYON, France; [2]Université Claude Bernard Lyon 1, INSERM U-1052, CNRS 5286, Cancer Research Center of Lyon, Lyon, France, LYON, France; [3]Centre Léon Bérard, Clinical Research Platform (DRCI), Lyon, France, LYON, France; [4]Centre Léon Bérard, Biopathology Department, Lyon, France , LYON, France; [5]Department of Medical Oncology, Institut Claudius Regaud/Institut Universitaire du Cancer de Toulouse - Oncopole, Toulouse, France, TOULOUSE, France; [6]Centre Léon Bérard, Department of Medical Oncology, 28 rue Laennec 69008 Lyon, France, LYON, France

Keywords: Pexidartinib, CSF1-R inhibitor, FLT3-L, dendritic cells, monocytes

The MEDIPLEX study investigated the clinical efficacy of combining pexidartinib (pexi), a TKI targeting CSF1-R (IC50 20nM), and anti-PDL1 (durvalumab) in advanced colorectal and pancreatic cancer with the aim to eliminate CSF1-dependent suppressive macrophages to enhance patient response to PDL1 blockade. No unexpected safety signal was identified and 800 mg pexi was declared as the recommended dose for phase 2. Activity, was however minimal with 1 partial response in an MSI-H CRC, 7 stable and 39 progressive diseases as their best response.

In the 47 patients (dose escalation + phase 2) treated, we confirmed the on-target effect of pexi with a dose-dependent increase of plasma CSF1 levels consequently to the CSF1-R signaling blockade. Flow cytometry analysis of phase 2 (n=28) revealed, at C1D15, a drop of circulating non-classical and inflammatory monocytes, but not classical ones, in agreement with the critical role of CSF1-R signaling in their survival.

No significant modulation of T cell frequency, phenotype nor function was detected. In sharp contrast, we observed in blood a rapid reduction (C1D15) in the number of various dendritic cell (DC) subsets. This was also associated with a decreased capacity of blood cells to secrete, upon TLR3 stimulation, IFN-I, a cytokine selectively produced by type-1 conventional DC.

As pexi can also target cKIT (IC50 10nM) and FLT3 (IC50 160nM) that are key growth factor receptors for DC differentiation and survival, we assessed changes in the plasma levels of their cognate ligand during treatment. Whereas SCF levels were not modified, we detected a sustained increase in FLT3-L levels confirming a systemic impact of pexi on FLT3 signaling.

Using in vitro bone marrow-derived murine DC differentiation models triggered by FLT3-L or GM-CSF, we demonstrated that the addition of pexi at early (D0, D2) but not at late (D6) time points strongly reduced the proportion of pDC and cDC subsets generated with FLT3-L but not GM-CSF, suggesting a major role of pexi on FLT3-L dependent DC differentiation but not on survival of differentiated DCs. The lack of impact of pexi on survival of differentiated DC was confirmed with human blood DC subsets from healthy donors.

Altogether, these results argue for a deleterious impact of pexi, through the inhibition of FLT3 signaling, on the differentiation of DC that are key players in the anti-tumor immune response, and may explain the limited anti-tumor clinical activity observed in this study.

P **01** - 012

DNASE1L3 DEFICIENCY IMPAIRS THE IMMUNOGENIC POTENTIAL OF CHEMOTHERAPIES IN BREAST CANCER

Pauline Santa[1], Anne Garreau[1], Severine Loizon[1], Dorothée Duluc[1], Vanja Sisirak[1]

[1]UMR5164 CNRS - Immunoconcept, Bordeaux, France

Keywords: Dnase, Interferon, breaste cancer, DNA sensing, chemotherapy

Detection of tumor-derived DNA (tDNA) by dendritic cells (DCs) plays a crucial role in activation of anti-tumor immunity by stimulating the production of type I interferons (IFN-I). IFN-I is associated with improved patients' outcomes and better efficacy of immunotherapies. In addition, chemotherapies and radiotherapy boost anti-tumor responses by increasing IFN-I production induced by tDNA. We have characterized a nuclease produced by DCs called DNASE1L3 that digests DNA released by dying cells and thus limits self-DNA abundance and its immunostimulatory potential. However, it remains unknown whether DNASE1L3 may be involved in the regulation of tDNA-induced anti-tumor immune responses. We aimed to characterize the impact of DNASE1L3 deficiency on cancer progression and responsiveness to cytotoxic tumor therapies. Dnase1l3-deficient mice were crossed with MMTV-PyMT mice that spontaneously develop mammary adenocarcinomas. In addition, transplantable orthotopic tumor models were established in Dnase1l3-deficient mice using the E0771 mammary carcinoma cell line. Tumor growth was followed weekly and the anti-tumor immune response was evaluated by flow cytometry at endpoint. Dnase1l3-deficient mice harboring either spontaneous or transplantable mammary tumors were also treated with immunogenic chemotherapies, such as Doxorubicin and Teniposide. After five consecutive treatments every other day, tumor growth was followed daily and the overall survival of mice was evaluated. Our preliminary

results show that Dnase1l3 deficiency did not affect the growth of either spontaneous or transplantable tumors, nor the tumor immune infiltrate. However, the therapeutic efficacy of the chemotherapies was strongly reduced in Dnase1l3-deficient mice bearing spontaneous and transplantable mammary tumors. Accordingly, Dnase1l3-deficient mice succumbed faster compared to control wild type mice upon immunogenic chemotherapies. Thus, DNASE1L3 likely processes tDNA to enhance its immunostimulatory potential. Further studies are needed, particularly of the mechanisms of action of DNASE1L3 in the regulation of anti-tumor immune responses induced by immunogenic therapies. Characterizing DNASE1L3 function in cancer may contribute to the development of novel therapeutic strategies to boost anti-tumor immunity and the efficacy of current therapies.

P **01** - 013

MOLECULAR AND PHYSICAL ANALYSIS OF THE TUMOR/MACROPHAGE INTERPLAY

Jovan Nikolic[1], Joseph Ackermann[2], Sarah Taheraly[1], François-Xavier Gobert[1], Mathieu Maurin[1], Jean-François Joanny[3], Martine Ben Amar[2], Philippe Benaroch[1]

[1]Institut Curie U932, Paris, France; [2]ENS , Paris, France; [3]Collège de France, Paris, France

Keywords: tumor spheroids, mathematical modeling, macrophages, adhesion forces

Myeloid cells are major players of the tumor microenvironment contributing to immune evasion mechanisms and providing pro-tumorigenic effects. Here we aim to analyze at the physical and molecular level, how myeloid cells can impact tumor growth and fate. Combining quantitative cell biology with the derivation of physical modeling of tumor growth, allow us to extract key parameters and make predictions that are testable both, by simulations in silico and by in vitro experiments.

We set up an in vitro system to follow the growth of tumor spheroids in 3D by real-time microscopy over time. Importantly, we replicate in this system the strong positive effect of macrophages on tumor growth. From the quantifications and growth curves obtained, a physical modeling of spheroid growth in 2 and 3D and with multiple cell types was derived. This model, tested in simulations, fits well with the data obtained in the absence or presence of macrophages. The model predicts that adhesion forces are key in the pro-tumoral effect observed. By blocking CD11c expressed by macrophages we indeed impede this effect. Anti-CD11c blocking antibodies diminish cell to cell adhesion forces, prevent spheroid nucleation and impaired spheroid growth.

Establishing that macrophages can have a direct impact on tumor growth by modifying key physical parameters represents an important discovery and may identify new approaches targeting myeloid cells within the tumor infiltrate to counteract their pro-tumoral activity.

P **01** - 014

RAPID LOSS OF CRUCIAL NK EFFECTOR FUNCTIONS UPON TUMOUR INFILTRATION DEBILITATES ANTI-TUMOUR RESPONSES

Isaac Dean[1], Zhi Li[1], Zewen Kelvin Tuong[2,4], Fabrina Gaspal[1], Claire Willis[1], Remi Fiancette[1], Gianluca Carlesso[5], Scott Hammond[5], Simon Dovedi[6], Menna Clatworth[2,3,4], Matthew Hepworth[7], James Arnold[8], Benjamin Willcox[1], David Withers[1]

[1]Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, United Kingdom; [2]Molecular Immunity Unit, University of Cambridge, Cambridge, United Kingdom; [3]Cambridge Institute for Therapeutic Immunology and Infectious Diseases, University of Cambridge, Cambridge, United Kingdom; [4]Cellular Genetics, Wellcome Sanger Institute, Cambridge, United Kingdom; [5]Early Oncology R&D, AstraZeneca, Boston, United States; [6]Early Oncology R&D, AstraZeneca, Cambridge, United Kingdom; [7]Lydia Becker Institute of Immunology and Inflammation, University of Manchester, Manchester, United Kingdom; [8]School of Cancer and Pharmaceutical Sciences, King's College London, London, United Kingdom

Keywords: NK cells, Innate immunity, Tumour microenvironment, Tumour immunity

Dysfunctional effector cells facilitate tumour immuno-evasion, however, the precise mechanisms behind ineffective anti-tumour responses remain unclear. CD8+ T-cells become exhausted from chronic antigen exposure, whereas alterations to other key effectors, including NK cells, are yet to be elucidated. NK cells directly target and kill cancer cells by secreting perforin and granzymes, and by engaging death receptors. Indirectly, NK-DC crosstalk supports crucial anti-tumour T-cell responses, recruiting cDC1s through CCL5 and XCL1, and priming DCs via IFN-γ and TNF-α. We established tumour models using photoconvertible Kaede mice, where temporal photo-labelling of the entire intratumoural immune compartment from native 'Kaede green+' to photo-converted 'Kaede red+' fluorescence enabled newly entering cells to be distinguished from those retained within tumours. To reveal transformation of NK cells within tumours over time, we performed scRNA-seq on infiltrating 'Kaede green+' and retained 'Kaede red+' lymphocytes in MC38 tumours 24- and 72-hours post photoconversion. This identified 3 distinct NK clusters; CD11b+CD49a- newly infiltrating 'NK-1', CD11b-CD49a- 'NK-2', and CD11b-CD49a+ resident-like 'NK-3' cells which accumulated over time, and strikingly were characterised by a transcriptomic signature indicating loss of functionality. Using flow cytometry, we validated these observations in ectopic, orthotopic, and primary tumour models, confirming a universal loss of NK function including CCL5, IFN-γ, and an augmented granzyme repertoire. In addition, we developed an endoscope guided model of colorectal cancer in situ, further confirming augmented NK cell responses in tumours. Our in-vitro data indicated NK cytotoxic deficiencies were TGF-β and PGE2 independent, and through depletion studies, we further confirmed that these changes occurred irrespective of FoxP3+ regulatory T-cell mediated suppression. Interestingly, although treatment with IL-15:IL-15Rα complexes partially restored NK functions, IL-15:IL-15Rα promoted systemic expression of inhibitory checkpoints NKG2A and KLRG1, suggestive of further negative feedback mechanisms constraining NK activation which may be targeted with checkpoint inhibitors. Collectively, our data reveals rapid loss of diverse NK cell functions within tumours, provides a unique temporal characterisation of NK cells adaptation to the TME, and investigates how potential therapeutic interventions impact this process.

P **01** - 015

ROLE FOR TL1A IN INNATE INTESTINAL INFLAMMATION AND COLITIS ASSOCIATED CANCER

Silvia Pires[1], Wei Yang[1], Lance Chou[1], Randy Longman[1]

[1]Weill Cornell Medicine , New York, United States

Keywords: inflammation, ILC3s, cancer, TL1A, Neutrophils

Inflammatory changes play an important role in tumorigenesis, and patients with chronic inflammatory bowel disease (IBD) are at an increased risk of developing colitis-associated cancer (CAC). However, the cellular contributions of these inflammatory changes are not well defined. The TNFSF15 locus harbors some of the most significant polymorphisms associated with IBD and recent work by our lab has demonstrated that its protein product (TNF-like cytokine 1a or TL1A) plays a central role in modulating group 3 innate lymphoid cells (ILC3s). The main objective of this work is to evaluate a potential mechanistic role of TL1A in regulating CAC and to define the cellular mechanisms linking chronic intestinal inflammation of IBD with cancer. Using the human atlas protein database, we found that high expression of TNFSF15 correlated with reduced survival in Colorectal cancer patients. To test the functional role for TL1A in tumorigenesis, we used the well established AOM/DSS model of CAC in mice deficient for the TL1A receptor (called death receptor 3-DR3). We found that DR3-deficient mice had a significant reduction in tumor number compared to heterozygous littermate controls. To evaluate the contribution of innate lymphocytes, we used DR3-deficient mice on a RAG-deficient background. Even in the absence of B and T cells, DR3-deficient mice had a significant reduction in tumor burden, suggesting innate cell contribution. Neutrophil infiltration, which is also a key process in IBD, can function as a potent driver of tumorigenesis due to its production of ROS and tissue damage via release of several proteases. We found a reduction of neutrophil infiltration in the colon of DR3-deficient mice

in our AOM/DSS model as well as on two different models of innate inflammatory colitis. In vitro co-culture of innate lymphoid cells and neutrophils revealed a role for ILC3s in regulating neutrophil biology in a TL1A dependent manner. Neutrophil depletion also resulted in a significant reduction of tumor numbers in our CAC model. Moreover, inflammation and activation of DR3 signaling resulted in increased ILC3 production of GM-CSF and an expansion of neutrophils in the bone marrow suggesting a potential role for TL1A-DR3 signaling on tissue ILCs in granulopoiesis. Our data reveals a functional role for TL1A in CAC and highlights a cellular role for ILCs in regulating neutrophils in this process. These findings highlight potential therapeutic importance of this pathway in CAC.

P **01** - 016

LOCAL MICROENVIRONMENT FINELY SHAPES THE FATE OF INFLAMMATORY MACROPHAGES AFTER LUNG SEVERE INFLAMMATION

Cedric Jacqueline[1], Marion Davieau[1], Florian Martin[1], Alice Molle[1], Victor Gourain[1], Alexis Broquet[1], Tanguy Chaumette[1], Cynthia Fourgeux[1], Jose Villadangos[2], Jeremie Poschmann[1], Antoine Roquilly[1,3]

[1]Nantes Université, Inserm, Center for Research in Transplantation and Translational Immunology, UMR1064, Nantes, France; [2]Department of Microbiology /Immunology, The University of Melbourne, Melbourne, Australia; [3]Surgical Intensive Care Unit, CHU Nantes, Nantes, France

Keywords: Innate immunity, inflammation, mononuclear phagocytes, Pneumonia

Objective: From a clinical point of view, patients suffering severe infections or trauma display a dramatically altered immunologic profile. Hospital-Acquired Pneumonia (HAP) is the most frequent cause of hospital-acquired infections, with 500,000 episodes of HAP being treated every year in Europe. Although self-sustaining resident alveolar macrophages (R-MAC) ensures normal host-pathogen interactions in healthy conditions, R-MAC are partially replaced by inflammatory macrophages over the course of lung inflammation. The specific contribution of tissue-resident alveolar macrophages and recruited inflammatory macrophages to the immunological scar induced after a severe inflammation remains unclear.

Methods & Results: First, we showed in WT and CCR2-/- mice that after primary pneumonia, inflammatory macrophages were recruited to the inflamed alveoli through the CCL2-CCR2 axis. Phagocytosis, production of cytokines (IL-4, IL-6, TNF), and iNOS were highly modulated in inflammatory macrophages during the resolution of inflammation (day3 and day7 after primary pneumonia). Adoptive transfer experiments demonstrated that signals remaining in the local microenvironment imprinted their final phenotype and functions. DGE-seq followed by GSEA analysis of inflammatory macrophages revealed that (i) type I and II interferons response-related signal pathways were highly enriched at day3 (ii) hallmark biological metabolic processes were significantly enriched at day7 suggesting a metabolic switch from glycolysis to lipid metabolism. These results have been confirmed by combined ChiP- and ATAC-seq analysis highlighting the potent role of specific transcription factors involved in these biological processes, notably the overexpression of PPARg, usually associated with R-MAC.

Conclusion: R-MAC are the first responders to pathogens and undergo a tolerogenic reprogramming after resolution of inflammation (1). Recruited inflammatory macrophages, coexisting with R-MAC within the inflamed alveoli, are finely shaped by local microenvironment, which allows a transient phagocytic and inflammatory profile within a lung tolerogenic environment. Understanding the role of these populations could lead to promising targets to improve the outcome of intensive care patients suffering from HAP.

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P **01** - 017

EFFEROCYTOSIS FACTORS ADMINISTRATION LIMITS CANCER PROGRESSION BY IMPROVING ANTI-TUMOR IMMUNE RESPONSE

Audrey Wetzel[1,2], Francis Bonnefoy[1,2], Cécile Chagué[1], Mathieu Vetter[1], Mélanie Couturier[2], Blandine Baffert[1], Olivier Adotévi[1,3], Philippe Saas[1], Sylvain Perruche[1,2]

[1]University of Bourgogne Franche-Comté, INSERM, EFS BFC, UMR1098, RIGHT Interactions Greffon-Hôte-Tumeur/Ingénierie Cellulaire et Génique, LabEx LipSTIC, Besançon, France; [2]MED'INN'Pharma, Besançon, France; [3]Department of Medical Oncology, University Hospital of Besançon, Besançon, France

Keywords: cancer, inflammation, efferocytosis, resolution, treatment

Cancers are the consequences of a cellular dysfunction characterized by an abnormal cellular multiplication and proliferation, which at least lead to metastasis formation. The main and natural defense against cancer cells is the inflammatory reaction, with immune cell recruitment. However, this inflammatory environment also favors cancer cell progression, their evasion from immune surveillance leading to cancer development. Current therapeutic strategies intend to enhance natural immune response in order to increase immunosurveillance, control and rejection of the tumor. However, these approaches represent a source of inflammatory mediators that can be used by cancer cells to grow, differentiate and migrate, encouraging metastasis formation. In consequence, limiting inflammation appears to be an innovative strategy to avoid the escape of cancer cells and potentially enhance the efficacy of antitumor therapies. Consequently, our studies aim to explore the control of tumor-associated inflammation by the administration of pro-resolutive factors on tumor progression. We have observed that pro-resolving mediators, issued from apoptotic cell efferocytosis by macrophages, injected in tumor bearing mice allowed the control of peritoneal cancer cell progression and dissemination to the blood and mesenteric lymph nodes. This was associated with macrophage mobilization at the tumor site and draining lymph nodes, with a specific phenotype related to IFN-g tumor-specific CD4 and CD8 T-cell response. Altogether, these results show that controlling tumor-associated inflammation represent an innovative opportunity to enhance cancer immunosurveillance.

P **01** - 018

THE EPCAM EPITHELIAL MARKER IS DETECTED ON TUMOR-ASSOCIATED NEUTROPHILS IN BREAST TUMORS

Manuela Pereira Abrantes[1], Pacôme Lecot[1,2], Sébastien Dussurgey[3], Maude Ardin[1], Lyvia Moudombi[1], Sara Belluco[4], Laurie Tonon[5], Alain Viari[5], Christophe Caux[1], Marie-Cécile Michallet[1]

[1]TERI (Tumor Escape, Resistance and Immunity) Department, Centre de Recherche en Cancérologie de Lyon, Centre Léon Bérard, Université de Lyon, Université Claude Bernard Lyon 1, INSERM 1052, CNRS 5286, Lyon, France, Lyon, France; [2]Present address: Buck Institute for Research on Aging, 8001 Redwood Blvd, Novato, California 94945, USA, California, United States; [3]Université de Lyon, SFR Biosciences, ENS de Lyon, Inserm US8, CNRS UMS3444, UCBL, Lyon, France, Lyon, France; [4]Université de Lyon, VetAgro Sup, ICE UPSP 2016.A104, Axe Cancérologie, Marcy l'Etoile, Lyon, France, Lyon, France; [5]Synergie Lyon Cancer, Plateforme de Bio-informatique 'Gilles Thomas', Lyon, France, Lyon, France

Keywords: Neutrophils, Breast Cancer, Immune Surveillance, EpCAM

Objective: Despite the development of efficient therapies, breast cancer remains the leading cause of cancer death in women. It is now well established that the tumor microenvironment (TME) influences the tumor progression and the clinical outcome of patients. Neutrophils have emerged as a promising therapeutic target since tumor-associated neutrophils (TANs) may affect cancer progression with pro or anti tumoral properties. In 2019, Zilionis et al. revealed neutrophil subsets in human lung cancer, testifying their heterogeneity. Our study aims to investigate TAN heterogeneity at early and late breast cancer stages to identify tumor-specific phenotypical and functional characteristics that could be further targeted by immunotherapies to increase antitumor immunity in breast cancer.

Methods: Due to the late diagnosis of breast cancer in women, we took advantage of the murine MMTV-Neu transgenic model expressing HER2/Neu oncogene developing spontaneous mammary tumors. We performed high content conventional and imaging flow cytometry to

characterize TANs at early and late cancer stages. We also achieved microbulk RNA sequencing to obtain neutrophil and epithelial cell transcriptomes across tumorigenesis to determine a tumor stage-specific interactome and pathways associated with neutrophils.
Results: We found that neutrophils are present in mammary glands and increase in the TME across tumorigenesis. Astoundingly, we discovered TANs with specific expression of the EpCAM epithelial marker, that constitute the major part of infiltrated neutrophils from early to late cancer stage. Especially, neutrophils seem to be the only EpCAM+ myeloid cells at the dysplasia stage. We deciphered by imaging flow cytometry that EpCAM detection at the neutrophil cell surface arises from epithelial pieces. Interestingly, TANs can also establish synapses with tumor and myeloid cells of the TME, involving a Ly6G neutrophil-specific marker polarization.
Conclusion: We identified a high proportion of TANs expressing EpCAM at the neutrophil cell surface, emanating from epithelial pieces, all along the tumorigenesis process. EpCAM+ TANs appear to establish contacts with a Ly6G polarization at cell and spot contacts. These observations suppose a novel function of neutrophils that is tumor-specific. A better understanding of the EpCAM+ TANs function may open the door to the identification of a TANs novel subtype and to potential therapeutical options based on the targeting of EpCAM+ TANs.

P **01** - 019

IDENTIFICATION OF THE DENDRITIC CELL LANDSCAPE IN HUMAN TUMORS BY MULTI-IMMUNOFLUORESCENCE AND TRANSCRIPTOMIC ANALYSIS AT SINGLE CELL LEVEL

Margaux Hubert[1,2], Elisa Gobbini[1], Anne-Claire Doffin[1,3], Sarah Barrin[1,2,3], Justine Berthet[1,2,3], Léo Laoubi[1], Yamila Rocca[1], Candice Sakref[1,4], Isabelle Treilleux[3], Christophe Caux[1,2,3,4], Jenny Valladeau-Guilemond[1,4]

[1]Univ Lyon, Université Claude Bernard Lyon 1, NSERM U1052, CNRS 5286, Centre Léon Bérard, Centre de Recherche en Cancérologie de Lyon, Lyon, France; [2]Laboratoire d'Immunothérapie des Cancers de Lyon (LICL), Lyon, France; [3]Centre Léon Bérard, Lyon, France; [4]LabEx DEVweCAN, Lyon, France

Keywords: Tumor immunology, Dendritic cells, Immunosurveillance, multi-immunofluorescence, single cell RNA-seq

Dendritic cells (DCs) represent promising targets of cancer immunotherapies owing to their central role in the initiation and the control of immune responses. Their functions encompass a wide range of mechanisms mediated by different subsets classically identified as: plasmacytoid DCs (pDCs), the two conventional DCs (cDCs) called cDC1 and cDC2, as well as the Langerhans cells (LCs). Several studies previously identified in situ human tumor-associated DC (TA-DC) populations by immunostaining or by flow cytometry (FACS) using a limited number of markers. The concomitant development of advanced technologies, such as complete transcriptome sequencing of a cell population or at a single cell level has unveiled previously unknown populations and highlighted potential functions. However, the spatial organization of the different DC subsets and their specific functions in tumor immune responses are still to be defined.
Here, we combined single cell RNA-sequencing (scRNA-seq) with multiplexed opal-based immunofluorescence (multi-IF) to identified the "tumor DC landscape" and its spatial organization. First, we performed a scRNA-seq (SORT-seq) analysis of all DC subsets in various tumors types. This allowed us to characterize their activation and maturation states. We highlighted the presence of two populations that may have a crucial role in antitumor immunity and composed of mixed cDC1 and cDC2: proliferating DCs and mature DCs. Interestingly, this last population reflects the convergence at the transcriptomic level of several DC subsets toward a final maturation stage. Then, we set up a 7 colors multi-IF to confirm the presence of all the previously identified DC subsets, as well to precisely localized them in tumors. Tissue stratification, cell phenotype attribution and quantification were performed with the INFORM software on cohorts of melanoma and breast cancer. We showed for the first time that pDC, cDC1 and mature DCs co-infiltrate tumors along with CD8+ T cells and were mostly located into the stroma compartment, in contrast to LCs found in the tumor compartment. Finally, an in-house bioinformatic pipeline was developed to define spatial communities that reflect cooperation between DC subsets and that may predict better survival or response to immunotherapies.
We believe that identification of crucial tumor infiltrating DC subsets, their markers and their spatial organization will pave the way for the identification of new therapeutic targets.

P **01** - 020

INFLUENCE OF ERALPHA SIGNALING ON THE EFFECTOR FUNCTIONS OF NK CELLS

Nina Anesi[1], Eve Blanquart[1], Audrey Mandonnet[1], Jean-Charles Guery[1], Sophie Laffont[1]

[1]Infinity-Inserm, Toulouse, France

Keywords: NK cell, Estrogen, ERa, Cancer, Sex biais

Epidemiologic data robustly show that sex influences both incidence and mortality linked to cancer. Women are at lower risk to develop cancer unrelated to sex like melanoma or leukemia (INCa 2019). Mechanisms underlying this sex dimorphism are poorly documented and probably complex. Sex differences could be explained by extrinsic factors, such as alcohol consumption or chemical exposure but also by sex-linked biological intrinsic factors. Indeed, x-linked genes and sex hormones can regulate both tumor microenvironment and immune cells. Estradiol, the main female hormone, has been shown to have pro-inflammatory properties by promoting development and functions of dendritic cells.
NKs cells, a subset of innate lymphoid cells (ILC) that express estrogen receptor alpha (ERα),are crucial to control tumor growth both in human and mice. These cells are able to kill tumor cells without injuring healthy cells, through their cytotoxic activities. They also contribute to anti-tumoral immunity by directly regulating the adaptive immune responses through the production of cytokines like IFN-g. Given the sexual bias and the central role of NKs cells in cancer, we hypothesized that estrogen signaling, viaits receptor ERα, could increase cytotoxic functions of NK cells. We generated mice with a selective deletion of ERα in NKs cells (NKp46- ERαKOmice). Lack of ERα, doesn't impair neither development nor maturation of NKs cells but decreases their capacity to control tumoral growth in different cancer models. We are now exploring the molecular mechanisms involved.
Several therapeutic strategies relying on the modulation of anti-tumoral activity of NKs cells are used in Human. It is therefore important to better understand how NK cell functions are regulated, especially because selective pharmacological tools (SERM, Selective Estrogen Receptor Modulators) are available to modulate estrogen receptors.

P **01** - 021

THE ONCOGENIC STRESS IS THE FIRST IMMUNOGENIC SIGNAL DURING TUMORIGENESIS LEADING TO EARLY IMMUNOSURVEILLANCE BY NEUTROPHILS

Antoine Debiesse[1], Lyvia Moudombi[1], Hélène Vanacker[1], Leïla Lefèvre[1], Emmanuelle Jacquet[1], Marie Laurent[1], Christophe Caux[1], Marie-Cécile Michallet[1]

[1]CRCL, Lyon, France

Keywords: Oncogenic Stress, Immunosurveillance, Unfolded Protein Response (UPR), Neutrophils

In the context of tumorigenesis, preneoplastic cells have to adapt to a cellular stress named "oncogenic stress" (OS) which corresponds to the overexpression of an oncogene leading to cellular transformation. The OS is well characterized regarding the oncogenic signaling pathway and the activation of intrinsic safeguard mechanisms (senescence or apoptosis) known to be potentially immunogenic. However, the immunogenicity of a cell at the early OS stage, as well as mechanisms of detection by the immune system is not known. In order to evaluate the immunogenicity of OS, we developed an original in vitro system based on immortalized Human Mammary Epithelial Cells (HMEC) in which the oncogene HRasG12V is inducible upon doxycycline treatment. We demonstrated that OS cells produce a specific secretome (SOS

for Secretome of OS) including chemokines like CXCL-1 and CXCL-3, cytokines like GM-CSF and IL8, and alarmins such as IL-1????. Moreover, OS cells express specific immune ligands notably the integrin ICAM-1 (CD54), but also immune regulatory molecules such as PD-L1 and NKG2D-ligands (ULBP2-5-6). We also demonstrated that supernatants of OS cells stimulate the recruitment and the activation of innate immune cells such as neutrophils, with the up-regulation of CD66b and CD63 that are markers of degranulation. At the intrinsic level, the OS cells activate the unfolded protein response (UPR) and more specifically the IRE1 branch that regulates the SOS but not the expression of the immune ligands. Altogether, these results demonstrated that preneoplastic cells are immunogenic as soon as the OS stage, and that the UPR is a major regulator of the SOS. This knowledge may improve existing UPR-targeted therapies and open the possibility to use them in novel therapeutic indications, such as early cancer stages or in combination with immunotherapy.

P **01** - 022

POST SEPTIC TRAINED-IMMUNITY SHAPES TISSUE RESIDENT T CELL ANTI-TUMORAL RESPONSE

Alexis Broquet[1,2], Victor Gourain[1], Thomas Goronflot[3], Virginie Le Mabecque[1], Sinha Debajyoti[1], Mitra Ashayeripanah[4,5], Cédric Jacqueline[1], Thomas Laurent[1], Cynthia Fourgeux[1], Tanguy Chaumette[1], Florian Martin[1], Cécile Poulain[1], Christelle Harly[6], Christelle Scotet[6,7], Nicolas Mauduit[8], Pierre Antoine Gourraud[3], Jose Villadangos[4,5], Karim Ashenoune[1], Jeremie Poschmann[1], Antoine Roquilly[1]

[1]Nantes Université, CHU Nantes, INSERM, Center for Research in Transplantation and Translational Immunology, UMR 1064, F-44000, Nantes, France; [2]CHU Nantes, INSERM, Nantes Université, Anesthesie Reanimation, CIC 1413, F-44000 , Nantes, France; [3]CHU de Nantes, Inserm, CIC 1413, Pôle Hospitalo-Universitaire 11: Santé Publique, Clinique des Données, Nantes, France; [4]Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Victoria 3010, Melbourne, Australia; [5]Department of Microbiology and Immunology, The University of Melbourne, The Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria 3000, Melbourne, Australia; [6]Université de Nantes, INSERM, CNRS, CRCINA, Nantes, France; [7]LabEx IGO "Immunotherapy, Graft, and Oncology", Nantes, France; [8]Service d'Information Médicale, Nantes University Hospital, Nantes, France

Keywords: trained-immunity, anti-tumoral response, tissue-resident T cells, CXCR6, IL-17-?? T cells

Sepsis induces functional alterations of innate immune cells which lasts for months after the cure. The impact of this immunological reprogramming on the long-term risk of cancer remains unclear. Here, we report that sepsis-cured mice were less susceptible to orthotopic lung cancer. Using single-cell transcriptomic analysis, we comprehensively described the immune composition of mouse lungs during and after sepsis. We identified an oligoclonal expansion of CXCR6+ tissue-resident T (TTR) cells, mainly composed of IL17+ RORgt+ gd6 T cells which were responsible for the resistance to lung cancer observed after sepsis. The post-sepsis lung enrichment of CXCR6+ TTR cells was due to augmented proliferation and decreased tissue egress and was induced by CXCL16+ secreted from sepsis-trained macrophages. In TCGA gene expression data, we observed that CXCR6/ TTR cell gene expression is associated with prolonged survival in several types of cancers. Finally, we confirmed in a French nation-wide cohort that sepsis-survivors had a lower risk of developing different types of cancers. Our results provide a comprehensive description of the long-term impact of sepsis on immunity and identify the capacity of trained macrophages to locally retain CXCR6+ TTR cells as a critical mediator of tumor immunosurveillance. Our study identifies a novel and therapeutically relevant anti-tumor consequence of sepsis-induced trained immunity involving the ability to regulate TRM cells numbers and functions.

P **01** - 023

LIVER INNATE LYMPHOCYTES ORCHESTRATE ENDOTHELIAL REGULATION OF ADAPTIVE IMMUNITY

Jia Xiang See[1,2], Sophia Papaioannou[1,2], Jeong Mingeum[1], Carolina De La Torre[3], Sandy Bauherr[4], Anja Erika Hauser[4,5], Michael Platten[6,7], Philipp-Sebastian Koch[8], Ana Stojanovic[1], Adelheid Cerwenka[1,2]

[1]Department of Immunobiochemistry, Mannheim Institute for Innate Immunoscience (MI3), Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany; [2]European Center for Angioscience (ECAS), Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany; [3]Center of Medical Research, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany; [4]Deutsches Rheuma-Forschungszentrum (DRFZ), an Institute of the Leibniz Association, Berlin, Germany; [5]Department of Rheumatology and Clinical Immunology, Charité-Universitätsmedizin, Berlin, Germany; [6]DKTK Clinical Cooperation Unit Neuroimmunology and Brain Tumor Immunology, German Cancer Research Center (DKFZ), Heidelberg, Germany; [7]Department of Neurology, MCTN, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany; [8]Department of Dermatology, Venereology and Allergology, University Medical Center and Medical Faculty Mannheim, Heidelberg University, and Center of Excellence in Dermatology, Mannheim, Germany

Keywords: Interferon gamma, ILCs, T cells, Liver sinusoidal endothelial cells, MHC II

Natural killer (NK) cells and distinct subsets of tissue-resident innate lymphoid cells (ILCs) in the liver are located in sinusoids and maintain intimate contact with the liver endothelium. Liver sinusoidal endothelial cells (LSECs) form the blood capillaries that connect the portal vein, the hepatic artery, and the hepatic vein in the liver. Although LSECs form a hub facilitating immune cell interactions, their functional role in immunology is still poorly understood.

Here, we demonstrate that LSECs represent an integral part of the liver immune network. We show that liver ILC1s, NK cells, and NKT cells produced IFN?γ in the liver in steady-state. The presence of IFN-γ and the master transcriptional co-activator CIITA was necessary to maintain the zonated expression of major histocompatibility complex class II (MHC II) on the cell surface of LSECs. Midzonal MHC IIhigh LSECs displayed a distinct transcriptomic profile compared to MHC IIlow LSECs, suggesting both spatial and functional segregation of LSECs in the liver. LSECs from ILC1- and T/B cell-deficient mice presented unaltered MHC II expression, while mice that lacked all lymphocytes displayed reduced MHC II expression, comparable to Ifnγ- and Ifnγr-deficient mice. Furthermore, the reduction of MHC II on LSECs in Ifnγ-deficient mice correlated with an increased CD4+ naïve over memory T cell ratio, indicating a role of IFN-γ in regulating LSEC-mediated CD4+ T cell differentiation in the liver. In summary, we identified lymphocyte populations expressing IFN-γ at steady-state that enables maintenance of MHC IIhigh expression on LSECs with high relevance for shaping adaptive liver T cell compartments.

P **01** - 024

VITAMIN A PROMOTES REGULATORY NK CELL FUNCTIONS

Mingeum Jeong[1], Jia-Xiang See[1,2], Carolina De La Torre[3], Ana Stojanovic[1], Adelheid Cerwenka[1,2]

[1]Department of Immunobiochemistry, Mannheim Institute for Innate Immunosciences (MI3), Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany; [2]European Center for Angioscience (ECAS), Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany; [3]Center of Medical Research, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

Keywords: All-trans retinoic acid, Natural killer cells, Interferon gamma, Dendritic cells, Regulatory T cells

Vitamin A, a lipid-soluble micronutrient, plays important roles in reproduction, embryonic development, and in the immune system of mammals. Vitamin A metabolites are digested and absorbed in the small intestine, and stored mainly in the liver and adipose tissue. Natural Killer (NK) cells, a group of innate lymphocytes, can be recruited to inflamed tissue to exert cytotoxicity against abnormal cells, as well as to tune immune responses in tissues, such as in liver and fat. In this study, we investigated the impact of the vitamin A metabolite, all-trans retinoic acid (atRA), on murine NK cell functions. We show that atRA induced transcriptional, phenotypic and functional reprogramming of NK cells. atRA altered mitochondrial fitness and enhanced mitochondrial respiration of NK cells, which differentially contributed to pro-inflammatory cytokine production. In response

to different stimuli, vitamin A-exposed NK cells displayed reduced release of the pro-inflammatory cytokine IFN-γ, which correlated with the impaired ability of NK cells to induce dendritic cell maturation. Furthermore, NK cells conditioned by vitamin A, supported regulatory T cell differentiation. Our findings suggest that atRA promotes regulatory functions of NK cells, which might contribute to tissue tolerance and reduced inflammatory responses in vitamin A-enriched environments.

P **01** - 025

CROSSTALK BETWEEN STROMAL COMPARTMENT AND MACROPHAGES LEAD TO CD169+ MACROPHAGES IN PANCREATIC DUCTAL ADENOCARCINOMA

Kevin Thierry[1,2,3], Sophie Bachy[1,2,3], Zhichong Wu[1,2,3], Pia Gamradt[1,2,3], Ana Hennino[1,2,3]

[1]Cancer Research Center of Lyon, UMR INSERM 1052, CNRS 5286, Lyon, France; [2]Université lyon 1, Lyon, France; [3]Centre Léon Bérard , Lyon, France

Keywords: Macrophages, Pancreatic cancer, Immune response

Pancreatic ductal adenocarcinoma (PDAC) is associated with an abundant stromal reaction, which accounts for up to 80% of the tumor mass. Within stromal reaction, immune cells communicate with several actors, from cancer associated fibroblast to soluble factors. Among immune cells, macrophages represent one of the major cell populations in the tumor microenvironment and have been previously shown to display both an immunosuppressive and pro-tumoral role. We sought to determine the respective roles of the tumoral and stromal compartment in impacting macrophages phenotype and function. We used two different primary cell lines isolated from the stromal and tumoral compartments respectively, from mice with neoplasia and PDAC and co-cultured them with bone marrow derived macrophages.
We report here that, in contrast to the tumoral compartment, the stromal compartment induces macrophage polarization towards an immunosuppressive phenotype by expressing both CD206 and CD169 markers as well as downregulating antigen presentation capacity. Those results have been validated through a pancreatic cancer orthotopic model. We show that this population is upregulated in pre-cancerous conditions as well as in human PDAC. We also address that the crosstalk between macrophages and the stromal compartment led to a significant increase of both immunosuppressive extracellular protein βig-h3 and CXCL12 chemokine production, both associated with inhibited CD8+ proliferation. We measured direct evidence showing that βig-h3 can induce CXCL12 production from the stromal compartment. Finally, we showed that both stromal compartment and macrophages can inhibit CD8+ T cells in a PD1 dependent and independent manner, respectively.
This work may lead to the identification of a new macrophage immunosuppressive population playing a key role in PDAC development.

P **01** - 026

IMPAIRED ANTI-TUMOR IMMUNE RESPONSE IN MYCN-AMPLIFIED NEUROBLASTOMA IS ASSOCIATED WITH LACK OF CCL2 SECRETION AND POOR DENDRITIC CELL RECRUITMENT

Jamila Kacher[1], Olivier Manches[1], Caroline Aspord[1], Hervé Sartelet[2], Laurence Chaperot[1]

[1]Institute for Advanced Biosciences, Inserm U 1209, CNRS UMR 5309, Université Grenoble Alpes Etablissement Français du Sang Auvergne-Rhône-Alpes, Grenoble, France; [2]Laboratoire de Biopathologie, CHRU de Nancy, Inserm U1256, Université de Lorraine, Nancy, France

Keywords: Neuroblastoma, chemokines, dendritic cells, tumor micro-environment, MYCN oncogene

In neuroblastoma (NB), MYCN amplification is associated with sparse immune infiltrate, and poor prognosis. Although DC are the pillars of efficient immune responses, little is known about their recruitment and function in NB. In the present study, by using in vitro models, we characterized human DC subsets migration and function in the context of NB, with a special emphasis on MYCN-amplified tumor. We observed a recruitment of monocytes, MDC and PDC towards AS and SH MYCN non-amplified but not DZ nor N91 MYCN-amplified NB cell lines supernatants. This migration was abrogated by the addition of anti-CCL2 antibodies, demonstrating the involvement of the CCR2/CCL2 axis in monocytes, MDC and PDC recruitment by these tumors. Using public RNAseq (GSE62564) and microarray (GSE3960 and GSE85047) data sets, we then describe lower level of expression of CCL2 in MYCN-amplified neuroblastoma tumors, and we propose a working model for T cell recruitment in neuroblastoma tumors in which CCL2 produced by NB cells initiates the recruitment of monocytes, MDC and PDC. We found correlations between CCL2 expression and pan-APC, MDC (CD1c+ and CD141+), monocytes/macrophages, and PDC (IRF7) infiltration, suggesting the involvement of CCL2/CCR2 in APC recruitment by NB tumors. Further analyses suggest that among these cells, the CD1C+ subset may recruit T cells by means of CCL19/CCL22 secretion. This was confirmed by in vitro experiments, showing that supernatants from dendritic cells co-cultured with NB cell lines and activated (R848) contain CCL22 and CCL19, and are chemotactic for both CD4+ and CD8+ T cells. We also looked at immunomodulation induced by NB cell lines in coculture with PBMC. Upon activation with R848, an increased secretion of IL-6 was observed in the cocultures performed with the MYCN non-amplified cell lines (AS and SH). Strikingly, secretion of IFNa, which is a cytokine specifically produced by PDC upon activation, was increased when immune cells were in contact with the MYCN non-amplified NB cell lines, suggesting that these cell lines, but not the MYCN-amplified ones, create a microenvironment favoring PDC activation. Overall, our findings highlight a major role for CCL2/CCR2 axis in monocytes, myeloid and plasmacytoid cells recruitment towards MYCN non-amplified neuroblastoma, allowing further immune cell recruitment, and show that these tumors present a microenvironment that can favor dendritic cell responses.

P **01** - 027

CONTEXT- AND SUBSET-DEPENDENT NK CELL ACTIVATION BY INTERLEUKIN-33 IN TUMORS

Valentin Picant[1], Yamila Rocca[1], Aurélien Voissière[1], Anaïs Eberhardt[1], Lara Revol-Bauz[1], Dominique Poujol[1], Christophe Caux[1], Nathalie Bendriss-Vermare[1]

[1]CRCL, Lyon, France

Keywords: Cancer, Natural Killer, Interleukin-33, Immunosurveillance, Dendritic cells

Natural Killer (NK) cells are key players in cancer immunosurveillance by directly killing tumor cells and supporting effective antitumor immune responses. However, they get progressively dysfunctional in developing tumors. Harnessing NK cell potential for cancer immunotherapy is thus a burning topic. A promising strategy consists in the identification of soluble factors or surface receptors that drive the activation and anti-tumor function of NK cells.
In this project, we investigated the role of the alarmin interleukin (IL)-33 in the activation of healthy donors (HD-NK) and cancer patients (T-NK) NK cells. First, we highlighted the importance of the dendritic cells (DC)/HD-NK crosstalk in the induction of IL-33-receptor (ST2), with a major role of conventional DC-derived cytokines. Moreover, we observed that IL-12 and IL-15 differentially regulated ST2 expression on NK cell subsets leading to cytotoxicity, cytokine secretion, and proliferation in response to IL-33. RNAseq analysis reinforced that IL-33 effect is specific to cytokinic contexts and NK cell subsets. Furthermore, T-NK cells isolated from various tumor types were able to produce IFN-γ in response to IL-33 combined to IL-12 and/or IL-15 ex vivo. Using in vivo tumor models, we reported that IL-33 was able to synergize with DC-activating TLR agonists to limit tumor development in therapeutic settings. Finally, we observed a higher tumor growth rate and a decreased antitumoral effects of DC-activating TLR agonists in IL33KO mice compared to wild type mice, both highlighting a role for IL-33 in cancer immune surveillance.
Thus, our work identifies IL-33 as a context- and subset-dependent NK cell activator that might be used to reverse intratumor NK cell dysfunctionality, paving the way to a refinement of immunotherapies targeting innate cells.

P **01** - 028

POST-NATAL MENINGEAL MACROPHAGES PROTECT AGAINST VIRAL NEUROINFECTION

Julie Rebejac[1], Rejane Rua[1]

[1]CIML, Marseille, France

Keywords: LCMV, meninges, brain, barrier, infection

The surface of the central nervous system (CNS) is seeded by two meningeal macrophage (MM) populations: MHC-II- MM which are abundant neonatally and MHC-II+ MM which appear over time. Using histocytometry, flow cytometry, and single-cell RNA sequencing approaches, we show that those two barrier macrophage populations have different behaviors in response to in vivo peripheral challenges such as LPS, SARS-CoV2 and lymphocytic choriomeningitis virus (LCMV). Focusing on LCMV peripheral infection, we found that in mice with Stat1- or Ifnar-deficient macrophages, the virus readily spread into the CNS. Unexpectedly, the post-natal MHC-II+ MM population efficiently blocked intrinsic viral replication and promoted a broad antiviral state in the meninges. Using innovative genetic and pharmacological depletion strategies, we show that in the absence of MM, specific areas of the meninges became highly infected, leading to fatal brain disease. Thus, MM represent a major line of protection against neuroinfection.

P **01** - 029

IFN-LAMBDA PRODUCED BY TYPE 1 CONVENTIONAL DENDRITIC CELLS (CDC1) INDUCES PDCS SURVIVAL AND ACTIVATION IN HUMAN TUMORS

Candice Sakref[1,2], Alexis Saby[1], Aurélien Voissiere[1], Margaux Hubert[1], Lyvia Moudombi[1], Elisa Gobbini[1,3], Anne-Claire Doffin[1], Maude Ardin[1], Rodriguez Céline[1], Christophe Caux[1,2,4], Jenny Valladeau[1,2]

[1]Centre de Recherche en Cancérologie de Lyon (CRCL), Lyon, France; [2]LabEx DEVweCAN, Lyon, France; [3]CHU Grenoble-Alpes, Grenoble, France; [4]Laboratoire d'Immunothérapie des Cancers de Lyon (LICL), Lyon, France

Keywords: IFN-III, pDCs, cDC1, Cross-talk, Tumor MicroEnvironment

Dendritic cells play a key role in the anti-tumoral immune responses. For example, the conventional cDC1 population play an important role by presenting Ag and recruiting T cells. In this context, we have previously shown that cDC1 produce high level of type III interferons (IFN-III). Nevertheless, the precise role of IFN-III produced by cDC1 in the tumor microenvironment is not known.

Here, we show that intra-tumoral plasmacytoid Dendritic Cells (pDCs) are the infiltrating immune cells that best respond to IFN-III. We demonstrate that the survival of isolated pDCs from PBMCs and tumors is increased after 24h treatment with IFN-III, along with the expression of PD-L1 and ICOS-L. Moreover, IFN-III stimulation of pDCs induced an intermediate activation phenotype based on CD80/CD86, BDCA2, CD123, HLA-DR, PD-L1, ICOS-L expression. We also performed RNA-sequencing of IFN-III treated pDCs and observed 1582 differentially expressed genes (DEG) compared to non-treated pDCs. To go further, we used Gene Set Enrichment Analysis (GSEA) revealing 19 enriched pathways in IFN-III treated pDCs.

Finally, thanks to a 7 colors immunofluorescence staining of the different infiltrating DC subsets performed on 70 TNBC patients, we also highlight a particular pDC– cDC1 cross-talk in situ.

P **01** - 030

HEPATIC INFLAMMATION ELICITS PRODUCTION OF PROINFLAMMATORY NETRIN-1 THROUGH EXCLUSIVE ACTIVATION OF TRANSLATION

Romain Barnault[1,2,3,4], Claire Verzeroli[1,2,3,4], Carole Fournier[5], Maud Michelet[1,2,3,4], Anna-Rita Redavid[2,3,4,6], Ievgeniia Chicherova[1,2,3,4], Marie-Laure Plissonnier[1,2,3,4], Annie Adrait[8], Fleur Chapus[9], Mathieu Richaud[2,3,4,6], Maëva Hervieu[2,3,4,6], Julie Lucifora[1,2,3,4], Michel Rivoire[2,4], Hesso Farhan[10], Thomas Decaens[5], Patrick Mehlen[2,3,4,6], Benjamin Gibert[2,3,4,6], Fabien Zoulim[1,2,3,4], Romain Parent[1,2,3,4]

[1]Pathogenesis of Chronic Hepatitis B and C Laboratory - LabEx DEVweCAN, Inserm U1052, Cancer Research Centre of Lyon, Lyon, France; [2]University of Lyon, Lyon, France; [3]CNRS UMR5286, Lyon, France; [4]Centre Léon Bérard, Lyon, France; [5]Institute for Advanced Biosciences, Inserm U1209, University of Grenoble-Alpes, La Tronche, France; [6]Apoptosis, Cancer and Development Laboratory - LabEx DEVweCAN, Inserm U1052, Cancer Research Centre of Lyon, Lyon, France; [7]Inserm U1052, Cancer Research Centre of Lyon, Lyon, France; [8]University of Grenoble-Alpes, Inserm, CEA, UMR BioSanté U1292, CNRS CEA FR2048, Grenoble, France; [9]Single Cell Dynamics Group, Epigenetics and Stem Cell Biology Laboratory, National Institute of Environmental Health Sciences, Research Triangle Park, Durham, United States; [10] Institute of Pathophysiology, Medical University of Innsbruck, Innsbruck, Austria; [11]Service of Hepato-Gastroenterology, Hospices Civils de Lyon, Lyon, France

Keywords: Netrin-1, Inflammation, Liver, translation, Staufen

Chronic liver diseases (CLDs) affect more than 1 billion people worldwide. Though CLDs exhibit a high degree of diversity in terms of causal effects, including infectious, metabolic, toxic, and genetic, they all converge toward hepatic inflammation and represent a pertinent model for its study. Inflammation in turn drives hepatocyte turnover, extracellular matrix accumulation, histological worsening, and the long- term induction of tumorigenic mediators, eventually leading to the development of HCC. Hence, the identification of factors involved in the harmful consequences of inflammation may lead to its clinical improvement.

Netrin-1 is well known for preventing cellular apoptosis, and we demonstrated that it is HBV- HCV induced and endowed hepatocytes with resistance to apoptosis during the UPR, a hallmark of CLDs. Owing to the causal link between inflammation, cancer in general, and HCC, we hypothesized that hepatic inflammation and netrin-1 may be reciprocal influencers in the liver. This prompted us to test its inducibility and inflammatory contribution in the liver in the context of previous descriptions in inflammatory bowel disease and colorectal cancer.

A panel of cell biology and biochemistry approaches on in vitro– grown primary hepatocytes, liver cell lines, mouse samples and clinical samples was used.

We identify netrin-1 as a hepatic inflammation- inducible factor and decipher its mode of activation through an exhaustive eliminative approach. We show that netrin-1 up- regulation relies on a hitherto unknown mode of induction, namely its exclusive translational activation. This process includes the transfer of NTN1 mRNA to the endoplasmic reticulum and the direct interaction between the Staufen-1 protein and this transcript as well as netrin-1 mobilization from its cell- bound form. Finally, we explore the impact of a phase 2 clinical trial-tested humanized anti-netrin-1 antibody (NP137) in two distinct, toll- like receptor (TLR) 2/TLR3/TLR6-dependent, hepatic inflammatory mouse settings. We observe a clear anti-inflammatory activity indicating the proinflammatory impact of netrin-1 on several chemokines and Ly6C+ macrophages.

These results identify netrin- 1 as an inflammation- inducible factor in the liver through an atypical mechanism as well as its contribution to hepatic inflammation.

P **01** - 031

COOPERATIVE EFFECTS OF RIG-I-LIKE RECEPTOR SIGNALING AND IRF1 ON DNA DAMAGE-INDUCED CELL DEATH

David Y Zander[1], Sandy Burkart[1], Sandra Wüst[1], Vladimir G Magalhães[1], Marco Binder[1]

[1]German Cancer Research Center (DKFZ), Heidelberg, Germany

Keywords: DNA damage, cell death, innate immunity, antiviral response, signaling

Properly responding to DNA damage is vital for eukaryotic cells,

including the induction of DNA repair, growth arrest and, as a last resort to prevent neoplastic transformation, cell death. Besides being crucial for ensuring homeostasis, the same pathways and mechanisms are at the basis of chemoradiotherapy in cancer treatment, which involves therapeutic induction of DNA damage by chemical or physical (radiological) measures. Apart from typical DNA damage response mediators, the relevance of cell-intrinsic antiviral signaling pathways in response to DNA breaks has recently emerged. Originally known for combatting viruses via expression of antiviral factors including interferons (IFNs) and establishing of an antiviral state, RIG-I-like receptors (RLRs) were found to be critical for adequate induction of cell death upon the introduction of DNA double-strand breaks. We here show that presence of IRF3 is crucial in this process, most likely through direct activation of pro-apoptotic factors rather than transcriptional induction of canonical downstream components, such as IFNs. Investigating genes reported to be involved in both DNA damage response and antiviral signaling, we demonstrate that IRF1 is an obligatory factor for DNA damage-induced cell death. Interestingly, its regulation does not require activation of RLR signaling, but rather sensing of DNA double strand breaks by ATM and ATR. Hence, even though independently regulated, both RLR signaling and IRF1 are essential for full-fledged induction/execution of DNA damage mediated cell death programs. Our results not only support more broadly developing IRF1 as a biomarker predictive for the effectiveness of chemoradiotherapy, but also suggest investigating a combined pharmacological stimulation of RLR and IRF1 signaling as a potential adjuvant regimen in tumor therapy.

P **01** - 032

SEVERE COVID-19 PATIENTS HAVE IMPAIRED PLASMACYTOID DENDRITIC CELL-MEDIATED CONTROL OF SARS-COV-2-INFECTED CELLS

Manon Venet[1], Margarida Sa Ribeiro[1], Elodie Decembre[1], Alicia Bellomo[1], Célia Nuovo[1], Garima Joshi[1], Marine Villard[1], David Cluet[2], Magali Perret[1], Rémi Pescamona[1], Helena Paidassi[1], Omran Allatif[1], Alexandre Belot[1], Sophie Assant[1], Emiliano Ricci[2], Marlène Dreux[1]

[1]CIRI, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, École Normale Supérieure de Lyon, Univ Lyon, F-69007, LYON, France; [2]Laboratory of Biology and Modeling of the Cell, Université de Lyon, ENS de Lyon, Université Claude Bernard, CNRS UMR 5239, Inserm U1293 , LYON, France

Keywords: SARS-CoV-2, plasmacytoid dendritic cells, interferon, synapse, TLR7

Type I and III interferons (IFN-I/λ) are key antiviral mediators against SARS-CoV-2 infection. Here, we demonstrate that plasmacytoid dendritic cells (pDCs) are the predominant IFN-I/λ source following their sensing of SARS-CoV-2-infected cells. Mechanistically, this short-range sensing by pDCs requires sustained integrin-mediated cell adhesion with infected cells. In turn, pDCs restrict viral spread by an IFN-I/λ response directed toward SARS-CoV-2-infected cells. This specialized function enables pDCs to efficiently turn-off viral replication, likely via a local response at the contact site with infected cells. By exploring the pDC response in SARS-CoV-2 patients, we further demonstrate that pDC responsiveness inversely correlates with the severity of the disease. The pDC response is particularly impaired in severe COVID-19 patients. Overall, we propose that pDC activation is essential to control SARS-CoV-2-infection. Failure to unfold this response could be key to understand severe cases of COVID-19.

P **01** - 033

LOW STING EXPRESSION IN LUNG CANCER CELLS CONTRIBUTES TO DISEASE PROGRESSION AND IMMUNE ESCAPE

Julien Faget[1], Chiara Ursino[1], Laurent Gros[1], Nathalie Bonnefoy[1]

[1]Immunity and Cancer Team, INSERM U1194 IRCM, Univ Montpellier, ICM, Montpellier, France, Montpelier, France

Keywords: STING, Sox2, Neutrophils, NSCLC, EMT

Introduction

Canonical STimulator of INterferon Gene (STING) signaling in tumor infiltrated immune cells 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. However, recent publications suggest that some type of tumors may rely on STING activation to fuel their growth. In this study, we evaluate the contribution of STING expression on Non-Small Cell Lung Cancer (NSCLC) progression.

Methods

Using the CRISPR/Cas9 system, we investigated the impact of endogenous STING expression trough different in vitro and in vivo approaches based on cancer cell lines derived from primary tumors of the KrasLSL-G12D/WT;p53fl/fl (KP) mouse model of lung cancer.

Results and discussion

Direct stimulation of STING pathway revealed an IFN-deficient STING signaling in our model. Monitoring the immune infiltrates in tumors and the response to anti-PD1 antibody, showed that STING expression can sustain tumor promoting neutrophil infiltration and immune-suppression. Coherently with these results, when treated with a-PD1 immunotherapy, STING proficient tumors were more resistant to therapy compared to STING knockout. RNAseq analysis on cancer cells sorted from ex vivo tumors confirmed these results and highlighted a contribution of STING in the differential expression of the Sox2 transcription factor, known to orchestrate the recruitment of tumor promoting neutrophils and cancer progression in squamous lung cancer. Consistently, we noticed that the absence of STING in the cancer cells significantly reduced their ability to engraft and form tumors in vivo. We performed bioinformatics analysis highlighting a link between STING expression and the Epithelial to Mesenchymal Transition (EMT) program across multiple human lung cancer cells. Migration assays and expression profiles of EMT markers in our models confirmed these findings. These results imply an original a role of STING in the EMT program that could influence tumor progression and shape the tumor microenvironment.

Conclusions

The evidences presented above delineate a complex role of STING in cancer cells. Our results are opening new intriguing scenarios to investigate the biological processes linked to the STING pathway, in a context where STING agonists are seen as an extremely attractive option to increase immune checkpoint blockade efficacy in NSCLC patients.

P **01** - 034

THE EXTRACELLULAR MATRIX OF ORAL SQUAMOUS CELL CARCINOMA MODULATES THE SPATIAL DISTRIBUTION AND THE PHENOTYPES OF TUMOR-ASSOCIATED MYELOID IMMUNE CELL POPULATIONS

Luciana Petti[1], Aida Meghraoui-Kheddar[1], Roxane Elaldi[1,2], Julie Cazareth[1], Ellen Van Obberghen-Schilling[3], Veronique Braud[1], Fabienne Anjuère[1]

[1]Molecular and Cellular Pharmacology Institute, Valbonne, France; [2]Centre Antoine Lacassagne, Nice, France; [3]Valrose Biology Institute, Nice, France

Keywords: Oral squamous cell carcinoma, tumor microenvironment, myeloid immune cells, extracellular matrix

Oral squamous cell carcinomas (OSCC) are cancers of the oral cavity and represents the sixth leading cancer worldwide. Only 50% of the OSCC patients survive up to 5 years after diagnosis due to disease relapse and loco-regional spread following treatment failure. Malignant cells escape immune surveillance by promoting the conversion of the physiological microenvironment into a pro-tumor state. These changes involve not only the direct recruitment and activation of immunosuppressive immune cell subsets, but also their in situ remodeling by the tumoral extracellular matrix (ECM). Recent studies showed that Tenascin-C (TNC) is one major ECM component upregulated in the stroma of OSCC lesions with immunosuppressive functions and a promoter of tumor malignancy (Spenlé, CIR2020). However, its role in the regulation of the phagocytic immune cells, main actors in the OSCC immune microenvironment has not been thoroughly investigated.

This study aims to delineate the OSCC tumor immune microenvironment focusing on the in-depth characterization of macrophages and dendritic cell subsets and their phenotypic and functional regulation by the TNC ECM protein. We combined multiparametric analyses in human OSCC and in the 4-nitroquinoline-1-oxide (NQO)-induced OSCC model in TNC-sufficient and TNC-deficient background using spectral flow cytometry and imaging mass cytometry.
This study reveals the unique spatial distribution and the heterogeneity of macrophages and dendritic cells subsets in human and mouse OSCC models compared to non-tumoral tissues. The TNC protein not only modulates the positioning of macrophage and dendritic cell subsets but also their phenotype and functions. Our work contributes significantly to define the landscape of the immune and matrix microenvironment of HNSCC and may help to identify novel therapeutic strategies to improve current treatments.

P **01** - 035

IDENTIFICATION OF LIPID-ASSOCIATED MACROPHAGES INVOLVED IN IMMUNE SUPPRESSIVE ACTIVITIES IN TRIPLE NEGATIVE BREAST CANCER

Eleonora Timperi[1], Paul Gueguen[1], Martina Molgora[2], Ilaria Magagna[3], Yann Kieffer[3], Silvia Lopez-Lastra[1], Philemon Sirven[1], Laura G. Baudrin[4], Sylvain Baulande[4], André Nicolas[5], Gabriel Champenois[5], Didier Meseure[5], Anne Vincent-Salomon[5], Anne Tardivon[6], Enora Laas[7], Vassili Soumelis[8], Marco Colonna[2], Fatima Mechta-Grigoriou[3], Sebastian Amigorena[1], Emanuela Romano[1]

[1]Department of Immunology, INSERM U932, PSL Research University, Institut Curie, 26, rue d'Ulm, Paris 75005, France; [2]Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO 63110, USA, St. Louis, United States; [3]Stress and Cancer Laboratory, Equipe labélisée par la Ligue Nationale contre le Cancer, INSERM U830, PSL Research University, Institut Curie, 26, rue d'Ulm, Paris 75005, France; [4]Institut Curie Genomics of Excellence (ICGex) NGS Platform, Institut Curie, 26, rue d'Ulm, Paris 75005, France; [5]Department of Pathology, Institut Curie, 26, rue d'Ulm, Paris 75005, France; [6]Department of Radiology, Institut Curie, 26, rue d'Ulm, Paris 75005, France; [7]Service of Breast and Gynecologic Surgery, Institut Curie, 26, rue d'Ulm, Paris 75005, France; [8]AP-HP, Laboratoire d'Immunologie et histocompatibilité, Hôpital St Louis, 1, Avenue Claude Vellefaux 75010, Paris

Keywords: single-cell RNA sequencing, macrophages, lipid-associated macrophages, cancer-associated fibroblasts, triple-negative breast cancer

Tumor-associated macrophages (TAM) play a detrimental role in triple negative breast cancer (TNBC). Nevertheless, their in-depth characterization and interactions with stromal cells, such as the cancer-associated fibroblast (CAFs), are lacking. We identify at the single-cell level a monocyte-derived-STAB1+TREM2high lipid-associated macrophage (LAM) subpopulation that bears immune suppressive capacities and is implicated in resistance to immune checkpoint blockade (ICB). Genetic depletion of mouse monocyte-derived-STAB1+TREM2high LAMs in TNBC-Trem2-/- mice partially controls the TNBC tumor growth. FACS and bulk-RNA sequencing data demonstrate that co-culture in vitro of TNBC-derived FAP+ CAFs reprogram blood monocytes towards STAB1+TREM2high suppressive LAM phenotype. Indeed, T cell activation and proliferation is inhibited in vitro in LAM-T cell co-cultures. Cell-to-cell interaction modeling and in vitro assays demonstrate the role of the inflammatory CXCL12-CXCR4 axis in inflammatory CAF-myeloid cell crosstalk and recruitment of monocytes at tumor site. Altogether, we propose an inflammation model, whereby monocytes – recruited to the tumor via the inflammatory CAF-driven CXCL12-CXCR4 axis – acquire pro-tumorigenic and suppressive LAM capacities supporting an immunosuppressive microenvironment amenable to therapeutic targeting.

P **01** - 036

THE TUMOR GLYCO-CODE IMPACTS HUMAN DC FUNCTIONALITY AND CORRELATES WITH THE CLINICAL OUTCOME OF MELANOMA PATIENTS

Eleonora Sosa Cuevas[1,2], Benoît Roubinet[3], Stephane Mouret[4], Michel Thépaut[5], Florence De Fraipont[6], Julie Charles[4], Franck Fieschi[5], Ludovic Landemarre[3], Laurence Chaperot[1,2], Caroline Aspord[1,2]

[1]Institute for Advanced Biosciences, Team: Epigenetics, Immunity, Metabolism, Cell Signaling & Cancer, Inserm U 1209, CNRS UMR 5309, Université Grenoble Alpes, Grenoble, France; [2]Etablissement Français du Sang Auvergne-Rhône-Alpes, R&D Laboratory, Grenoble, France; [3]GLYcoDiag, 2 rue du Cristal, Orléans, France; [4]Dermatology, Allergology&Photobiology Department, CHU Grenoble Alpes, Grenoble, France; [5]Univ. Grenoble Alpes, CNRS, CEA, Institut de Biologie Structurale, Grenoble, France; [6]Medical Unit of Molecular genetic (hereditary diseases and oncology), Grenoble University Hospital, Grenoble, France

Keywords: Human dendritic cells, immune subversion, glycans, melanoma, clinical outcome

Subversion of immunity by tumors is a hallmark of cancer development. Dendritic cells (DCs) are strategic immune cells that connect innate and adaptive immunity, and trigger and shape subsequent anti-tumor immune responses. Tumor cells exploit DCs' versatility through suppressive pathways to subvert their functions. Given their high mutational rate, tumor cells present unusual glycosylations of surface proteins and lipids that can be recognized by C-type lectin receptors (CLR) expressed by DCs. Thus, the aberrant glycosylation of tumor surface glycoproteins and glycolipids could modify DC activity through CLR signaling and subsequently reduce anti-tumor immune responses. There is a major perturbation of the expression of enzymes responsible for glycosylation (glycosyl-transferases, glycosidases) in melanoma. Furthermore, we recently demonstrated that the three major DC subsets (BDCA1+ cDC2s, BDCA2+ pDCs and BDCA3+ cDC1s) infiltrated melanoma tumors and that their functionality was differentially impacted by tumor cells. Moreover, the expression of several CLRs was modulated on circulating and tumor-infiltrating DC subsets and impacted clinical outcome of melanoma patients. Yet, the impact of the tumor glyco-code on DC subsets has not been explored in melanoma.
The goal of our study is to characterize the melanoma tumor glyco-code, and to decipher its impact on patients' clinical outcome and DC subsets' functionality. First, we established an in-vitro model of interaction between primary tumor cell lines (derived from melanoma patients) and purified DCs (derived from healthy donor blood), and depicted the three DC subsets' functionality by flow cytometry. We observed different alterations of DCs' cytokine production depending on tumor cell lines. Next, we depicted the tumor glyco-code of the melanoma tumor cell lines through the GLYcoPROFILE methodology (lectin arrays), and found that expression of specific glycans correlated with the clinical outcome of melanoma patients. Strikingly, tumor cell lines that differently impacted DCs harbored distinct glycoprofiles.
Thus, the glycan/CLR/DC axis constitutes a new immune subversion pathway in melanoma. Grasping DC's CLR/glycan profile in patients could lead to the conception of new therapeutic strategies exploiting DCs' potentiality while preventing hijacking by the tumor in order to restore anti-tumor immunity.

P **01** - 037

HUMAN CORONAVIRUS INFECTED LUNG CELLS RECRUIT COMPLEMENT INHIBITORS VITRONECTIN AND CLUSTERIN AND DELAY COMPLEMENT-MEDIATED LYSIS

Candace Fox[1], Griffith Parks[1]

[1]University of Central Florida, ORLANDO, United States

Keywords: coronavirus, complement, parainfluenza virus

The complement (C') system is a critical pathway of the innate immune response which viruses and infected cells must evade for survival. We sought out to better understand the innate immune response to a highly prevalent and reoccurring RNA human coronavirus (CoV)-OC43. CoV-OC43 is a major public health concern that imposes a huge burden on the economy and health care industry. Using real-time

cell viability assays and flow cytometry, we have shown the kinetics of C'-mediated lysis of CoV-OC43 infected lung cells was substantially delayed as compared to the rapid and potent C'-mediated killing of cells infected with parainfluenza virus type 5 (PIV5). In the case of CoV-OC43 infected cells that were co-infected with PIV5, the C'-mediated lysis was delayed to the same extent as CoV-OC43 infected cells alone, suggesting that CoV-OC43 infection can overcome highly activating signals induced by PIV5 infection. Our prior work has shown respiratory virus infection can upregulate expression of C' inhibitors such as CD59. Vitronectin (VN) and clusterin (CLU) are key regulators of the terminal C' pathway found in normal human serum (NHS) that can prevent membrane attack complex formation and cell death. Here we investigated the hypothesis that human respiratory virus infected cells can utilize the C' inhibitors VN and CLU. VN and CLU could be recruited from exogenous serum to the surface of CoV infected cells. VN wasn't bound to OC43-infected cells after treatment with antibody-depleted serum. Reconstitution experiments with purified IgG and VN showed that human antibodies are both necessary and sufficient for VN recruitment to OC43-infected cells. Similarly, SARS-CoV-2 infected lung cells also recruited VN from NHS to the infected cell surface. Taken together, we propose a novel role for VN and CLU recruitment by coronavirus infected cells to delay C'-mediated death – findings that have implications for viral pathogenesis and tissue tropism.

P **01** - 038

UNIQUE CLR PROFILING OF DC SUBSETS CORRELATES WITH CLINICAL OUTCOME IN MELANOMA PATIENTS

Eleonora Sosa Cuevas[1,2], Jenny Valladeau-Guilemond[3], Stephane Mouret[4], Florence De Fraipont[5], Julie Charles[4], Nathalie Bendriss-Vermare[3], Laurence Chaperot[1,2], Caroline Aspord[1,2]

[1]Institute for Advanced Biosciences, Team: Epigenetics, Immunity, Metabolism, Cell Signaling & Cancer, Inserm U 1209, CNRS UMR 5309, Université Grenoble Alpes, Grenoble, France; [2]Etablissement Français du Sang Auvergne-Rhône-Alpes, R&D Laboratory, Grenoble, France; [3]Univ Lyon, Université Claude Bernard Lyon 1, INSERM 1052, CNRS 5286, Centre Léon Bérard, Centre de Recherche en Cancérologie de Lyon, Lyon, France; [4]Dermatology, Allergology & Photobiology Department, CHU Grenoble Alpes, Grenoble, France; [5]Medical Unit of Molecular genetic (hereditary diseases and oncology), Grenoble University Hospital, Grenoble, France

Keywords: DCs subsets, CLR, melanoma, clinical outcome

Subversion of immunity by tumors is a hallmark of cancer and a crucial step for cancer development. Dendritic cells (DCs) are strategic immune cells that connect innate and adaptive immunity. Given their unique ability to uptake antigens, perform cross-presentation, and activate antigen-specific adaptive immunity, DCs trigger and shape subsequent anti-viral/anti-tumor immune responses. Furthermore, immune checkpoints (ICP) expressed by DCs are critical players for the triggering, orientation and evasion of immunity. Despite recent improvements in cancer treatment, around 60% of melanoma patients do not respond to immunomodulation by ICP blockers. Yet, the infiltration of the tumor by innate and adaptive specific immune cells has been linked to good clinical outcomes and is correlated with the responsiveness to immunotherapies.

Tumor cells exploit DCs' versatility through suppressive pathways that subvert their functions. Given their high mutational rate, tumor cells present unusual glycosylations of surface proteins and lipids that can be recognized by C-type lectin receptors (CLR) expressed by DCs and are considered as novel immune checkpoints in cancer. Thus, the aberrant glycosylation of tumor surface glycoproteins and glycolipids could modify DC activity through CLR signaling and reduce subsequently anti-tumor immune responses. We recently demonstrated that the three major DC subsets (BDCA1+ cDC2s, BDCA2+ pDCs and BDCA3+ cDC1s) infiltrated melanoma tumors and that their functionality was differentially impacted by tumor cells. Yet, the CLR expression by DC subsets has not been explored in melanoma.

Using a multiparametric flow cytometry approach, we assessed in this study the expression of several CLRs by circulating and tumor-infiltrating DC subsets in melanoma patients. Our work revealed a modulation of CLR expression in DC subsets from patients when compared to healthy donors depending on the CLR and/or the DC subset. Intra-group clustering was shown through principal component analysis and changes in specific CLR expression correlated with patients' clinical outcome. These results highlight the interest of studying the CLR/glycan axis in the context of melanoma. Grasping DC's CLR/glycan profile in melanoma patients could lead to the conception of new therapeutic strategies which exploit DC potentiality while preventing hijacking by the tumor resulting in the restoration of anti-tumor immunity and improvement of patient clinical outcome.

P **01** - 039

REGULATION OF THE INFLAMMASOME BY NLRP3 POST-TRANSLATIONAL MODIFICATIONS

Tingting Niu[1,2], Amaury Rey[1], Marine Groslambert[1], Danish Patoli[1], Charlotte De Rosny[1], Séverine Chautard[1], Camille Cosson[1], Romane Riou[1], Zhirong Zhang[3], Orane Visvikis[4], Sabine Hacot[5], Maggy Hologne[6], Olivier Walker[6], Romeo Ricci[3], Thomas Henry[1], Laurent Boyer[4], Virginie Petrilli[5], Benedicte Py[1]

[1]CIRI, Centre International de Recherche en Infectiologie, Univ Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, Lyon, France; [2]Shanghai Key Laboratory of Regulatory Biology, Institute of Biomedical Sciences and School of Life Sciences, East China Normal University, Shanghai, China; [3]IGBMC, Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS, UMR7104, Inserm, U964, Université de Strasbourg, Illkirch, France; [4]Université Côte d'Azur, Inserm, C3M, Nice, France; [5]CRCL, Centre de Recherche en Cancérologie de Lyon, INSERM U1052, CNRS UMR5286, Université de Lyon, Université Lyon 1, Centre Léon Bérard, Lyon, France; [6]Institut des Sciences Analytiques (ISA), Univ Lyon, CNRS, CNRS UMR5280, Université Claude Bernard Lyon 1, Lyon, France

Keywords: Innate immunity, inflammasome, cell signaling, inflammation

Aim/Objective: The NLRP3 cytosolic pattern recognition receptor supports inflammation in response to infections or tissue malfunction. Upon activation, NLRP3 assembles an inflammasome complex resulting in the activation of caspase-1 which controls the maturation of cytosolic inflammatory cytokines IL-1beta and IL-18, and processes GasderminD forming then pores across the plasma membrane leading to pyroptosis cell lysis. Inflammasome assembly is tightly controlled and usually requires macrophage priming by other pattern recognition receptors and activation by various cell stress. The molecular mechanism of NLRP3 activation remains incompletely understood. Following our results revealing the critical deubiquitination of NLRP3 by BRCC3, NLRP3 post-translational modifications is now widely recognized to play a key role in inflammasome assembly.

Design: We used mass spectrometry to identify modified site in NLRP3, and generated knock-in mice bearing substitutions of these residues to investigate their functional impacts on the inflammasome activity in primary macrophages and in mouse in vivo models.

Results: In a first axis, we discovered that phosphorylation of a particular serine controls NLRP3 deubiquitination by BRCC3, inflammasome assembly and mouse sensitivity to endotoxic shock. In a second axis, we revealed a lysine ubiquitinated upon LPS priming, which prevents the activation of a LPS-dependent structurally alternative inflammasome resulting in GasderminD-independent IL-1beta secretion in the absence of cell death. Knock-in mice with substitution of this lysine show reduced survival to endotoxic shock. Its substitution in patient is associated with auto-inflammation.

Conclusion: Together, our results identify two new inhibitory checkpoints in inflammasome activation, and reveal a novel pathologically relevant alternative inflammasome.

P **01** - 040

PYRIN INFLAMMASOME IN HEALTH AND DISEASE

Flora Magnotti[1], Thomas Henry[1]

[1]CIRI, Inserm U1111, CNRS UMR5308, ENS Lyon, Univ. Lyon, Lyon, France

Keywords: inflammasome, autoinflammatory diseases, Pyrin

Inflammasomes are innate immune platforms assembled in the cytosol in response to the sensing of microbial molecules or danger signals. Activation of caspase-1 within inflammasomes leads to the release of the inflammatory cytokines IL-1β and IL-18 and to a fast inflammatory cell death termed pyroptosis.

Pyrin is an inflammasome sensor acting as a guard of RhoA GTPases. Numerous bacteria have virulence factors inhibiting RhoA and are thus detected by the pyrin inflammasome. Yet, mutations in the

pyrin-encoding gene, MEFV, cause two distinct autoinflammatory diseases, familial Mediterranean fever (FMF) and Pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND). Our data demonstrate that the pyrin inflammasome is activated by a two step mechanism and that the two autoinflammatory diseases affect differentially each of the activation step. Finally, through a chemical screen, we identified endogenous steroid catabolites activating pyrin activation step 2. These results demonstrate a novel pyrin activation mechanism and suggest that deregulation of these endogenous metabolites could contribute to inflammation in patients suffering from autoinflammatory diseases.

P **01** - 041

RECRUITMENT AND ACTIVATION OF TYPE 3 INNATE LYMPHOID CELLS PROMOTE ANTITUMOR IMMUNE RESPONSES

Mélanie Bruchard[1], Mannon Geindreau[1], Frédérique Végran[1], Francois Ghiringhelli[1]

[1]INSERM U1231, Dijon, France

Keywords: ILC, Cancer, chemotherapy, T cells, chemokines

T lymphocyte infiltration to the tumor bed is a major prognostic factor in many cancer types. Immune checkpoint blockade (ICB) is able to trigger long-lasting antitumor immune responses resulting in clinical benefit in various cancer types, but only in a limited number of patients. Tumors that lack lymphocyte infiltrate (also called 'cold' tumors) are resistant to ICB monotherapy and some cytotoxic chemotherapies used in a neoadjuvant setting. This led to the hypothesis that increasing T cell infiltration of cold tumors may enhance their sensitivity to checkpoint inhibitors and chemotherapies, as well as the number of responding patients.

ILC3s express the transcription factor RORγt and produce the cytokines interleukin-17 (IL-17) and IL-22. ILC3s include natural cytotoxicity receptor (NCR)+ ILC3s, chemokine receptor type 6 (CCR6)+ ILC3s and fetal lymphoid tissue inducer (LTi) cells involved in the development of secondary lymphoid organs. Conflicting roles for ILC3s have been described in the development of cancer. Accumulation of ILC3s is associated with tumor growth and poor outcome in breast and colorectal cancer. In contrast, such accumulation is associated with a better outcome in non-small cell lung carcinoma (NSCLC) and melanoma. Notably, in NSCLC, ILC3s have also been positively associated with the presence of tertiary lymphoid structures, a known predictor of a favorable clinical outcome, suggesting that ILC3s in tumors might possess lymphocyte recruitment capacity, a typical feature of LTi cells. Using murine models, we found that CCR6+ type 3 innate lymphoid cells (ILC3s) can trigger an increase in the number of T cells infiltrating a tumor. Shortly after administration of cisplatin chemotherapy, production of the chemokine CCL20 and proinflammatory cytokine IL-1β at the tumor site led to the recruitment and activation of ILC3s. Within the tumor, ILC3 production of the chemokine CXCL10 was responsible for the recruitment of CD4+ and CD8+ T lymphocytes to the tumor. ILC3-dependent infiltration of T cells was essential for antitumor immune responses and increased the efficacy of checkpoint inhibition. Thus, we reveal an essential role of CCL20 and IL-1β, which promote ILC3-dependent antitumor immunity and enhance tumor sensitivity to immunotherapy.

P **01** - 042

ANTI-PD-1 REINVIGORATE VG9VD2+ T CELLS IN HUMAN BREAST CANCER

Stephane Fattori[1,2], Laurent Gorvel[1,2], Samuel Granjeaud[3], Amira Ben Amara[1,2], Nicolas Boucherit[1,2], Marie-Sarah Rouvière[1,2], Philippe Rochinieux[1,2,4], Anthony Goncalves[4,8], Carole Tarpin[4], Jihane Pakradouni[5], Eric Lambaudie[6,7], Gilles Houvenaeghel[6,7], François Bertucci[4,8], Jacques A. Nunès[1], Anne-Sophie Chrétien[1,2,7], Daniel Olive[1,2,7]

[1]Team Immunity and Cancer, Centre de Recherche en Cancérologie de Marseille (CRCM), Inserm, U1068, CNRS, UMR7258, Institut Paoli-Calmettes, Aix-Marseille University, UM105, Marseille, France; [2]Cancer Immunomonitoring Platform, Centre de Recherche en Cancérologie de Marseille (CRCM), Inserm, U1068, CNRS, UMR7258, Institut Paoli-Calmettes, Marseille, France; [3]Systems Biology Platform, Centre de Recherche en Cancérologie de Marseille (CRCM), Inserm, U1068, CNRS, UMR7258, Institut Paoli-Calmettes, Aix-Marseille University, UM105, Marseille, France; [4]Department of Medical Oncology, Institut Paoli-Calmettes, Marseille, France; [5]Department of Clinical Research and Innovations, Institut Paoli-Calmettes, Marseille, France; [6]Department of Surgical Oncology, Institut Paoli-Calmettes, Marseille, France; [7]Faculty of Medical and Paramedic Sciences, Aix Marseille University, UM105, Marseille, France; [8]Team Predictive Oncology, Centre de Recherche en Cancérologie de Marseille (CRCM), Inserm, U1068, CNRS, UMR7258, Institut Paoli-Calmettes, Aix-Marseille University, UM105, Marseille, France

Keywords: single-cell RNA sequencing , Vg9Vd2+ T cell , breast cancer, cancer immunotherapy, pembrolizumab

Vγ9Vδ2+ γδ (Vγ9Vδ2+) T cells are promising therapeutic targets with efficient antitumor properties both in vitro and in preclinical models of breast cancers. Yet, little information has been reported regarding their functional state in human breast cancers, owing to scarce Vγ9Vδ2+ T cell infiltrates. Here, we employed single-cell transcriptomic and proteomic analytic tools to provide a comprehensive landscape of Vγ9Vδ2+ T cells from untreated and anti-PD-1-treated human breast cancers. We report skewed Vγ9Vδ2+ T cell differentiation profiles trending towards early memory Tc1 phenotypes, along with no detectable terminally differentiated phenotypes, both in untreated and pembrolizumab-treated breast tumors as opposed to normal tissues. Importantly, pembrolizumab monotherapy increased activating hallmarks in breast tumor-infiltrating Vγ9Vδ2+ T cells. Our data may provide central information for the development of Vγ9Vδ2+ T cell-based immunotherapies in breast cancer.

P **02** - 001

CYCLOSPORIN H REGULATES T CELL METABOLISM AND MITIGATES EXHAUSTION

Ilaria Castiglioni[1], Giulia Unali[1], Laura Cassina[2], Federico Rossari[3], Vittoria Matafora[4], Giovanni Crivicich[1], Ivan Cuccovillo[1], Monah Abou Alezz[7], Ivan Merelli[7], Julie Haines[5], Angelo D'Alessandro[5], Angela Bachi[4], Luigi Naldini[6], Nadia Coltella[3], Alessandra Boletta[2], Anna Kajaste-Rudnitski[1]

[1]San Raffaele Telethon Institute for Gene Therapy, Retrovirus-host interactions and innate immunity to gene transfer, MIlan, Italy; [2]IRCCS San Raffaele Scientific Institute, Molecular Basis of Cystic Kidney Disorders, Division of Genetics and Cell Biology, MIlan, Italy; [3]San Raffaele Telethon Institute for Gene Therapy (SR-TIGET), Targeted Cancer Gene Therapy Unit, Milan, Italy; [4]FIRC Institute of Molecular Oncology (IFOM), Milan, Italy; [5]Department of Biochemistry and Molecular Genetics, University of Colorado Anschutz Medical Campus, Aurora, Colorado, United States; [6]San Raffaele Telethon Institute for Gene Therapy (SR?TIGET), Gene transfer technologies and new gene therapy strategies, Milan, Italy; [7]San Raffaele Telethon Institute for Gene Therapy, Bioinformatics Core, Milan, Italy

Keywords: metabolism, CAR-T, HSC, immunity

We have previously described the non-immunosuppressive Cyclosporine H (CsH) as a potent enhancer of lentiviral vector (LV) transduction in human hematopoietic stem cells (HSC) and T cells. Exploring the impact of CsH on the global proteome of primary human HSC, we observed an upregulation of pathways that are mainly involved in metabolic processes such as Kreb-cycle and fatty acid oxidation. Conversely, genes related to lipid metabolism and T cell receptor signaling pathway were downregulated in cells exposed to CsH as compared to controls. Accordingly, mass spectrometry metabolomics revealed a significant CsH-induced increase in acylcarnitines and free fatty acids at steady state in HSC, thus confirming a potential effect of CsH in inducing relevant metabolic alterations. We next evaluated the impact of CsH in CD4+ and CD8+ T cells, in which a metabolic reprogramming is crucial for their activation and differentiation to ultimately mount an efficient response. Interestingly, preliminary results showed that both CD4+ and CD8+ T cells transduced in the presence of CsH have significantly reduced levels of both basal and maximal respiration, as well as ATP production, in Seahorse assays as compared to controls. In line with these metabolic changes, genes related to fatty acid β-oxidation and mitochondrial activity such as CPT1a and NRF1, were downregulated. We excluded an impact of CsH on T cell subset distribution and proliferation. Instead, T cells transduced in presence of CsH resulted less exhausted in culture as compared to controls. This effect was confirmed also in the context of murine T cells. Growing evidence indicates that exhausted T cells undergo metabolic alterations, leading to poor responsiveness to immune-checkpoint-blockade and lower efficacy of CAR T cell therapies. Therefore, the reprogramming of CAR T cell metabolism is an attractive approach to improve their antitumour activity, also promoting their resistance within the tumor microenvironment. Metabolomic and lipidomic analysis on CAR T cells generated in presence of CsH are currently ongoing, aimed at dissecting whether and how the CsH-induced metabolic changes are linked with lower T-cell exhaustion, and whether it could represent a potential beneficial effect in the context of CAR T cell therapy.

P **02** - 002

USING PANGENOMIC CRISPR/CAS9 SCREENS TO IDENTIFY REGULATORS OF MTORC1 IN HUMAN NK CELLS.

Uzma Hasan[1], Quentin Sueur[1], Michelle Ainouze[1], Antoine Corbin[1], Omran Allatif[1], Thierry Walzer[1], Antoine Marcais[1]

[1]CIRI, Lyon, France

Keywords: mTOR, pS6, size, CRISPR screen, NK cells

Natural Killer (NK) cells are innate lymphoid cells that kill infected or tumour cells and secrete cytokines. We showed that both functions are controlled by the mammalian Target of Rapamycine complex 1 (mTORC1). Indeed, this complex controls both the acquisition of effector functions during NK cell ontogeny and the encounter with a target cell. We further determined that mTORC1 is sensitive to a large range of external immunologic and metabolic cues affecting NK cells. Yet the molecular pathways that control mTORC1 activity in NK cells are poorly defined. Here, we present a CRISPR screening strategy to identify the mTORC1 regulators in NK cells. We engineered a human NK cell line (NK-92) to express the Cas9 enzyme and infected it with a pooled genome-wide sgRNA lentiviral library. Two complementary strategies were devised to identify mTORC1 regulators.

1. By monitoring mTORC1 activity using the measurement of ribosomal S6 phosphorylation (pS6) classified as being high or low.
2. We adopted cell size as a functional read-out as it has been shown to correlate to mTORC1 activity.

Following IL-2 stimulation, we isolated cells that failed to fully induce pS6 or remained small as well as cells that had higher pS6 levels or were bigger.

Cells were sorted by flow cytometry and all parameters were discussed as well as optimised with SFR128-AniRA - Cytometry platform. Cell size sorting parameters were confirmed by visualizing cells using the ISX. DNA was then extracted and sequencing was performed at the IGFL's sequencing platform,

Identification of enriched vs depleted sgRNAs and genes that regulate mTORC1 in NK cells is currently ongoing.

P **02** - 003

SEX-SPECIFIC IMPACT OF OBESITY ON THE EFFICACY OF CANCER IMMUNOTHERAPY

Eloïse Dupuychaffray[1,2], Hélène Poinot[1,2], Montserrat Alvarez[1,2], Betül Taskoparan[1,2], Michael Groessl[4], Carole Bourquin[1,2,3], Aurélien Pommier[1,2]

[1]School of Pharmaceutical Sciences, University of Geneva, Geneva, Switzerland; [2]Institute of Pharmaceutical Sciences of Western Switzerland, Geneva, Switzerland; [3]Department of Anaesthetics, Pharmacology, Intensive Care and Emergencies, Faculty of Medicine, University of Geneva, Geneva, Switzerland; [4]Deparment of Clinical Research, Division of Nephrology and Hypertension, University of Bern, Bern, Switzerland

Keywords: Cancer, Immunotherapy, Obesity, Sex, Estrogen

Recent clinical studies suggest that obese cancer patients, especially men, have a better outcome when treated with immune checkpoint inhibitors (ICI) compared to non-obese patients. However, whether obesity and sex are determinant factors influencing the antitumor immune response in the context of ICI is unknown. Male and female mice fed with a high-fat diet to induce obesity or with a control diet were subcutaneously injected with B16-F10 melanoma cells. ICI efficacy was assessed upon anti-PD-1 treatment by measuring tumor growth and immune cell activation markers. In non-obese groups, ICI efficacy was observed in females but not in males. Interestingly, obese males became sensitive to ICI treatment while obesity did not affect the ICI response in females. RNA sequencing analyses prior ICI therapy revealed that obesity induced a female-like tumor gene expression profile in males characterized by the enrichment of differentially expressed genes involved in epithelial-mesenchymal transition, angiogenesis processes and estrogen gene signature. We hypothesized that steroid hormones such as estrogens and androgens may play a major role in the response to ICI as their level is known to be sex- and BMI-dependent. Steroid quantification confirmed a higher level of estrogens in obese males compared to non-obese. Obese males were treated with the aromatase inhibitor letrozole to prevent the obesity-induced increase in estradiol (E2) synthesis. This treatment abolished the efficacy of ICI initially observed in obese males, demonstrating the pivotal role of estrogens in the ICI sensitivity of obese males. In vitro experiments demonstrated that E2 enhances the tumor immune response mainly by stimulating the antigen presentation capacity of dendritic cells. These results support the beneficial role of E2 on the response to ICI treatment that appears to be modulated by both obesity and sex. This work shows that obesity may improve the response to ICI in male mice and revealed unexpected pathways differentially regulated by obesity in males versus females.

P **02** - 004

IMPACT OF EXTRACELLULAR PYRUVATE DEPLETION HIGHLIGHTS THE CENTRAL ROLE OF THE ONE CARBON METABOLISM IN HUMAN BLOOD CD56DIM NATURAL KILLER CELLS

Nicolas Kern[1], Thierry Walzer[1], Antoine Marçais[1]

[1]INSERM, Lyon, France

Keywords: Pyruvate, Glycolysis, NK cells, One Carbon metabolism, Metabolism

Human Natural Killer (NK) cells are innate lymphocytes able to recognize and eliminate abnormal cells. In peripheral blood, two subsets of NK cells coexist, identified on the basis of CD56 expression. CD56bright NK cells have precursor functions, whereas CD56dim are terminally differentiated cytotoxic cells. Cellular metabolism is now recognized as an important factor that contributes to successful immune responses. However, what fuels basic NK cell metabolism is unknown. In the general situation, glucose is oxidized to pyruvate that fuels mitochondrial tricarboxylic cycle (TCA), or can be reduced to lactate and excreted in the medium. This last step allows rapid regeneration of NAD+ required for GAPDH activity in the upstream part of glycolysis. We have discovered that acute depletion of extracellular pyruvate negatively impacts both glycolysis and Oxidative Phosphorylation in human CD56dim NK cells. This correlated with the fact that extracellular pyruvate is needed to maintain both NAD+ and ATP levels in human NK cells. These metabolic changes ultimately prevented activation of the metabolic regulator mTORC1. This pyruvate auxotrophy results from the incapacity of NK cells to fully oxidize glucose to pyruvate as shown by metabolomic analysis. We hypothesized that NK cells are unable to generate pyruvate from glucose due to a leak of glycolysis into the One Carbon Metabolism pathway (1CM). Indeed, pharmacological inhibition of the first limiting enzyme of this pathways, the phosphoglycerate dehydrogenase (PHGDH) rescues NK cells metabolism in the absence of extracellular pyruvate. Extracellular pyruvate thus seems to play a pivotal role in NK cells metabolism. We are now in the process of characterizing the impact of extracellular pyruvate on human NK cell functions.

P **02** - 005

LIPID-LOADED MACROPHAGES IN CANCER: ROLE IN RESISTANCE TO THERAPY

Michela Masetti[2], Roberta Carriero[2], Federica Portale[2], Giulia Marelli[2], Nicolò Morina[1,2], Marta Pandini[1,2], Paolo Kunderfranco[2], Massimo Lazzeri[2], Paolo Casale[2], Rodolfo Hurle[2], Piergiuseppe Colombo[2], Gianluca Basso[2], Cibella Javier[2], Subhankar Mukhopadhyay[3], Ronald Depinho[4], Siamon Gordon[5], Diletta Di Mitri[1,2]

[1]Humanitas University, Pieve Emanuele, Italy; [2]IRCCS Humanitas Clinical And Research Center, Rozzano, Italy; [3]MRC Centre for Transplantation, Peter Gorer Department of Immunobiology, School of Immunology and Microbial Sciences, King's College London, London, United Kingdom; [4]Department of Cancer Biology, The University of Texas MD Anderson Cancer Center, Houston, United States; [5]Sir William Dunn School of Pathology, Oxford, OX1 3RE UK, Oxford, United Kingdom

Keywords: Tumor Associated Macrophages, Cancer, Innate Immunity, Lipids, Immunotherapy

Immunotherapies have revolutionized cancer outcomes, yet these therapies are ineffective in most patients and predicting which patients will respond and how to enhance efficacy remain urgent clinical needs. We and others have shown that myeloid cells promote cancer progression, orchestrate immune evasion and hinder response to therapies in most tumors. On this line we have discovered a population of macrophages infiltrating prostate cancer that shows a deregulated lipid metabolism and a prominent lipid-egulfment (Lipid-Macs). Lipid-Macs infiltrate several tumor types,including ovarian and melanoma. Importantly, Lipid-Macs promote cancer cells proliferation and invasiveness thus contributing to tumor progression. Moreover, preliminary evidence indicates that Lipid-Macs hinder response to chemotherapy in prostate cancer and are associated with resistance to immunotherapies in melanoma. Mechanistically, lipid accumulation drives the upregulation of immune-regulatory proteins on intratumoral myeloid subsets. Accordingly, the abundance of Lipid-Macs inversely correlates with the infiltration of CD8+ T lymphocytes. Together, our findings identify a heterotypic signalling involving lipid-loaded TAMs and cancer cells that drives cancer aggressiveness and provides novel therapeutic targets to improve therapy response.

P **02** - 006

BOOSTING CAR T CELLS WITH MANNOSE

Macarena Lucia Fernandez Carro[1], Adam Hurlstone[1]

[1]The University of Manchester, Manchester, United Kingdom

Keywords: mannose, CAR T, immune therapy, solid tumour, microenviornment

Despite success with B-cell malignancy, CAR T cells have yet to make an impact in the clinic against solid cancer. Multiple factors make the solid tumour microenvironment a hostile place for T cells. Tumour and myeloid-derived cells are avid competitors with T cells for nutrients and create toxic waste due to their increased metabolic activity that contribute to T cell exhaustion and malfunction. Notably tumour microenvironments are glucose depleted, hypoxic and acidic. In exploring interventions that selectively antagonise tumour cell metabolism, we find that supplementation with the hexose mannose boost T cell function. We have confirmed that mannose suppresses viability of a range of cultured human cancer cells through creating an ATP deficit resulting in their death by necrosis. Intriguingly, and in contrast, T cells treated with mannose upregulate key glycolytic enzymes and maintain lactate secretion even at low glucose concentration, and do not lose viability. We are currently examining the impact of mannose on a range of CAR T cell responses to incubation with target cells, in normal and low glucose concentrations, and will be testing mannose as a supplement to accompany CAR T cell adoptive therapy in mouse. Our findings highlight a simple, inexpensive intervention with potential to boost CAR T cell and TIL activity in the solid tumour microenvironment.

P **02** - 007

THE HEXOKINASE ISOENZYMES CONTROL SUSCEPTIBILITY OF HUMAN HEPATOCARCINOMA (HCC) CELLS TO NATURAL KILLERS THROUGH MODULATION OF ICAM-1 AND PVR EXPRESSION

Anne Aublin-Gex[1], Olivier Diaz[1], Pierre-Olivier Vidalain[1], Clemence Jacquemin[1], Vincent Lotteau[1], Laure Perrin-Cocon[1]

[1]CIRI - Team VIRal Infection, Metabolism and Immunity, Lyon, France

Keywords: Glycolysis, Hepatocellular Carcinoma, Natural Killer cells, Cytotoxicity, Hexokinases

Aim/Objective- Hexokinases (HKs) control the first step of glucose catabolism, fueling glycolysis as well as glycogen, pentose phosphate and triglyceride synthesis. In humans, five hexokinase isoenzymes display distinct enzymatic kinetics and tissue distributions. A switch from the liver HK (glucokinase, GCK) to the tumor isoenzyme HK2 occurs during the transition from primary to tumor hepatocytes. HK2 expression level has been correlated with disease progression and dedifferentiation of hepatocytes. We discovered that HK isoenzyme expression not only controls hepatic metabolic functions but also interferes with intrinsic innate immunity of hepatocytes and their sensitivity to NK cell-cytotoxicity (Perrin-Cocon et al., 2021). The aim of our study is to understand how metabolic modifications occurring in HCC impacts innate immunity and the anti-tumoral defense.

Design- Huh7 cells expressing GCK, HK2 or both were generated and compared for their immunologic and metabolic profiles.

Results- Compared to the parental cell line Huh7-GCK-/HK2+, Huh7-GCK+/HK2- cells presented a rewired central carbon metabolism, an increased mitochondrial respiration and a restoration of essential hepatocyte functions such as lipogenesis, VLDL secretion and glycogen storage. Innate immune responses to RIG-like receptor stimulation were also restored. HK2 expression at the expense of GCK in HCC reduced the cell surface expression of the adhesion molecule ICAM-1 and NK cell receptors ligand PVR, correlating with a resistance to NK cell-induced cell death.

Conclusion- Our results show that HK2 expression in HCC cells downregulates innate immunity and promotes resistance to NK cell-cytotoxicity.

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P **03** - 001

IMMUNE EVOLUTION DURING TUMOR INVASION IN EARLY STAGE ENDOMETRIAL ADENOCARCINOMA

Nawel Zouggari[1], Prasad Chaskar[1], Jean-Christophe Tille[2], Zeinab Dehghani Ghobadi[1], Ariane De Agostini[3], Petros Tsantoulis[1,4], Intidhar Labidi-Galy[1,4]

[1]Department of Medicine, Division of Oncology, Faculty of Medicine, University of Geneva, Geneva, Switzerland; [2]Department of Diagnosis, Division of Clinical pathology, Hôpitaux Universitaires de Genève, Geneva, Switzerland; [3]Department of Pathology and Immunology, Faculty of Medicine, University of Geneva, Geneva, Switzerland; [4]Department of Oncology, Hôpitaux Universitaires de Genève, University of Geneva, Geneva, Switzerland

Keywords: endometrial, cancer, immune, microenvironment, invasion

Endometrial cancer (EC) is the most frequent gynecological cancer. It is mainly diagnosed at low grade and early stage (I). Exploring the largely uncharted biology of tumor invasiveness requires deciphering the molecular processes of early stage lesions.

Here, we aimed to characterize the tumor immune microenvironment (TiME) of low grade, early stage EC with different patterns of myometrial invasion: i) Broad Front (BF) pushes the myometrium border and is the least aggressive subset; ii) Infiltrating Glands (IG) is associated with lymphovascular invasion; and iii) Microcystic Elongated and Fragmented Glands (MELF) is associated with a higher "metastasis-likelihood".

We applied a new method of spatial transcriptomics that combines laser-capture microdissection and RNA-sequencing technology to clinically annotated low grade, early stage EC samples (N=25), to precisely target the TiME at the tumor front where the tumor invades the myometrium.

We found that TiME of MELF was characterized by activation of innate immune response (type I interferon and NK cell signaling), as compared to BF and IG. Microenvironment cell populations-counter algorithm suggested an enrichment of MELF's TiME in NK cells, macrophages and CD8+ T cells. In situ validation of our transcriptomics analyses is ongoing through sequential multiplexed immunohistochemistry analysis (NKp46-Foxp3-CD4-CD45-CD163-CD8-CD68-CD20 and Cytokeratin).

Our work will help for a better comprehension of the molecular alterations involved in myometrial invasion, the first step of tumor invasion in EC, and could have potential implications for new immunotherapy strategies in this disease.

P **03** - 002

PRESENTING THE ACTIVITIES OF THE LYON IMMUNOTHERAPY FOR CANCER LABORATORY: ILLUSTRATION THROUGH THE STUDY OF IMMUNE SYSTEM MODULATIONS IN MELANOMA PATIENTS TREATED WITH ANTI-PD1 MONOCLONAL ANTIBODIES

B Dubois[1,2], C Ménétrier-Caux[1,2], J Caramel[2], J Berthet[1,2], A Nkodia[1,2], A Eberhardt[2,3], C Rodriquez[2], E Verronese[1,2], S Barrin[1,2], C Bardin-dit-Courageot[1,2], N Bendriss-Vermare[1,2], U Hasan[1,2], C Caux[1,2], S Dalle[2,3]

[1]LICL, Lyon, France; [2]CRCL, Lyon, France; [3]CHU Lyon, Pierre Benite, France

Keywords: Immunotherapy, Melanoma, mIF, Blood, Immunology

Cancer immunotherapy is now part of the standard care for multiple tumor types at diverse disease stages. This provides improved survival and reduced disease recurrences. Currently, the two most important challenges in cancer immunotherapy are to identify immune biomarkers predictive of response to treatment, and to understand the mechanisms of resistance to checkpoint blockade to propose alternative immunotherapy treatment in non- responding patients. To take on these challenges, powerful research platforms are needed, and novel fundamental knowledge must be integrated into the development of novel immunotherapies improving clinical responses. The newly created Lyon Immunotherapy for Cancer Laboratory (LICL) has been devised in this objective. LICL is equipped with state-of-the-art equipment in the field of immune monitoring. The main objective of the LICL is to provide the CRCL/CLB and public hospitals in Lyon with all the skills and expertise required to monitor innovative immunotherapy clinical trials in the field of cancer, to identify resistance mechanisms and new targets, for which new drugs could be developed in partnerships.

To achieve these objectives the LICL is built on four major axes: i) Blood monitoring of immune cells and markers ii) In situ analysis of tumor-infiltrating immune cells through multiplex fluorescence tissue imaging, iii) Ex vivo preclinical evaluation of immunotherapies in patient samples, and iv) In silico identification of resistance mechanisms and new immunotherapy targets. These axes work in tight collaboration with existing established platform at the CRCL/CLB sites.

As, an example of LICL activities we present here a translational study exploring biomarkers that could determine the efficacy of anti-PD-1 (Nivolumab) therapy in patients with relapsed metastatic melanoma. Here our preliminary analysis in the blood profiling by PI3 revealed that patients that respond to anti PD-1 increased their T cell polyfunctionality as measured by intracytoplasmic cytokine detection by multiparametric flow-cytometry. Furthermore, multiparametric spectral IF analysis by MIFIS of the tumor immune infiltrate showed that responding patients displayed an increased number of tertiary lymphoid structures (TLS) and associated B cells. Further molecular and cellular characterization of the TLS and blood phenotyping is currently on going.

P **03** - 003

TARGETING CHI3L1 BY A SMALL MOLECULE MODULATES IMMUNE CELLS

Katarzyna Drzewicka[1], Katarzyna Krysztofiak[1], Aleksandra Rymaszewska[1], Katarzyna Piwowar[1], Wojciech Czestkowski[1], Agnieszka Bartoszewicz[1], Adam Golebiowski[1], Zbigniew Zaslona[1]

[1]Molecure SA, Warsaw, Poland

Keywords: Chi3l1, immunomodulation, macrophages, small molecules, cancer

Chitinase 3-like 1 (Chi3l1) is a 40 kDa chitinase-like protein (CLP) linked to prognosis, progression, and severity of numerous types of cancer. The protein is produced and secreted by immune cells (especially macrophages) and various structural cells within the cancer microenvironment. Chi3l1 has been demonstrated to bear anti-inflammatory effects on immune cells as well as a direct pro-oncogenic effect by inducing proliferation and survival of cancer cells. Therefore, there has been a lot of interest in targeting Chi3l1 as a therapy in the immune-oncology field. In this study, we have developed first in class small molecule binders of Chi3l1. We showed that targeting Chi3l1 in macrophages could modulate inflammatory processes, suggesting the potential of our binders in fighting cancer. Further studies are conducted to validate our findings.

P **03** - 004

EVALUATION OF THE THERAPEUTIC POTENTIAL OF TCR ENGINEERED T CELLS TARGETING HUMAN ENDOGENOUS RETROVIRUSES IN TRIPLE NEGATIVE BREAST CANCER

Célia Gardet[1], Marion Mallet[5], Julie Grandsire[3,5], Paola Bonaventura[3], Vincent Alcazer[6], Virginie Mutez[3], Yann Estornes[3], Nicolas Chuvin[3], Stéphane Depil[1,2,3,4]

[1]Centre de Recherche en Cancérologie de Lyon, Lyon, France; [2]Centre Léon Bérard, Lyon, France; [3]ErVaccine Technologies, Lyon, France; [4]Université Claude Bernard Lyon 1, Lyon, France; [5]AgroParisTech, Paris, France; [6]Hospices Civils de Lyon, Lyon, France

Keywords: TCR, HERV, immunotherapy, breast cancer, cell therapy

Background. About 8% of the human genome is composed of Human Endogenous Retroviruses (HERVs). These sequences correspond to remnants of ancient infections by exogenous retroviruses. In normal cells, HERVs are silenced by epigenetic mechanisms while they can be aberrantly expressed in some tumors, including breast tumors, and may thus represent a source of tumor antigens. In a previous study, we identified HERV-derived peptides as a new class of tumor epitopes that are shared between patients in Triple Negative Breast Cancer (TNBC). We showed that these HERV epitopes induce a strong CD8+ T cell response ex vivo and we demonstrated the presence of HERV-specific tumor infiltrating lymphocytes in TNBC patient samples. HERV-specific T cells from healthy donors are highly cytotoxic against MDA-MB231

HLA-A2+ TNBC cells but spare normal primary breast epithelial cells. These results paved the way for the development of T cells engineered with a T cell receptor (TCR-T cells) targeting HERV-derived epitopes.
Methods. The TCR sequences of HERV-specific T cells were partially sequenced and both alpha and beta chains were reconstructed for each T cell clones using the International Immunogenetics Information System (IMGT) online database. The TCR constant regions of each of these TCRs were replaced by the murine constant region containing an additional bi-sulfide bond to improve transgenic TCR expression and pairing when expressed into human primary T cells using lentiviruses.
Results. We successfully generated HERV-specific TCR-T cells and validated their ability to secrete effector cytokines in response to antigenic stimulation. They also target T2 cells artificially presenting the epitope on HLA-A2 molecules. Most importantly, HERV TCR-T cells specifically recognize and eliminate TNBC tumor cells MDA-MB231 in vitro.
Conclusion. Altogether, these results support the therapeutic potential of HERV-specific TCR-T cells against TNBC.

P **03** - 005

TUMOR CELLS INFECTED BY AN ONCOLYTIC VIRUS ACTIVATE GAMMA-DELTA T CELLS

Morgane Fouet[1,2], Fabienne Konczak[1,2], Chirine Rafia[1,2], Frédéric Tangy[3], Emmanuel Scotet[1,2], Nicolas Boisgerault[1,2]

[1]Nantes Université, Inserm UMR 1307, CNRS UMR 6075, Université d'Angers, CRCI2NA, F-44000 Nantes – France, Nantes, France; [2]Labex IGO, Immunology Graft Oncology, Nantes, France , Nantes, France; [3]Institut Pasteur, Université de Paris, Laboratory of Innovative Vaccines, 75015, Paris, France., Paris, France

Keywords: oncolytic virus, cancer, gamma-delta T cell

Oncolytic virotherapy is an emergent approach in oncology that relies on viruses able to specifically replicate in and kill tumor cells. The immune cell death induced by oncolytic virus infection allows the recruitment and the activation of immune cells at the tumor site but many immune mechanisms still need to be characterized. Gamma-delta T cells (GDT) are a subset of non-conventional T cells with innate and adaptive immune properties. They have important functions against virus-infected and tumor cell, which make them attractive in the context of oncolytic virotherapy. Recent results from our team show an activation of a fraction of human gamma-delta2 T cell after coculture with human tumor cells infected with an oncolytic strain of measles virus (MV). This activation seems specific to MV as MDA-MB-231 breast cancer cells infected with other oncolytic viruses such as vesicular stomatitis virus, vaccinia virus or myxoma virus did not result in GDT activation. Furthermore, this activation is contact-dependent as it is prevented when using transwell inserts for coculturing GDT with infected MDA-MB-231 cells. To verify whether GDT activation involves TCR binding, we inhibited butyrophilin 3A (Gamma9-delta2 TCR ligand) in MDA-MB-231 cells with a shRNA or a blocking antibody. This did not block oncolytic MV-induced GDT activation. Similarly, we investigated the expression of NKG2D ligands in tumor cells infected or not with MV and no difference was observed. Altogether, our results show an activation of a fraction of GDT in presence of tumor cells infected with oncolytic MV. This activation seems specific to MV, contact-dependent and not involving the TCR/butyrophilin and NKG2D axes. Further characterizations are ongoing to identify the cellular pathways involved in this activation.

P **03** - 006

PERSONALIZED CANCER VACCINE TG4050 INDUCES CD8 AND CD4 ANTI-TUMOR RESPONSES

Ana Ines Lalanne[1], Camille Jamet[1], Jean-Pierre Delord[2], Christophe Le Tourneau[1], Gisele Lacoste[3], Annette Tavernaro[3], Maud Brandely[3], Nathalie Silvestre[3], Benoit Grellier[3], Maude Delost[1], Eliane Piaggio[1], Eric Quemeneur[3], Kaïdre Bendjama[3], Olivier Lantz[1]

[1]Institut Curie, Paris, France; [2]IUCT Oncopole, Toulouse, France; [3]Transgene SA, Illkirch-Graffenstaden, France

Keywords: neo-antigen, cancer, vaccine, neo-epitope

Background:
Cancer mutations acquired during carcinogenesis are not subjected to central tolerance, opening the possibility to use the adaptive immune responses against the tumors. Therapeutic cancer vaccines aim to generate new T cell responses against tumor antigens or to amplify naturally occurring ones.
Methods:
Mutations were identified by DNA and RNA sequencing of the tumors. Putative epitopes were selected on their likelihood to elicit a class I or II response based on HLA binding, allelic frequency, prediction of processing and RNA expression level. The vaccine was a modified Vaccinia Ankara (MVA) strain encoding for up to 30 sequences of 29 amino acids centred on the predicted epitope. The viral vaccine was produced under GMP conditions and administered to patients after curative intent treatment in two phase I trials either in high risk of recurrence patients (HNSCC) or during biochemical relapse (CA-125 in ovarian cancers).
Immunomonitoring in peripheral blood mononucleated cells (PBMC) consisted in deep phenotypical analysis by flow cytometry, ex vivo IFNγ ELISPOT using vaccine peptides and tetramer staining.
Results:
After 6 doses of vaccine, a decline in naïve CD4 and CD8 subsets and the onset of effector CD27- CD4 and CD8 T cells was observed in all treated patients as compared with pre-vaccine samples. While the other cell populations were stable, CD16 expression decreased in the CD56dim NK cell subset, suggesting a stimulation of antitumor innate immunity.
We measured immunoreactive T cells in PBMCs against each of the targeted epitopes using ex vivo IFNγELISPOT. Robust responses were observed in all evaluable patients. Positive responses to vaccination were observed for a total of 46 epitopes over 115 tested with a median of 11 epitopes (6 to 18) per patient. Responses were directed against both class I and II epitopes and were either de novo responses (64%) or amplification of pre-existing responses (36%).
In one ovarian cancer patient, these immune changes correlated with a normalization of CA-125 that lasted 7 months
For one HNSSC patient, tumor specific cells by flow cytometry using pMHC tetramers were analyzed. A population of tumor antigen specific CD8 T cells appeared after vaccination while it was absent at baseline. These cells did not express PD-1, CD39 or CD27, indicating a non-exhausted effector phenotype.
Conclusion: These data show that the personalized vaccine TG4050 is immunogenic.

P **03** - 007

IL-10 REPROGRAMS CAR T CELLS FATE TO IMPROVE THEIR CYTOTOXIC RESPONSES

Manuel Van Gijsel Bonnello[1], Adeline Cozzani[2], Silvia Gaggero[2], Claire Gorby[1], Jonathan Martinez-Fabregas[1], Tiziano Ingenere[2], Suman Mitra[2], Ignacio Moraga[1]

[1]University of Dundee, Dundee, United Kingdom; [2]Institut pour la Recherche sur le Cancer - CANTHER, Lille, France

Keywords: IL-10, CAR T Cells, Metabolism, Immunotherapy

IL-10 is a powerful regulator of the inflammatory response. In addition to its anti-inflammatory activities, IL-10 increases the cytotoxic function of CD8 T cells, augmenting their ability to target tumours. These important immunomodulatory properties make IL-10 a central molecule to control inflammation and anti-cancer responses. However, a dearth of knowledge regarding how IL-10 achieves its functional diversity has hindered our ability to translate its properties into the clinic. IL-10 therapies yielded poor efficacy and produced toxicity.
IL-10 dimerizes IL-10Rα and IL-10Rß subunits to trigger activation of the JAK/STAT3 signalling pathway. Here we hypothesize that IL-10's poor clinical activities result from its weak binding affinity for the IL-10Rβ subunit. We have used yeast surface display to generate a new IL-10 variant that binds IL-10Rb with 1000-fold higher affinity than IL-10 wild-type. Our engineered IL-10 variant exhibited enhanced receptor heterodimerization in live cells and triggered more potent STAT1 and STAT3 activation than IL-10 wild-type. In agreement with its enhanced

signalling profile, our engineered IL-10 variant induced more robust gene expression programs in CD8 T cells than IL-10 wild-type at all doses tested. We showed that CAR-modified T cells cultured with our engineered IL-10 variant displayed more robust cytolytic activities in vitro against a target cell line. Interestingly, CAR-T cells cultured in the presence of IL-10 variants exhibited a “stemlike memory T cells with cytotoxic properties, suggesting IL-10 can reprogram CAR-T cells into a hybrid “state” consist of both the “memory” as well as “effector” T cell functions. Mechanistically, IL-10 downregulated key rate limiting glycolytic enzymes while upregulating large subsets of mitochondrial proteins involved in the electron transport chain at the protein level, which may contribute towards enhanced cytotoxicity.
Overall, this newly engineered IL-10 variant represents a promising alternative to revitalize failed IL-10 therapies for treating cancer including generation of superior effector memory T cells capable of mediating durable remission.

P **03** - 008

GLOBAL ANALYSIS OF INTERCELLULAR COMMUNICATION IN CLEAR CELL RENAL CELL CARCINOMA TUMOR MICROENVIRONMENT

Lucile Massenet-Regad[1,2], Justine Poirot[1,2], Caroline Hoffmann[3,4], Elise Amblard[1], Fanny Onodi[1], Malika Djouadou[5], Jérôme Vérine[1,6,7], Idir Ouzaid[8], Evanguelos Xylinas[8], Jasna Medvedovic[1], Vassili Soumelis[1,9]

[1]Université de Paris, INSERM U976, F-75006 , Paris, France; [2]Université Paris-Saclay, F-91190, Saint Aubin, France; [3]INSERM U932, Cancer et Immunité, 75005, Paris, France; [4]Institut Curie, Département de Chirurgie oncologie, 75005, Paris, France; [5]AP-HP, Hôpital Saint-Louis, Département d’Urologie, 75010, Paris, France; [6]CEA/DRF/ Institut François Jacob, IRSL, Hôpital Saint-Louis, Paris, France; [7]AP-HP, Hôpital Saint-Louis, Département de Pathologie, 75010, Paris, France; [8]AP-HP, Hôpital Bichat-Claude Bernard, Département d’Urologie, 75019, Paris, France; [9]AP-HP, Hôpital Saint-Louis, Département d’Immunologie-Histocompatibilité, 75010, Paris, France

Keywords: Cell communication, Renal carcinoma, Immunotherapy

Cells are dynamic systems that integrate multiple signals. They communicate through a wide variety of molecules to adapt and respond specifically to their environment. In the tumor microenvironment (TME), tumor cells, infiltrating immune cells and stromal cells express communication molecules in order to interact. Depending on the molecules, the emitting cells and the receiving cells, cellular crosstalk can participate in immunosuppression or anti-tumor response.
In this study, we used single-cell RNA sequencing (scRNAseq) to characterize communication networks in the context of human clear cell renal cell carcinoma (ccRCC). We generated a scRNAseq dataset from enriched cell types of ccRCC human tumors and juxtatumor control samples to capture and profile rare cells in the TME (n= 27 963 cells, from 3 patients).
The analysis of intercellular communication using ICELLNET identified about 30 interactions specifically used in silicoby ccRCC tumor cells to communicate with the other cell types, and significantly upregulated at transcriptomic level in the TME (both ligand and receptor): Some of them are currently targeted by daily practice therapies (VEGF and its receptors), or have been already tested in ccRCC (interactions involving EGFR) or are investigated in current clinical trials (such as CD70/CD27). Among the top relevant interactions, we found EDN1/EDNRB as putative novel tumoral/stromal cell interaction, and ANG/PLXNB2 as putative new autocrine loop in ccRCC tumor cells. Both interactions might represent promising new therapeutic targets and have, to our knowledge, never been investigated in ccRCC.
The newly identified interactions are being experimentally validated by immunofluorescence or flow cytometry on human ccRCC samples from independent donors. Our findings are an important step toward our understanding of kidney cancer biology. Large-scale systemic analysis of cell-cell communications may provide new biological insights into other tumor types and may shed light on new ligand/receptor interactions as potential targets for immunotherapies.

P **03** - 009

INHIBITION OF GASDERMIN-D MEDIATED IL-1ß RELEASE USING NECROSULFONAMIDE FOR THE TREATMENT OF BREAST CARCINOMA

Bart Boersma[1,2], Karin Möller[3], Lisa Haddick[3], Viola Puddinu[1,2], Sébastien Fauteux-Daniel[4,5], Carole Bourquin[1,2,6], Thomas Bein[3], Gaby Palmer-Lourenco[4,5]

[1]Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, Genève, Switzerland; [2]School of Pharmaceutical Sciences, University of Geneva, Genève, Switzerland; [3]Department of Chemistry and Center for NanoScience, University of Munich, Munich, Germany; [4]Division of Rheumatology, Department of Medicine, Faculty of Medicine, University of Geneva, Genève, Switzerland; [5]Department of Pathology-Immunology, Faculty of Medicine, University of Geneva, Genève, Switzerland; [6]Department of Anesthesiology, Pharmacology and Intensive Care, Faculty of Medicine, University of Geneva, Genève, Switzerland

Keywords: gasdermins, cancer, cytokines, nanoparticles, IL-1B

The gasdermins (GSDMs) are a family of pore-forming proteins. They are activated by proteolytic cleavage, which liberates their N-terminal domain to assemble into 10–14 nm pore-forming structures in the cell membrane. Cleavage of gasdermin-D (GSDMD) causes pyroptosis and allows the release of pro-inflammatory mediators, like IL-1b and IL-18, HMGB1, and ATP. Previous studies have shown that the growth of orthotropically implanted EO771 cells, a murine breast carcinoma model is IL-1β dependent and therefore relies on pyroptosis. The aim of this study is to investigate the effect of blocking GSDMD-mediated IL-1β secretion using a necrosulfonamide (NSA) loaded nanoparticles. NSA prevents GSDMD pore formation by directly binding to the p30 GSDMD fragment, thereby inhibiting IL-1β release. Nanoparticles were used to facilitate the delivery of the extremely hydrophobic NSA. Cyclodextrin, mesoporous silica and magnesium-phosphate citrate nanoparticles, either bare or coated with a lipid bilayer, were loaded with NSA. To test the efficacy of NSA delivery, mouse bone marrow-derived macrophages were stimulated with lipopolysaccharide and exposed to NSA-loaded nanoparticles before GSDMD cleavage and IL-1β release was induced by the addition of ATP. All types of NSA-loaded particles were able significantly prevent IL-1β secretion. Similar results were obtained in the human monocytic THP-1 cell line. Additionally, fresh splenocytes were cultured with fluorescently labeled nanoparticles. FACS showed that cyclodextrin (CD) particles, without a lipid layer, were delivered most effectively in vitro to myeloid cells in a pool of splenocytes. CD particles also appeared to be non-toxic to myeloid cells after 24hours. cells. Myeloid cells are known to be the main producers of IL-1β in the tumor microenvironment. In future experiments, we will establish if orthotopic growth of EO771 tumors is dependent on GSDMD in tumor-infiltrating cells by using GSDMD-KO mice. Then, NSA-loaded nanoparticles will be administered to determine if EO771 tumor growth can be inhibited with NSA. Overall, we propose that GSDMs may represent a novel target to improve the treatment of cancers, which are dependent on pro-inflammatory cytokines for their growth.

P **03** - 010

CHARACTERIZATION OF THE COMBINED EFFECTS OF EXERCISE AND IMMUNO CHEMOTHERAPY TREATMENTS ON TUMOUR GROWTH IN MC38 COLORECTAL CANCER MICE

Manon Gouez[1,2,3], Etienne Gouraud[2], Charles Dumontet[4], Cédric Chaveroux[4], Mathis Damon[2], Amandine Thomas[2], Béatrice Fervers[1,3], Vincent Pialoux[2]

[1]Department of Cancer and Environment, Centre Léon Bérard, Lyon, France; [2]Inter-university Laboratory of Human Movement Sciences (LIBM) EA7424, University of Lyon, Lyon, France; [3]Cancer Research Center of Lyon, INSERM UA1296, Centre Léon Bérard, Lyon, France; [4]INSERM 1052, CNRS 5286, University of Lyon, Lyon, France

Keywords: cancer, immunotherapy, chemotherapy, physical exercise, perfusion

Context : It is well established that physical exercise improves global health and is an important component of therapeutic strategies in oncology. Indeed, several studies have shown a higher survival in patients practicing exercise during cancer treatment. Interestingly, it has been shown recently that a single physical exercise bout transiently increases tumor perfusion in mouse models and can induces changes

in immune cell infiltration in the tumor microenvironment. Both parameters are known to modify tumor development and therapy responsiveness, and this highlights the beneficial effect of an acute exercise associated to the treatment. Among therapies, immuno-chemotherapy (ICI) has demonstrated a high potential in the treatment of aggressive cancers by its direct action on tumor-associated immune cells. Until now, the potential of acute exercise during ICI remains to be tested. The purpose of this pre-clinical study is to evaluate if an acute exercise conducted immediately before an ICI can improve its efficacy and consequently to increase tumor size regression in an orthotopic mouse model of colon cancer. Moreover, to evaluate underlying mechanisms we assessed whether this acute exercise pre-treatment 1) increase tumoral perfusion and 2)modify the phenotype of immune cell infiltrate.METHODS: 6 week old mice injected with colorectal cancer cells (MC38) performed incremental maximal aerobic speed (MAS) test to be randomized in 4 groups (20/grp): control(CTRL), immune-chemotherapy(TRT), exercise(EXE) and combined intervention(TRT-EXE). Both TRT and TRT-EXE received ICI (anti-PD1-1 + capecitabine + oxaliplatine) 5 times a week for 1 week. TRT-EXE and EXE groups were submitted to 50' of a treadmill exercise at 60% of MAS before each treatment administration (<15'). Along the protocol, tumor size has been monitored daily. After 1 week, tumor perfusion was assessed after exercise or at rest as well as tumor weight and immune microenvironment by flow cytometry. RESULTS: Preliminary results have shown a decrease in tumor size in the TRT-EXE group compared to the other groups. This stronger tumor size reduction for the TRT-EXE mice is associated with lower percentages of PD-1 expression on T cells and Treg on CD4+ cells measured by flow cytometry. We expect that exercise should increase intratumoral perfusion and could explain the increased efficacy of the treatment in the TRT-EXE group compared to the TRT group. Full results will be available in April 2022.

P **03** - 011

CHARACTERIZATION OF THE IMMUNE/INFLAMMATORY MICROENVIRONMENT OF WILMS TUMOR IN CHILDREN

Mathilde Penel-page[1,2], Alexia Gazeu[2,3], Benoit Dumont[1,2], Yamila Rocca[2], Valentin Picant[2], Aurélien Voissiere[2], Isabelle Rochet[3], Laurie Tonon[4], Alain Viari[4], Pierre Saitigny[5], Valery Attignon[5], Justine Berthet[2], Christophe Bergeron[1], Christophe Caux[2], Frédérique Dijoud[3], Nathalie Bendriss-Vermare[2]

[1]Pediatric Hematology and Oncology Institute IHOPe, Léon Bérard Cancer Center, Lyon, France; [2]UMR Inserm 1052 CNRS 5286, Cancer Research Center of Lyon, Lyon, France; [3]Department of Pathology-Groupement Hospitalier Est, Hospices Civils de Lyon, Lyon, France; [4]Synergie Lyon Cancer – Bio-Informatics Platform, Léon Bérard Cancer Center, Lyon, France; [5]Department of Translational Research and Innovation, Léon Bérard Cancer Center, Lyon, France

Keywords: Wilms tumor, immune microenvironment, intratumor heterogeneity

Background: Wilms tumor (WT), composed of 3 histological subsets (blastema, stroma, epithelium), is a good model to study intratumoral heterogeneity. WT is known to be infiltrated by immune cells, but only a few studies have addressed the impact of tissue heterogeneity on immune infiltrate. Here we performed a comprehensive analysis of the immune microenvironment of WT in each histological component.
Methods: 56 FFPE samples from 31 WT patients, treated in SIOP2001 protocol in our comprehensive cancer center were retrospectively collected. Manual microdissection of each histological subset was performed. We analyzed the expression of 2,560 transcripts involved in tumorigenesis or immune responses by targeting sequencing (HTG EdgeSeq®, "Oncology Biomarkers" panel). We also performed muticolor immunofluorescence staining to identify immune cell populations and their activation states in situ. Fresh tumors and peritumoral renal tissue were prospectively collected for phenotypic analysis of tumor-infiltrating immune cells by multicolor flow cytometry and for quantification of cytokines and chemokines by MSD multiplex technology in tumour-derived supernatants.
Results: Overexpression of MYCN-activated genes and downregulation of IL6/JAK/STAT and IFN-γ pathways, along with a trend to lower inflammatory and immune response signatures were observed in the blastema. We identified in situ 3 immunological profiles characterized respectively by: i) a strong innate and adaptive immune infiltrate in peri-tumoral areas, including tertiary lymphoid structures (TLS) and PDL1 expression on macrophages, that are evocative of inflamed tumors; ii) moderate infiltrate mainly dominated by macrophages in the tumor area; iii) very low immune infiltrate that is reminiscent of non-inflamed tumors. As expected, macrophages were well represented in all tumor samples. We also identified strong infiltrate of T cells, B cells, NK cells, neutrophils, and dendritic cells. Distinct cytokine and chemokine profiles were observed between the normal and the tumor kidney.
Conclusion: We identified a rich immune infiltrate composed of both innate and adaptive immune cells, sometimes organized in TLS, and the expression of PDL1 and inflammatory cytokines/chemokines in WT microenvironment. Blastemal areas are associated with an immunosuppressive microenvironment. Further analysis to correlate immunological parameters with clinical data will determine which WT patients might benefit from immunotherapy.

P **03** - 012

CHARACTERIZATION OF TUMOR RESISTANCE FACTORS TO THE ONCOLYTIC MEASLES VIRUS IN A 3D CULTURE MODEL

Marion Grard[1]

[1]CRCI²NA U1307 Team 1, Nantes, France

Keywords: Oncolytic virus, spheroid, Measles, RNA senquencing, Mesothelioma

Our team is studying the use of oncolytic viruses that will specifically infect tumor cells and induce immunogenic cell death thus stimulating the anti-tumor immune response in malignant pleural mesothelioma (MPM). In particular, we are studying the mechanisms of tumor cell sensitivity and resistance to attenuated measles virus (MV) and their effects on the anti-tumor immune response.
The team has developed a model to study different MPM lines as spheroids in order to elucidate the mechanisms of replication and propagation of OVs in tumors. We have demonstrated the establishment of a 3D resistance to MV in the classical 2D culture model in a majority of MPM lines (7/8). This resistance is not overcome by an inhibitor of IFN I signaling (Ruxolitinib), the main cellular antiviral defense.
In order to identify the nature of the restriction factors to MV in 3D, we performed a 2D versus 3D transcriptomic analysis. The results of the analysis revealed an absence of significantly differentially expressed genes upon MV infection of spheroids for resistant lines, suggesting that viral restriction factors are not activated by infection but are present basally. Meta-analysis of the main signaling pathways commonly found to be deregulated in 3D for resistant lines compared to sensitive lines revealed fibrosis of the extracellular matrix associated with an inflammatory environment unfavorable to MV replication in MPM spheroids with a notable reduction in the cell cycle.
This study should lead to a better understanding of the oncolytic activity of MV against MPM in order to improve it.

P **03** - 013

CANCER CELLS SECRETED TGF-B INCREASES CD8 T CELLS TCR ACTIVATION THRESHOLD ALLOWING IMMUNE ESCAPE

Ossama Labiad[1], Elodie Guillemot[1], Alexandra Laine[1], Hélène Tarayre[1], Apostol Apostolov[1], Julien Marie[1]

[1]Department of immunology virology and inflammation CRCL, Inserm 1052, UMR CNRS 5286, Centre Léon Bernard Lyon-1, Lyon, France

Keywords: TCR, TGF-b1, Cancer

The low immunogenicity of established solid tumors can be explained by the weak ability of CD8 T cells to efficiently monte a response to cancer cell antigens. This impairment of CD8 T cell functions is thought to be conditioned for a large part by the tumor micro-environment (TME). One particularity of the TME of solid tumors is to be highly enriched in Transforming Growth Factor Beta (TGF-ß1) secreted mainly by the cancer cells. However, whether TGF-ß present in the TME affects the ability of CD8 T cells to react to cancer cell antigens remains unknown. Here, using gain and loss of TGF-ß signaling selectively in CD8 T cells

as well as cancer cells bearing antigens with different affinity for a given CD8 TCR, we reveal that TGF- ß signaling in CD8 T cells controls their TCR activation threshold. TGF-ß signaling promotes the LCK conformation in its inactive form by stabilizing CSK. Subsequently, the strength of the signal given to the TCR to activate CD8 t cells cytotoxic function in presence of TGF-ß needs to be stronger. CD8 T cells escaping TGF-ß signaling control efficiently eliminate cancer cells even thus bearing weak affinity antigen for their TCR, whereas CD8 T cells receiving TGF-ß signaling fail to respond to weak antigens expressed cancer cells, leading to a selective advantage of these cells. Importantly, we identified cancer cells as the main source of TGF-ß1 controlling TCR activation threshold in the TME. Thus, this study reveals that secreted TGF- ß1 by cancer cells increase TCR activation threshold of CD8 T cells present in the TME, and allow the immune escape of the cancer cells bearing antigens with weak affinity.

P **03** - 014

HIGHLIGHTING IMMUNE CHECKPOINT HETEROGENEITY IN HEAD AND NECK SQUAMOUS CELL CARCINOMA USING SINGLE-CELL RNA SEQUENCING

Yannick Le Meitour[1], Lucas Michon[1], Pierre Saintigny[1], Karène Mahtouk[1]

[1]Univ Lyon, Claude Bernard Lyon 1 University, INSERM 1052, CNRS 5286, Centre Léon Bérard, Cancer Research Center of Lyon, Lyon, France. , Lyon, France

Keywords: Immune checkpoint, Head and neck, Single cell RNAseq

Head and neck squamous cell carcinoma (HNSCC) is a frequent cancer associated with a poor prognosis. Although immunotherapy with PD-1/PD-L1 inhibitors offers new therapeutic options, it remains inefficient in most patients, paving the way for combinatorial blockade of alternative immune checkpoint (ICP) molecules. An in-depth characterization of ICP expression could be an important step in the design of combined immunotherapies targeting non-redundant pathways. Single-cell RNA sequencing (scRNA-seq) has emerged as a powerful approach to dissect human tumors at the individual cell resolution, allowing the analysis of diverse cell types and tumor heterogeneity. Since no comprehensive characterization of ICP expression has been established so far in HNSCC, we addressed the expression of 13 ICP molecules and 22 ligands on 1,237 T cells from 14 patients and 2,176 malignant cells from 10 patients, respectively, using publicly available scRNA-seq data. 15/22 ICP ligands (HMGB1, LGALS9, CEACAM1, LGALS3, CD276, IDO1, PVR, PVRL2, TNFRSF14, CD274, CD86, CD40, TNFSF9, CD70, TNFSF18) were expressed in tumor cells while 13/13 ICP molecules were expressed in T cells. Moreover, UMAP generation based on the expression of 15 ICP genes, followed by cluster analysis, partitioned tumor cells into three main clusters. Cluster 2 comprised 737 cells from four patients that overexpressed ICP genes compared to clusters 1 and 3. Interestingly, patients' cells from cluster 2 were characterized by a distinctive transcriptional profile related to response to interferon gamma, antigen processing and immune cell migration, while metabolism-related pathways were recurrent themes in clusters 1-3. Thus, 4/10 patients having tumor cells with an immune phenotype and strong ICP expression were identified, highlighting significant inter-patient heterogeneity. A more detailed analysis of these four patients revealed that they expressed both "shared ICP molecules" common to all patients and "restricted ICP molecules" unique to each patient. Finally, we addressed intra-tumor heterogeneity. Although some ICP were heterogeneously expressed, cluster analysis failed to identify any sub-cluster associated with a particular immunomodulatory status within tumor cells of each patient. In conclusion, this study, which offers a global picture of ICP gene expression in single tumor cells from HNSCC, could contribute to the broader issue of designing new biologically-based combined immunotherapies.

P **03** - 015

ANTITUMOR EFFECT OF GTN +/- DOXORUBICIN IN TRIPLE NEGATIVE BREAST CANCER: INVOLVEMENT OF THE IMMUNE SYSTEM

Nesrine Mabrouk[1], Cindy Racoeur[1], Jingxuan Shan[2], François Ghiringhelli[3], Lotfi Chouchane[2], Ali Bettaieb[1], Catherine Paul[1]

[1]EPHE-Université de Bourgogne, Dijon, France; [2]Weill Cornell Medicine-Qatar, Doha, Qatar; [3]CGFL, Dijon, France

Keywords: breast cancer, immunotherapy, NO donor, chemotherapy, G-MDSC

Breast cancer (BC) is a highly complex, heterogeneous and multifactorial disease. Compared to other subtypes, triple-negative (TNBC) is characterized by a strong inter- and intra-tumor heterogeneity, making treatments very difficult. The absence of estrogen and progesterone receptor expression, and the absence of HER2 receptor overexpression, make chemotherapy (eg. doxorubicin) the prior treatment. Unfortunately, the complete anatomopathological response rate, in response to these chemotherapies, rarely exceeds 40%. This project aimed to (1) determine whether the association of glyceryl trinitrate (GTN), a nitric oxide (NO) donor, with doxorubicin can improve the therapeutic efficacy of this chemotherapy in TNBC and to (2) understand the mechanism by which this combination acts by emphasizing the immune microenvironment.

We showed that the association of GTN with doxorubicin significantly improved the anti-tumor efficacy of this chemotherapy in a TNBC model. This effect is due, in part, to the ability of GTN to increase the polarization of CD4+ T lymphocytes (TL) to the anti-tumor subtype, Th1, and the intra-tumor recruitment of TCD8+ / PD-1+ cells and G-MDSCs down-expressing PD-L1. Nevertheless, the main mechanism by which GTN seems to acts is essentially based on its ability to reprogram these G-MDSCs towards a less immunosuppressive phenotype.

P **03** - 016

IDENTIFICATION AND CHARACTERISATION OF THE IMMUNE RESPONSE TO SALMONELLA-MEDIATED CANCER THERAPY AND ITS USE IN DEVELOPING COMBINATION CANCER THERAPY

Severina Peneva[1], Jason Williams[1], Adam Higgins[2], Paul Dyson[1], Lee Parry[2], Nick Jones[1]

[1]Institute of Life Science1, Swansea University, Swansea, United Kingdom; [2]European Cancer Stem Cell Research Institute, School of Biosciences, Cardiff University, Cardiff, United Kingdom

Keywords: cancer, Salmonella, immunity, PD-L1, c-MYC

As of 2018, colon cancer is the fourth most common cancer worldwide whereas cancer of the rectum is ranked eighth overall. Together, colorectal cancers (CRC) comprise 11% of all cancer cases and are the third most commonly diagnosed form of cancer in the world. Bacterial cancer therapy is an emerging method for targeted tumour treatment, including CRC tumours. Despite the great success of oncogene silencing and improved subject survival rates achieved thus far, the wider implications bacterial therapy has on the immune response are yet to be established. Previous work conducted by our group has shown a successful Salmonella typhimurium SL7207-mediated delivery of RNA interference for the oncogene c-MYC to tumour cells, resulting in the increased longevity of mice. This project's aim is to address the key differences in the immune response resulting from this bacterial cancer therapy alone and in combination with PD-L1 checkpoint silencing. Studies on the effect of c-MYC knockdown on PD-L1 expression indicated that while c-MYC is involved in PD-L1 regulation, it cannot efficiently silence the PD-1 ligand in CRC cell lines. A Salmonella strain carrying PD-L1 shRNA has since been developed and shown an improved PD-L1 gene and protein silencing in vitro. Additionally, mouse in vivo analyses are currently being performed in order to characterise the immune cell populations found in the colon tumour microenvironment following Salmonella treatment; to study the systemic effects of the different treatment options; and to determine whether a shift towards a pro-inflammatory milieu is observed. Further studies will be conducted to establish the metabolic profile

of key cancer immunity players such as T cells and NK cells and the factors that drive their activation and proliferation. The outcomes will contribute towards our general understanding of the effects bacteria-based cancer therapy has on the immune response and will also aid the progression of developing a novel, more effective treatment option for CRC patients.

P **03** - 017

IMPLEMENTATION OF THE PATIENT-DERIVED EXPLANT (PDE) MODEL FOR PREDICTING THE EFFICACY OF IMMUNE CHECKPOINT INHIBITORS (ICI)

Naila Abid[1], Gareth Miles[1], Giuditta Viticchie[1], Tamihiro Kamata[1], Howard Pringle[1], Anne Thomas[1], Catrin Pritchard[1]

[1]Leicester Cancer Research Centre, University of Leicester, Leicester, United Kingdom; [2]MRC Toxicology Unit, University of Cambridge, Cambridge, United Kingdom

Keywords: ICI, PDE, multi-immunofluorescence, TME, Melanoma

Melanoma accounts for the highest number of deaths from skin cancer. Although primary tumours are treated by surgery, some patients develop metastasis and resistance to treatment. Ninety five percent of oncology agents fail to reach market authorisation, partly due to a lack of relevant pre-clinical models predictive of patient outcome. Accurate pre-clinical models with the potential to reduce drug attrition rates and better predict patient outcomes are urgently sought, particularly for ICIs. We have established a metastatic melanoma (MM) PDE pre-clinical model, which we have shown represents the “live” culture of MM tumour fragments and retains tumour architecture intact.

Tumours obtained from surgery were sectioned into explants (1-3mm3) and cultured for 16-24 h. The next day, PDEs were transferred into fresh media containing ICIs. On day 3, PDEs were fixed and formalin fixed paraffin embedded (FFPE) blocks generated. Proliferation marker (Ki67) and cell death (cPARP) in the tumour and stroma areas were assessed by tyramide signal amplification (TSA) based multi-immunofluorescence (mIF) coupled with digital pathology quantitation to assess cell viability in response to ICIs. Biomarkers of interest (CD4, CD8, FOXP3) were also used for mIF to monitor the density and location of key immune cells in response to the ICIs.

mIF analyses showed differential levels of CD4+FOXP3+ T regulatory cells (Tregs) and CD8+ Cytotoxic T Lymphocytes (CTLs) infiltration into stroma and tumour areas of the MM-PDEs. Approximately 50% of MM tumours are “hot” tumours i.e. high levels of infiltrating CTLs. We have also observed differential apoptotic responses to ICIs across the MM-PDEs, with ~30% of tumours responding to anti-PD1 inhibition (pembrolizumab/nivolumab) or anti-CTLA4 inhibition (ipilimumab). We are currently evaluating whether there is a correlation between CTL infiltration and apoptosis induction in response to the ICIs and if this is linked to clinical outcomes. These data will be presented at the conference.

PDEs contextually preserve the tumour microenvironment. We have been able to identify tumours that have differential levels of T cell infiltration and are differentially sensitive/resistant to anti-PD1/anti-CTLA4 therapies in ex vivo culture. Further analysis of these data with clinical comparisons, will allow us to understand the extent to which the MM-PDEs are predictive of patient outcomes in the preclinical testing of ICIs.

P **03** - 018

BIG-H3-STRUCTURED COLLAGEN ALTERS MACROPHAGE PHENOTYPE AND FUNCTION IN PANCREATIC CANCER

Sophie Bachy[1]

[1]STROMACARE, lyon, France

Keywords: PDAC, macrophage, collagen, bigh3, T cell

Macrophages play an important role in immune and matrix regulation during pancreatic adenocarcinoma (PDAC). Collagen deposition massively contributes to the physical and functional changes of the tissue during pathogenesis. We investigated the impact of thick collagen fibers on the phenotype and function of macrophages. We recently demonstrated that the extracellular protein bigh3/TGFbi (Transforming growth factor-b-induced protein) plays an important role in modulating the stiffness of the pancreatic stroma. By using atomic force microscopy, we show that big-h3 binds to type I collagen and establishes thicker fibers. Macrophages cultured on big-h3-structured collagen layers display a different morphology and a pro-tumoral M2 phenotype and function compared to those cultured on non-structured collagen layers. In vivo injection of those instructed CD206+CD163+ macrophages was able to suppress T cell responses.

These results reveal for the first time that the collagen structure impacts the phenotype and function of macrophages by potentiating their immunosuppressive features.

P **03** - 019

UNRAVELLING THE ROLE OF EARLY DISSEMINATION IN COLORECTAL CANCER

Tinhinan Lahlou[1], Zeinab Homayed[1], Guillaume Belthier[1], François Gerbe[1], Philippe Jay[1], Jean-François Bourgaux[2], Andrei Turtoi[3], Frederic Lagarrigue[4], Julie Pannequin[1]

[1]IGF, Univ. Montpellier, CNRS, INSERM, Montpellier, France; [2]Service d’Hépato-Gastroentérologie, CHU Carémeau, Nîmes, France; [3]Cancer Research Institute of Montpellier, INSERM U1194, Montpellier, France; [4]IPBS - CNRS UMR5089, Toulouse, France

Keywords: Early dissemination , Colorectal cancer, Premetstatic niche

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 make up for 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.These mice make it possible from the very first stages of tumorigenesis,via the expression of TdTomato,to follow the dissemination of these early cells eDTC in the liver.The impact of eDTCs in the liver was assessed using CyTOF/hyperion and immunolabelling where we can detect a strong enrichment of macrophages suggesting a microenvironmental remodelling.Moreover at the systemic level,a cytokine profile was observed,which would be incriminated in this massive enrichment of macrophages

Our primary aims are deciphering the mechanisms explaining the enrichment of macrophages in response to early dissemination. Secondly the validation of certain results on patients with intestinal polyps.

Identification of macrophage subpopulations was first performed by qPCR on the livers of APC WT /Mutated mice, further characterization will be performed by scRNA-seq.To identify genes and cytokines that are essential for macrophage subset enriched in the liver,co-culture assay of intestinal polyp cells purified from APC mutated mice with HoxB8-derived macrophages were carried out.The cytokine profile of patient plasmas was carried out using a cytokine array.

The qPCR results obtained on the livers of APC WT/Mutated mice suggest an overall polarization towards an M2-like immunosuppressive phenotype.HoxB8-derived macrophages cultured for 3 days in the presence of intestinal polyps adopt a state of polarization mimicking macrophages in the liver of mice with mutated APC.Cytokine array analysis on patient plasmas demonstrated that 3of the top candidate cytokines found in mice are overrepresented compared to healthy individual plasma.

This project demonstrated that early dissemination occurs in CRC,and drastic hepatic remodeling is induced including macrophage enrichement. In addition,macrophages tend to have an immunosupressive profils, which strongly suggests that this early dissemination has a causal role in the establishment of a premetastatic niche.

P **03** - 020

THE EBV LATENCY III TUMORAL B CELL MODEL ALLOW TO POINT FUCOIDANS AS A POTENTIAL ADJUVANT OF ANTI-PD-L1 IMMUNOTHERAPY

Jennifer Saliba[1], Chanez Manseur[2], Hugo Goult[2], Hussein Akil[1], Mona Tannoury[3], Danielle Troutaud[1], Jean Feuillard[1], Ingrid Arnaudin[2], Chantal Jayat-Vignoles[1]

[1]CRIBL Laboratory, Limoges University, UMR CNRS 7276 INSERM 1262, Limoges Cedex, France; [2]LIENSs Laboratory, La Rochelle University, UMR CNRS 7266, La Rochelle, France; [3]Lebanese University, Faculty of Sciences II, Al Fanar, Lebanon

Keywords: EBV latency III B cells, B lymphomas, fucoidans, PD-L1, immunotherapy

The Epstein Barr virus (EBV) infects more than 95% of the world's population and persists latently in the body. It has the ability to immortalize B cells and is associated with many lymphomas. We have shown that EBV latency III B cells inhibit the anti-tumor T cell response by mimicking regulatory B cells. They secrete immunosuppressive cytokines and overexpress the immune checkpoint PD-L1 (Programmed Death-Ligand 1) responsible for anergic regulatory T cell (T-regs) expansion and effector T cell inhibition, through the PD-L1/PD-1 interaction. Regarding immunotherapy challenges and limitations, we project the restoration of the anti-tumoral T cell response, by decreasing PD-L1 expression.

The work was carried out on three Lymphoblastoid Cell Lines (LCLs, EBV latency III B cells). We were interested by fucoidans, which are sulfated polysaccharides extracted from brown seaweed that gained attention in the last years because of their in vitro and in vivo anticancer activities, depending on their polymerization degree. Very few data have been reported on lymphoma cells or on immune checkpoints. Cells have been treated with native fucoidans of Fucus vesiculosus or with two original depolymerized fractions.

We observed a decrease in cell proliferation and an induction of apoptosis with the two depolymerized fractions. We also highlighted a PD-L1 decrease at both transcriptional and cell surface levels of viable cells. Surprisingly, intracellular expression remained high. We showed that depolymerized fractions altered actin network. Therefore, this is in agreement with our previous work, which emphasized that PD-L1 is stored in secretory lysosomes and need actin network to promote their fusion with cell membrane. Preliminary results on autologous co-cultures of EBV latency III B cells and activated T cells showed that depolymerized fractions of fucoidan increased tumoral B cells apoptosis and can potentiates a treatment with an anti-PD-L1 monoclonal antibody. We did not observe any toxic effect on peripheral blood mononuclear cells, on normal B cells, or on isolated T cells (activated or not).

Altogether, our results showed that fucoidans could contribute to restore anti-tumoral immune response, alone or as an adjuvant of anti-PD-L1 immunotherapy, due to its proapoptotic effects combined with its ability to decrease PD-L1 membrane expression.

P **03** - 021

IDENTIFICATION OF MULTIPLE IMMUNE ESCAPE MECHANISMS INVOLVED IN WALDENSTRÖM MACROGLOBULINEMIA EMERGENCE AND TRANSFORMATION INTO DIFFUSE LARGE B CELL LYMPHOMA

Quentin Lemasson[1], Jean Feuillard[1], Christelle Vincent-Fabert[1]

[1]UMR CNRS 7276 / INSERM 1262 CRIBL, Limoges, France

Keywords: B cell lymphomas, Waldenström Macroglobulinemia, Diffuse large b cell lymphoma, MYD88, Immmunomodulation

B cell lymphomas are subdivided into aggressive and indolent forms. Diffuse Large B Cell Lymphoma (DLBCL) is an aggressive lymphoma associated with a rapid evolution, while Waldenström's macroglobulinemia (WM) is an indolent LymphoPlasmacytic Lymphoma (LPL). To note, L265P mutation of MYD88 is commonly found in these two lymphomas (90% for WM, 40% for ABC-DLBCL).

In our team, we developed a mouse model that expresses the Myd88L252P mutation (the ortholog of the human L265P mutation). In most cases, mice developed an indolent lymphoma with some features of WM (Ouk et al. 2021). Interestingly, some aged LPL mice developed a more aggressive form resembling to ABC-DLBCL.

To understand the mechanisms involved in LPL development in our model, but also to explain its transformation into an aggressive form; we are focusing our work on Immune Escape Mechanisms (IEMs). A previously published review from our team carefully highlighted the implication of IEMs in lymphoma development through engineered mouse models (Lemasson et al. 2021).

In the present work, we are showing many IEMs and their consequences:

- Tumor B cells exhibit an immunosuppressive phenotype with high expression of PD-L1 associated to a decrease of MHC II surface expression.
- Concomitantly, Tumor infiltrated T cells (TILs) show an exhausted phenotype with an overexpression of PD-1, TIM3 and LAG3. T cells exhaustion is higher in DLBCL tumors compared to LPL.
- Regulatory T cells proportion increases in LPL lymphomas, and then decrease to a normal value in DLBCL tumors.
- Tumor Associated Macrophages (TAMs) population decreases drastically in LPL, due to the loss of CD80 and MHC II expression.

Taken together, these results show an evident implication of multiple IEMs in the development of lymphomas in our mouse model of WM. In addition, these mechanisms seem to be implicated in its transformation into an aggressive form (DLBCL).

Finally, we are also using our mouse model as a powerful pre-clinical model thanks to its ability to develop LPL and DLBCL. In consequence, we will test innovent targeted therapies for WM and DLBCL treatment (anti-TIM3, ruxolitinib, anti-PD-1, …) which are able to act not only on the tumor clone but also on its microenvironment.

P **03** - 022

UNVEILING THE ROLES OF CD8 T CELL SUBSETS IN ANTITUMOR IMMUNITY

Christina Metoikidou[1], Pierre-Emmanuel Bonte[1], Eleonora Timperi[1], Christel Goudot[1], Sylvain Baulande[1], Sonia Lameiras[1], Emanuela Romano[1], Sebastian Amigorena[1]

[1]Institut Curie, PARIS, France

Keywords: CD8, immunotherapy, cancer

Context: Immune checkpoint blockade (ICB) has emerged as a promising treatment option for many cancer types, however, resistance mechanisms, such as a suboptimal activation of T cells, limit the response to ICB. Hence, a comprehensive characterization of CD8+ tumor-infiltrating lymphocyte (TIL) populations is crucial. Recently in the team, we characterized in non-small -cell lung cancer (NSCLC) two distinct CD8+ subpopulations, expressing memory-like gene modules: one derived from blood (circulating precursors) and one from juxta-tumor tissue (tissue-resident precursors)1. In tumors, these precursors converge into terminally differentiated cells, often referred to as dysfunctional cells. We showed that differentiation is associated with TCR expansion, and transition from precursor to late-differentiated states correlates with intratumor T cell cycling.

Objective: Better understanding how these CD8+ TIL subsets are correlated with ICB responses in human cancer.

Methods: To address this question we are currently studying triple-negative breast cancer (TNBC) patients, included in the SYNERGY clinical trial (NCT03616886). Patients are stratified in two groups, based on the treatment they receive (chemotherapy + anti-PDL1/ ± anti-CD73). Tumor biopsies are collected at two timepoints: baseline and week-3 on-treatment. Total cell suspension (including immune, tumor and stromal cells) is analyzed by a combination of single-cell RNA and T cell receptor (TCR) sequencing, using the 10X 5' VDJ chromium technology.

Results: We observe distinct CD8+ T cell populations in TNBC. CD8+ T cells follow a continuum differentiation from memory-precursor cells (TCF7high) towards terminally differentiated cells, that express markers related to exhaustion, such as PD-1,TIM3 and TOX. Upon ICB treatment, we observe an overall expansion of TILs. Terminal differentiated CD8+ T cells found to be enriched in responders both before and upon treatment. In parallel, TCR analysis reveals higher

clonal expanison in responder compared to non-responder patients at baseline state.

Conclusions: Single cell transcriptome and immune profiling analysis unveils CD8+ T cell subsets, T cell markers, and distinct clonal expansion patterns associated with ICB response. Together these results identify immune targets of clinical interest for reactivation of tumor-infiltrating CD8+ T cells.

1. Gueguen*, Metoikidou* et al., 2021, Science Immunology. 6, no. 55.

P **03** - 023

NOVEL PERSONALIZED IMMUNOTHERAPY WITH CELL-ENCAPSULATION TECHNOLOGY FOR ADVANCED REFRACTORY HEAD AND NECK SQUAMOUS CELL CARCINOMA

Eugenio Fernandez[1], Remi Vernet[1], Emily Charrier[1], Olivier Von Rohr[1], Marie-Claude Belkouch[1], Muriel Urwyler[1], Valentin Saingier[1], Virginie Ancrenaz[1], Olivier Rubin[1], Nicole Granjean[1], Elisabeth Lafferma[1], Emmanuel Lavaliere[1], Jean Villard[1], Jessica Renaux[2], Julien Grogg[2], Nicolas Mach[1]

[1]HUG, Geneva, Switzerland; [2]MaxiVAX SA, Geneva, Switzerland

Keywords: Personalized, cell-encapsulation, Adjuvant, autologous, tumor cells

Despite current cancer therapies, the vast majority of patients with advanced disease die within 5 years. Ag specific immunization induces strong, specific response in many murine models but translation into clinical applications has failed over the last 2 decades. Learning from these failures and capitalizing on the critical need for potent adjuvants in addition to tumor specific Ag, we developed a unique personalized cell-based cancer immunotherapy addressing three critical features: large specific antigenic repertoire, very strong immunostimulatory signal and robust technology.

This strategy can be applied to any cancer types and in all setting (adjuvant, advanced 1st line, 2nd line)

Best Ags: Each tumor is distinct and no single tumor associated Ag has proven its efficacy as single target; therefore the antigenic repertoire needs to be diverse and specific. A good way to obtain a wide antigenic repertoire while maintaining specificity is by 'using' the patient's own cancer cells as source of Ags.

Best adjuvant: Protective immunity can be achieved when GM-CSF is applied locally, in small dose in a sustained manner. It triggers a very strong immune response, leading to protective immunity in all cancer tested in preclinical murine models and also infectious diseases such as HIV, Ebola, Hepatitis B & C, Malaria, TB. Excess GM-CSF and/or systemic activity leads to tolerance, stressing the critical delivery method for this cytokine.

Best delivery method: Allogeneic human cells, engineered to produce GM-CSF are loaded into a biocompatible macrocapsule, delivering GM-CSF locally at the vaccine site over 7 days The IMP is the combination of irradiated autologous tumor cells and 2 biocompatible macrocapsules releasing stable level of GM-CSF, implanted subcutaneously at the vaccination site, distant from any tumor deposit

We recently completed a Phase I first in human trial showing both feasibility and very good safety profile.

A multicenter phase II clinical trial is currently ongoing for patients with metastatic Head&Neck carcinoma progressing after at least one line of systemic therapy. 75% of these patients failed prior immune checkpoint therapy. No systemic adverse event has been reported so far and 70% of pts are alive at 6 months including complete response.

Ongoing efforts are focusing on immunomonitoring to better define the mechanism of actions looking at both T cell parameters and seromics. Clinical data and immune monitoring will be presented.

P **03** - 024

CHARACTERIZATION OF IMMUNE RESPONSE AGAINST TUMOR SPECIFIC AND TUMOR-ASSOCIATED ANTIGENS IN PATIENTS WITH LOCALIZED AND METASTATIC UVEAL MELANOMA

Francesca Lucibello[1], Manuel Rodrigues[2,3], Marion Salou[1], Francois Legoux[1], Pascale Mariani[4], Nathalie Cassoux[4,5], Sophie Gardrat[2,6], Marc-Henry Stern[2], Ana Lalanne[7], Olivier Lantz[1,7,8]

[1]U932 Institut Curie, Paris, France; [2]INSERM U830, DNA Repair and Uveal Melanoma (D.R.U.M.), Equipe labellisée par la Ligue Nationale Contre le Cancer, PSL University, Institut Curie, Paris, France; [3]Department of Medical Oncology, Institut Curie, Paris, France; [4]Department of Surgical Oncology, University of Paris, Institut Curie, Paris, France; [5]BIOLOGIE CELLULAIRE ET CANCER (UMR144), Paris, France; [6]Departments of Pathology and Translational Research, Institut Curie, Paris, France; [7]Laboratoire d'immunologie clinique, Institut Curie , Paris, France; [8]Centre d'investigation Clinique en Biothérapie, Institut Curie (CIC-BT1428), Paris, France

Keywords: neo-antigen, tumor-associated antigen , SF3B1, Uveal melanoma

Uveal melanoma (UM) is the most frequent ocular primary tumor in adults. Metastatic UM display a poor overall survival. The splicing factor SF3B1 is mutated in 22% of UM. Disruption of splicing patterns in tumors represents a potential source of shared and tumor-specific neoantigens (TSA). We previously showed that mutations of SF3B1 in UM generate immunogenic neoantigens. Herein, we analyzed the immune response locally and systemically at different stages of the disease.

We analyzed tumor infiltrating lymphocytes (TILs) in 20 primary UM tumors. In 9 tumors, we observed CD8+ T cells expressing CD39 and PD1 suggesting local antigen reactivity. Among them, 1 to 10% were specific for A2-Melan-A. These findings are compatible with a local immune response against a tumor associated antigen (TAA). Notably, the specific Melan-A CD8 T cells in 7 of these patients were still naïve (CCR7+CD45RA+) in the peripheral blood indicating an absence of systemic response.

Using ex vivo tetramer staining, we then characterized the circulating T cells specific for SF3B1-related neo-antigens. In patients with localized SF3B1mut tumor the neoantigen-specific CD8+ T cells were enriched in antigen-experienced cells as compared to patients bearing SF3B1wt tumors. This specific enrichment could reflect the recognition of TSA at early stages of the disease. In patients with metastatic SF3B1mut tumor, we confirmed the presence of memory cells among neoantigen specific CD8 T cells. In addition, the metastatic patients bearing a SF3B1mut tumor displayed a higher level of memory Melan-A specific CD8+ T cells in the peripheral blood as compared with patients bearing SF3B1wt tumors.

Thus, in patients bearing metastatic UM SF3B1mut tumors, the immune responses against TSA and TAA were correlated, suggesting a coordinated anti-tumor immune response.

Finally, we cloned specific-neoantigen and Melan-A CD8+ T cells from PBMCs of patients bearing metastatic SF3B1mut UM. Measurement of the TCR:MHC-peptide binding K-off in the resulting clones indicated that TCRs with low and high affinity were mobilized during immune response against both TA and TS Antigens, suggesting an absence of clonal deletion. In addition, TCR repertoire analysis evidenced conserved TRAV usage between donors suggesting a possible public repertoire for some TSA.

These results allow new insight of the immune response in uveal melanoma tumor which is important to develop new type of immunotherapies such as vaccination.

P **03** - 025

IMCS DIRECTLY IMPAIR HOMOLOGOUS DNA REPAIR PATHWAYS IN HORMONE-DEPENDENT BREAST CANCERS PROMOTING SENSITIVITY TO PARP INHIBITION

Siddhartha Mukherjee[1], Angela Elia[1], Laura Di Rito[1], Simone Mosole[1], Isabella Giacomini[1], Maria Luisa Gasparri[2], Andrea Rinaldi[1], Marco Bolis[1], Francesco Meani[2,3], Simona Di Lascio[3], Arianna Calcinotto[1]

[1]Institute of Oncology Research (IOR), Bellinzona, Switzerland; [2]Clinic Gynecology and Obstetrics, Regional Hospital of Lugano - Civic and Italian, Via Tesserete 46, 6900 Lugano, Switzerland., Lugano, Switzerland; [3]EOC Senology Center of Italian Switzerland, Via Pietro Capelli 1, 6900 Lugano, Lugano, Switzerland

Keywords: Immature myeloid cells, PARP inhibitors, Breast cancers, DNA repair pathways, Metabolite

About 80% of breast cancers are hormone-dependent and defined as Luminal-A and Luminal-B tumours. Although anti-hormonal therapy remains a mainstay treatment, most of them do not respond or become resistant to the therapy. Luminal-B disease has worse baseline distant recurrence-free survival at 5- and 10-years compared to Luminal-A, however clear biological culprits that define differences between Luminal-A and Luminal-B tumours are missing. Acquiring a better understanding of the mechanisms that control the development of Luminal-B tumours remains an unmet clinical need. The well-established dependency of cancer cells on the tumour microenvironment indicates that the microenvironment might have a role in the higher aggressiveness of Luminal-B tumours. Here, we identified a novel subset of immature myeloid cells (IMCs) specifically enriched in patients affected by Luminal-B tumours that modulate the tumour DNA repair machinery through epigenetic reprogramming. Mechanistically, a lipid metabolite secreted by IMCs directly inhibits homologous recombination, promoting error-prone DNA repair through non-homologous end-joining regulated by PARP-1. Consequently, the breast cancer cells acquire genomic instability promoting tumour progression and therapy resistance. Intra-tumour IMC score correlates with copy number alterations and a mutational signature driven by homologous recombination deficiency in breast cancer patients. Selective inhibition of these pathways induces the killing of the tumour cells both in vitro and in vivo. Treatments that block PARP-1, oppose the IMCs' pro-tumorigenic effects and synergize with standard endocrine therapies. Our discovery will pave the way to use PARP inhibitors also in tumours not driven by germline mutations and describe an unexpected new feature for this immune subset as a cellular mediator of synthetic lethality.

P **03** - 026

COMPLEMENT ACTIVATION PROMOTED BY THE LECTIN PATHWAY MEDIATES C3AR-DEPENDENT SARCOMA PROGRESSION AND IMMUNOSUPPRESSION

Elena Magrini[1], Sabrina Di Marco[1], Sarah Mapelli[1], Chiara Perucchini[1], Fabio Pasqualini[2], Alessia Donato[2], Maria de la Luz Guevara Lopez[3], Roberta Carriero[1], Andrea Ponzetta[1], Piergiuseppe Colombo[1], Ferdinando Cananzi[1,2], Domenico Supino[1,2], Edimara S. Reis[4], Clelia Peano[1,5], Antonio Inforzato[1,2], Sebastien Jaillon[2], Andrea Doni[1], John D. Lambris[4], Alberto Mantovani[1,2,3], Cecilia Garlanda[1,2]

[1]IRCCS Humanitas Research Hospital, Rozzano (Milan), Italy; [2]Department of Biomedical Sciences, Humanitas University, Pieve Emanuele (Milan), Italy; [3]The William Harvey Research Institute, Queen Mary University of London, London EC1M 6BQ, United Kingdom; [4]Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, United States; [5]Institute of Genetic and Biomedical Research, UoS Milan, National Research Council, Rozzano (Milan), Italy

Keywords: tumor, inflammation, macrophages, complement, immunotherapy

Complement has emerged as an important component of tumor promoting inflammation. The present study was developed to conduct a systematic assessment of the role of complement activation and effector pathways in sarcomas. Experiments of mesenchymal 3-methycolanthrene (3-MCA)-induced carcinogenesis and two transplantable sarcoma models were performed in mice deficient for the key complement molecule C3, for molecules selectively necessary for the activation of each complement activating pathway (classical, lectin and alternative) and for the receptors of C3a and C5a produced downstream C3 cleavage. C3-/-, MBL1/2-/-and C4-/-mice showed reduced susceptibility to 3-methylcholanthrene sarcomagenesis and transplanted sarcomas, whereas C1q and factor B deficiency had marginal effects. Complement 3a receptor (C3aR), but not C5aR1 and C5aR2, deficiency mirrored the phenotype of C3-/-mice. C3 and C3aR deficiency were associated with reduced accumulation and functional skewing of tumor-associated macrophages, increased T cell activation and response to anti-PD-1 therapy. Transcriptional profilingof sarcoma infiltrating macrophages and monocytes revealed the enrichment of MHC II-dependent antigen presentation pathway in C3-deficient cells. In patients, C3aR expression correlated with a macrophage population signature and C3 deficiency-associated signatures predicted better clinical outcome.Thus, by providing the genetic evidence of the pro-tumoral role of the lectin pathway and the C3a-C3aR axis, this study unveils novel links between complement activation and the establishment of a macrophage-dependent immunosuppressive microenvironment in sarcomas.

P **03** - 027

PROKINETICIN FAMILY MEMBERS CONTROL THE MICROENVIRONMENT OF HIGH-GRADE SEROUS OVARIAN CANCER

Constance Collet[1,2,3], Nicolas Lemaitre[1,2,3], Roland Abi Nahed[1,2,3], Marc Vassal[3], Frederic Sergent[1,2,3], Laurie Girard[1,2,3], Tiphaine Raia-Barjat[6], Vincent Lavoué[7], Marie Cohen[5], Thierry Michy[1,3], Mohamed Benharouga[1,2,3], Pierre-Adrien Bolze[4], Nadia Alfaidy-Benharouga[1,2,3]

[1]Institut National de la Santé et de la Recherche Médicale U1292, Biologie et Biotechnologie pour la Santé, Grenoble , France; [2]Commissariat à l'Energie Atomique et aux Energies Alternatives (CEA), Interdisciplinary Research Institute of Grenoble, Grenoble, France; [3]Service Obstétrique & Gynécologie, Centre Hospitalo-Universitaire Grenoble Alpes, University Grenoble-Alpes, Grenoble , France; [4]Department of Gynecological Surgery and Oncology, Hospices Civils de Lyon, University Hospital Lyon Sud, University of Lyon 1, Lyon, France; [5]Department of Pediatrics, Gynecology and Obstetrics, University Hospitals of Geneva, University of Geneva, Geneva, Switzerland; [6]Department of Gynecology and Obstetrics, Centre Hospitalier Universitaire de Saint-Étienne, Saint-Etienne, France; [7]Department of Gynecology, Rennes University Hospital, France. INSERM 1242, COSS., Rennes, France

Keywords: prokineticins , ovarian cancer , tumor microenvironment, syngeneic mouse model , antagonists

High-grade serous ovarian cancer (HGSOC) is one of the most frequent and lethal cancer among gynaecological ones. Resistance to conventional therapies contributes to recurrence and death. Prokineticin 1 (PROK1) and prokineticin 2 (PROK2) are two multifunctional secreted proteins that act via two G-protein coupled receptors, PROKR1 and PROKR2. PROKs are implicated in several processes including angiogenesis and immune modulation. PROK1 is highly secreted by the placenta and the ovary and was shown to be involved in physiological and pathological functions of these organs. A recent study from our group demonstrated that PROK1 participates to the growth and progression of gestational choriocarcinoma by promoting proliferation of malignant trophoblast cells. The role of PROKs in the aetiology of the ovarian cancer remains unclear and controversial. To characterize the role of this protein family in HGSOC, we used five different human ovarian cancer cell lines, two cohorts of HGSOC patients and an immunocompetent mouse model of ovarian cancer.

We first demonstrated that the HGSOC cell lines express both receptors and that treatment with PROK1 or PROK2 slightly affected their proliferation and migration, in vitro. At the clinical level, we demonstrated that PROK1 circulating levels were elevated in the serum of HGSOC patients compared to age matched controls and that this protein is abundant in the patient's ascites at advanced stages. Analyses of tumor tissues collected from same patients showed that PROKR2 is mainly expressed by tumor cells, whereas PROKR1 is strongly expressed by neutrophils infiltrating the tumor. We also demonstrated that PROKRs expression is highly heterogeneous across patients, suggesting a potential use of their degree of expression to stratify cases. In the perspective of conducting an in vivo study to better

characterize the involvement of the prokineticins in the control of the HGSOC immune environment, we injected female immunocompetent mice with the murine ovarian cancer cell line (ID8). Once tumors developed, mice were treated or not with PROKR1 antagonist (PC1). We observed a trend to a decrease in tumor growth in the treated group. Ongoing studies are in progress to better understand how the PROKs/PROKR1 system controls the immune landscape. Overall, our results strongly suggest that the prokineticin family is tightly associated with HGSOG and that PROKR1 antagonisation could be considered as a therapeutic option.

P **03** - 028

DECIPHERING PD-1-INDUCED T CELL IMMUNE SYNAPSE INHIBITION

Noémie Paillon[1,2,3], Violette Mouro[1,2], Mathieu Maurin[1], Stéphanie Dogniaux[1], Andrés Zucchetti[1], Claire Hivroz[1,2]

[1]Institut Curie, INSERM U932, PSL Research University, Paris 75005, France, Paris, France; [2]Fondation ARC, Paris, France, Paris, France; [3]ITMO Cancer Aviesan, Paris, France, Paris, France

Keywords: PD-1, T cell activation, immune synapse, actin

Despite the great advance immune checkpoint blockade represents in cancer treatment, the mechanisms underlying PD-1-induced T cell inhibition remain only partially understood. PD-1 is a receptor expressed by T lymphocytes upon activation. The binding to its ligand PD-L1 inhibits several T cell effector functions such as cytokine production and cytotoxicity. Efficient T cell activation relies on the formation of an immune synapse (IS) with an antigen presenting cell. Actin remodeling is essential for IS formation and more generally for T cell activation: the formation of a dense actin meshwork at the periphery of the contact and a depleted area at the center enables exchanges of cytokines and cytolytic granules. Our hypothesis is that PD-1 inhibits actin remodeling at the IS, thus accounting for the pleiotropic inhibitory effects observed on T cell activation. To test this hypothesis, we developed artificial antigen presenting cells able to activate T cells and engage PD-1 simultaneously. Using this tool, we were able to highlight that PD-1 inhibits T cell actin remodeling at the IS. Upon PD-1 ligation, T cell deformation and actin reorganization was lost. Thus, we propose that the remaining actin at the contact zone forms a physical barrier blocking cytolytic granule secretion, thus accounting for PD-1-induced cytotoxicity defect. Upon pharmacological opening of the actin meshwork at the IS, we were able to rescue PD-1-induced cytotoxic defect. Altogether, our results reveal T cell actin dynamics as a novel target of PD-1 inhibition. We will characterize the pathways and molecules that could be co-targeted together with PD-1 blockade to improve cancer immunotherapy.

P **03** - 029

BORTEZOMIB RESISTANCE OF MULTIPLE MYELOMA IS DUE TO UNFOLDED STRESS RESPONSE MODULATION

Julien Gigan[1], Philippe Pierre[1], Rafael Arguello[1]

[1]CIML-Aix marseille université, Marseille, France

Keywords: ER stress, Bortezomib, Unfloded protein response, Cytometry, Chemotherapy

The Unfolded Protein Response (UPR) is associated to the sensitivity of cancer cells to bortezomib treatment. Bortezomib is proteasome inhibitor used in therapeutic against multiple myeloma and lymphoma as a cytotoxic agent. While the mechanism behind bortezomib efficacy remains undefined, it is commonly accepted that UPR plays a role in its cytotoxicity. UPR is the answer to the accumulation of unfolded protein endoplasmic reticulum (ER) compartment. This accumulation leads to the activation of three ER sensors; PERK, IRE-1, and ATF6. In this study, we investigate on the role of different UPR sensors in Bortezomib toxicity. To address this question, we generated a method named SNUPR to measure the activation of the different branches of UPR using single nuclei suspension by flow cytometry. SNUPR helped to uncover the heterogeneity of UPR responses in different cell lines in response of ER stress inducers and bortezomib. Indeed, bortezomib treatment induced ER sensors activation, but this activation did not contributed to BTZ cytotoxity. More importantly, we discovered that IRE-1 function is required in a subpopulation of multiple myeloma cells that are resistant to treatment. Taken together, those data place UPR modulation as resistance marker and a complementary chemotherapy target to optimise bortezomib treatment.

P **03** - 030

DISPLAY OF NATIVE ANTIGEN ON CDC1 WITH SPATIAL ACCESS TO B AND T CELLS UNDERLIES EFFECTIVE HUMORAL RESPONSE TO VACCINATION

Thiago M. Steiner[1,3], Kato Yu[1], Mireille H. Lahoud[2], Caminschi Irene[1], William Heath[1,3]

[1]Department of Microbiology and Immunology, The Peter Doherty Institute, University of Melbourne, Parkville, Victoria 3010, Australia, Melbourne , Australia; [2]Department of Immunology, Monash University, Melbourne, Victoria 3004, Australia, Melbourne , Australia; [3]The ARC Centre of Excellence in Advanced Molecular Imaging, University of Melbourne, Parkville, 3010, Australia, Melbourne, Australia

Keywords: conventional dendritic cell, B cell, Clec9A, humoral immunity

The use of monoclonal antibodies to deliver antigen (Ag) to dendritic cells (DC) has shown to be a novel immunisation strategy of strong potential for vaccine development. DC are primarily known to support antibody responses by priming CD4+ T cells and contributing for the generation of T follicular helper cells (TFH), which support B cell proliferation and differentiation into antibody-secreting cells. In contrast to CD4+ T cells that can only recognise linear peptide epitopes presented on MHC II; B cells recognise antigen in their native form. It is becoming increasingly clear that DC not only contribute to humoral immunity by activating CD4+ T cells, but also by directly priming B cells. Targeting Ag to Clec9A has been long known to induce potent humoral responses even in the absence of adjuvants. We have previously shown that Clec9A-targeting induces potent TFH responses. We now show that the same immunisation strategy not only contributes for efficient presentation of antigen on MHCII by cDC1, but also allows for the retention of native antigen on their surface. With the use of in vivo imaging, we have observed that display of native antigen on cDC1 facilitates a direct interaction between them and Ag-specific B cells in regions bordering B cell follicles in the lymph-node and spleen. cDC1 native Ag presentation to B cells enables an efficient B cell activation and migration to the T/B border for MHCII restricted Ag-presentation to TFH for acquisition of help. We have also observed that MHCII-restricted presentation by B cells to TFH further supports the latter. These findings suggest that Clec9A-targeting represents an efficient mechanism for B cell activation and generation of humoral immunity, which can be exploited by novel vaccination approaches.

P **03** - 031

MVdeltaC A NEW THERAPEUTIC VACCINE IN IMMUNO-ONCOLOGY

Frédéric Tangy[1,2], Nicolas Boisgerault[3], Aleksandr Barinov[1], Chantal Combredet[1,2], Marc Grégoire[1,3]

[1]Oncovita, Paris, France; [2]Institut Pasteur, Paris, France; [3]INSERM UMR 1307 , Nantes, France

Keywords: vaccine, measles, intra tumoral

Oncovita, in collaboration with Institut Pasteur (Paris) and Inserm (Nantes), is developing a therapeutic vaccine based on Measovir® technology derived from the safe and highly immunogenic measles attenuated vaccine virus (MV), which has clinically proven natural oncolytic potential. This specific activity is due to hCD46 entry receptor of attenuated MV which is overexpressed in most cancer cells to protect them from antitumor innate immune response mediated by complement. We generated MVdeltaC, a genetically modified virus that demonstrated improved immuno-oncolytic capacity in vitro against a series of human tumoral cell lines, including mesothelioma, lung adenocarcinoma, bladder cancer, cervical cancer, hepatocarcinoma and ovarian cancer. MVdeltaC was active in 70-80% of these cell lines. In vivo, a single low dose administered intraperitoneally in NOD SCID mice provoked the rejection of human malignant pleural mesothelioma (MPM) cells intraperitoneally grafted. We confirmed this strong activity after intra-tumoral administration in 4 mesothelioma and

one bladder PDX models in nude mice.
Not only does the virus specifically target and destroy cancer cells, but it also has the potential to elicit strong and long-lasting anti-tumor immune responses. In ex-vivo experiments with human autologous cells, we have demonstrated that the oncolytic infection of cancer cells with MVdeltaC elicits release of danger signals and tumor associated antigens (TAA) from infected cells, activation of mDC and pDC, phagocytosis of dying infected cancer cells, and cross-presentation of TAA to T lymphocytes. We observed that MVdeltaC replication in cancer cells produces over a million times more defective interfering genomes (DIG) than standard MV. These short viral RNA molecules are strong ligands of RIG-I and MDA5 cytosolic receptors of innate immunity. Their binding rapidly triggers apoptosis and activates the expression of inflammatory and type I interferon genes. This activity in infected cells provokes death signaling on neighboring cells and the attraction of inflammatory molecules and cells inside the tumor.
We plan to initiate a FIH trial in patients with solid tumors. The activity in CPI resistant tumors will be of particular interest to investigate.

P **03** - 032

ANTIGEN RECEPTOR PROFILES IN NON-MUSCLE INVASIVE BLADDER CANCER AND ASSOCIATIONS WITH OUTCOMES

Magdalene Joseph[1,2], Yin Wu[1,2], Florian Rubelt[3], Hosseinali Asgharian[3], Dilduz Telman[3], Richard Bryan[4], Douglas Ward[4], Jan Berka[3], Nick James[5], Adrian Hayday[1,2]

[1]King's College London, London, United Kingdom; [2]Francis Crick Institute, London, United Kingdom; [3]Roche Sequencing Solutions, Pleasanton, United States; [4]University of Birmingham, Birmingham, United Kingdom; [5]Institute of Cancer Research, London, United Kingdom

Keywords: Bladder cancer, T cell receptor, B cell receptor, Gamma Delta T cells, Next Generation Sequencing

Non-muscle invasive bladder cancer (NMIBC) constitutes 75% of bladder cancer diagnoses and, despite its name, often relapses after curative resection. Post-surgical intravesical Bacillus Calmette-Guerin (BCG) is a well-established and accepted adjuvant therapy for high-risk NMIBC. Nonetheless, BCG therapy is associated with significant side effects and failure rates can approach 50%. Moreover, there are no validated biomarkers to guide its use. It is widely accepted that BCG works through inducing a protective anti-tumour immune response, but the underlying mechanisms are poorly understood. Here, we make use of ImmunoPETE, a next-generation antigen receptor sequencing platform, to explore the role of the adaptive immune response.
ImmunoPETE is a UMI-based, target amplification and sequencing assay concurrently assessing the representation and diversity of all three adaptive lineages of lymphocytes (ab T cells, gd T cells and B cells) in a high fidelity, quantitative manner. By applying ImmunoPETE to peripheral blood and tumour samples from 24 patients prior to any intravesical therapy, we have mapped the baseline antigen receptor characteristics of patients with NMIBCs. In a subset of patients, we have also applied ImmunoPETE to paired urine to determine to what extent urine may act as a surrogate for tumours.
We found that those patients who subsequently exhibited worse recurrence-free survival (RFS) after intravesical BCG showed a significantly lower ab T cell : B cell ratio in their initial, pre-treatment TIL populations. Conversely, patients with improved RFS had fewer T and B cells overall, but significantly higher T:B cell ratios, and additionally, antigen receptor diversity tended to be higher, as if reflecting competence to respond to high antigenic diversity. Collectively, these findings argue for the prognostic potential of assessing lymphocyte repertoires at the initial point of sampling. Finally, we found that urine samples yielded TCRb, and IGH chain sequences with higher Jaccard similarity indices with paired tumours compared with peripheral blood. This suggests the value of this non-invasive modality in continuously monitoring the immune status of the bladder during and after BCG therapy.

P **03** - 033

LOCAL ADMINISTRATION OF ANTI-BACTERIAL ANTIBODY PROVIDES LONG-TERM PROTECTION AGAINST PSEUDOMONAS AERUGINOSA RESPIRATORY INFECTION

Aubin Pitiot[1], Marion Ferreira[1], Christelle Parent[1], Mélanie Cortes[1], Chloé Boisseau[1], Christophe Paget[1], Nathalie Heuzé-Vourc'h[1], Thomas Sécher[1]

[1]Centre d'études des pathologies respiratoires, Inserm U1100, Université de Tours, Tours, France

Keywords: therapeutic antibody, mucosal administration, respiratory infection, Pseudomonas aeruginosa

Respiratory infections, due to the colonization of the airways by pathogenic agents are the 4th cause of death worldwide. Increase in antibiotic-resistant bacterial strains and seasonal virus outbreaks, require alternative treatments. Therapeutic antibodies (mAbs) targeting specifically pathogens have already proven efficacy, allowing pathogen's neutralization by the host immune system. Recent studies, also suggest a novel immunomodulatory modality for systemically-administered mAbs with the development of long-term immune protection.
The aim of this project was to make the proof-of-concept of the development of long-term immune protection, associated with an inhaled antibody treatment against a bacterial infection and to decipher the different mechanisms involved in this effect.
We use a mouse model of Pseudomonas aeruginosa (P. aeruginosa) lung infection, resembling to human acute pneumonia. Primary infection was cured using an airway-delivered anti-P. aeruginosa mAb. To investigate long-term protection, mice surviving primary infection were challenged a month later with P. aeruginosa without additional mAb treatment. Local and systemic humoral and cellular immune responses were analyzed thereafter.
Local mAb treatment was able to protect mice from primary P. aeruginosa-lethal infection, and allowed complete and rapid control of the bacterial load present in the airways. After a convalescent period of 30 days, and when PK analysis of the mAb revealed its disappearance in blood and BAL, 80 to 90% surviving primed mice were protected from a subsequent lethal challenge. This long-term protection was shown to be dependent to both the dose of the antibody, the size of the inoculum and the presence of the cognate antigen. Further studies revealed the development of a sustained and protective humoral response after priming, expended rapidly after challenge. Interestingly, this long-lasting response protected against secondary infections due to heterologous P. aeruginosa strains.
We have demonstrated that, in the context of a pulmonary bacterial infection, local mAb treatment was able to induce the development of a long-term immune protection. Our results suggested the importance of the anti-P. aeruginosa humoral response in the development of this effect. Further studies will be required to provide a complete understanding of the cellular and molecular partners accounting for the induction of cross-protective long-term anti-P. aeruginosa humoral response.

P **03** - 034

A NEW SYNTHETIC CIRCUIT FOR B CELL REPROGRAMMING TO CURE CANCER

Audrey Page[1], Floriane Fusil[1], François-Loïc Cosset[1]

[1]CIRI-Centre International de Recherche en Infectiologie, Univ Lyon, Université Claude Bernard Lyon 1, Inserm, U1111, CNRS, UMR5308, ENS Lyon, Lyon, France

Keywords: B cell, Immunotherapy, Cell transfer, Synthetic circuit

The expansion of techniques for genetic engineering has brought a new dimension for synthetic immunology. Immune cells are perfect candidates for such approaches because of their ability to patrol, to interact with many cell types, to proliferate upon activation and differentiate in memory cells. In particular, for cancer cure, they can be modified to eradicate tumor cells while counterbalancing the immunosuppressive tumor microenvironment.
The goal of our study is to implement a new synthetic circuit in B cells

by lentiviral vector transduction, allowing the expression of therapeutic molecules in a temporally and spatially restricted manner controlled by the presence of tumor antigens. These molecules will increase inflammation locally and promote the differentiation of effector immune cells, thereby mediating tumor clearance.
We developed a synthetic circuit encoding a "sensor" (a membrane-anchored B-cell receptor, BCR, targeting a tumor antigen), a "transducer" (a promoter that can be induced by activated BCR) and "effector" molecules (Interleukine 18). We isolated a 734 bp-long fragment of the NR4A1 promoter, which is specifically activated by the BCR signaling cascade in a fully reversible manner. By co-delivery of lentiviral vectors encoding respectively the transducer/effector and the sensor targeting ovalbumine, we achieved antigen-specific induction of the circuit. Indeed, the recognition of ovalbumine on the ectopic sensor specifically induced the activation of the NR4A1 promoter and the expression of the effector, demonstrating the circuit functionality in vitro. We are currently evaluating the ability of reprogrammed B cells to mediate tumor clearance in immunodeficient mice bearing human melanomas.

P **03** - 035

ACTIVIN-A IMPAIRS CD8 T CELL-MEDIATED IMMUNITY AND IMMUNE CHECKPOINT THERAPY RESPONSE IN MELANOMA

Katarina Pinjusic[1], Olivier Dubey[1], Olga Egorova[1], Sina Nassiri[1,2], Etienne Meylan[1,3,4,5], Julien Faget[1,6], Daniel Constam[1]

[1]Ecole Polytechnique Fédérale de Lausanne , Lausanne, Switzerland; [2]SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland; [3]Institut Jules Bordet, Anderlecht, Belgium; [4]Université Libre de Bruxelles, Gosselies, Belgium; [5]ULB Cancer Research Center , Brussels, Belgium; [6]INSERM U1194, Montpellier, France

Keywords: melanoma, Activin-A, cancer immunotherapy, immune evasion, chemokines

Activin-A encoded by the TGFβ-related INHBA gene is frequently upregulated and predicts poor outcomes in various cancer types. Gain of Activin-A expression in B16-F1 melanoma promotes tumor growth and reduces the intratumoral frequency of cytotoxic T cells, independently of systemic and pro-angiogenic effects. However, the underlying mechanisms and their relevance for cancer therapies are unclear.
Here, we identify that BRAF-driven melanomas most commonly upregulate INHBA gene expression and characterize the effects of Activin-A in mouse BRAF mutated melanoma models YUMM3.3 and iBIP2. By comparing changes in the tumor microenvironment (TME) across different models, we found that Activin-A signaling consistently expands the myeloid cell compartment at the expense of CD8 T cell infiltration and activation. Using antibody depletion strategies, we investigate the dependence of Activin-A tumor-promoting effect on different immune cells. Immune-regulatory effects of Activin-A were further characterized ex vivo and by the adoptive transfer of T cells. Finally, we assessed INHBA expression in melanoma patients who received immune checkpoint therapy and tested whether it impairs the response in preclinical models. We show that Activin-A secretion by melanoma cells inhibits adaptive anti-tumor immunity irrespective of BRAF status by inhibiting CD8 T cell infiltration indirectly and even independently of CD4 T cells, at least in part by directly attenuating the production of CXCL9/10 by myeloid cells. In addition, we show that INHBA expression correlates with anti-PD1 therapy resistance in melanoma patients and impairs the response to dual anti-CTLA4/anti-PD1 treatment in preclinical models.
Our findings suggest that strategies interfering with Activin-A induced immune-regulation offer new therapeutic opportunities to overcome CD8 T cell exclusion and immunotherapy resistance.

P **03** - 036

IRON AS AN ADJUVANT FOR ANTI-TUMOR RESPONSES

Sarah Porte[1,2], Carole Peyssonnaux[1,4], Sophie Vaulont[1,4], Bruno Lucas[1,3], Bruno Martin[1,3]

[1]Institut Cochin, Paris , France; [2]Université Paris Cité, Paris, France; [3]CNRS UMR8104, Paris, France; [4]INSERM U1016, Paris, France

Keywords: Iron, T cell, Cancer, Immunotherapy, Interferon gamma

The ability of the immune system to respond to tumor antigens has naturally led to the development of therapeutic trials in humans. In the 1990s, the first trials of immunotherapy, such as the use of IL-2 or IFN-α, were not particularly encouraging due to mitigated results and collateral toxicities. However, we know from these early therapeutic trials that the tolerogenic environment induced by tumors is dominant and prevents the immune system from eliminating them. One of the many discovered mechanisms of tumor escape is the induction of an inability of effector T cells to respond to a tumor antigen, a mechanism called "exhaustion". This mechanism has since become the target of therapeutic trials. There are, in fact, control points of the immune system, called "Immune Checkpoints", corresponding, among others, to the expression by T cells of inhibitory molecules that block their effector functions. The inhibition of these checkpoints allows the restoration of effector T-cell functions. Revolutionizing the treatment of many cancers such as melanoma, these new immunotherapies (anti-CTLA-4 and anti-PD-1 in particular) increase the survival of patients. Unfortunately, only 20-30% of patients respond successfully to these treatments by developing an effective anti-tumor immune response.
We have recently obtained results showing that iron significantly increases T-cell responses in vivo and in vitro. This "adjuvant" effect results in a strong slowing down of tumor-cell growth after transplantation in mice. In this context, iron promotes the differentiation/expansion of type 1 effector T cells which are characterized by the expression of the transcription factor T-bet and by their ability to produce high amounts of IFN-γ. Furthermore, we observed that growths of tumor-cell lines deficient either for ACSL4-enzyme production or either lacking IFN-γ-receptor expression, which are key drivers of ferroptosis induction, were not affected by iron supplementation after transplantation in mice. Thus our results strongly suggest that the iron-induced anti-tumor T-cell response would lead to cancer cell death by ferroptosis induction. In the light of these findings, we hypothesise that the effectiveness of immunotherapy could be improved by enhancing and shaping the T-cell response with one of the most classical chemical elements, iron.

P **03** - 037

TERTIARY LYMPHOID STRUCTURES REQUIRE A SPECIFIC IMMUNE MICROENVIRONMENT AND IMPROVE PROGNOSIS IN CERVICAL CANCER

Laurent Gorvel[1], Stephane Fattori[1], Marylou Panouillot[2], Jumaporn Sonongbua[1], Nicolas Boucherit[1], Marie-Sarah Rouvière[1], Samuel Granjeaud[3], Clara Degos[1], Amira Ben Amara[1], Xavier Carcopino[4], Eric Lambaudie[5], Renaud Sabatier[6], Jacques Nunes[1], Anne-Sophie Chretien[1], Marie-Caroline Dieu-Nosjean[2], Daniel Olive[1]

[1]Tumor Immunology laboratory, INSERM U1068, Immunomonitoring platform, Cancer Research Center in Marseille, Paoli-Calmettes Institute, Marseille, France; [2]Centre d'Immunologie et des Maladies Infectieuses, Cimi-Paris, INSERM U1135, Paris, France; [3]CRCM Integrative Bioinformatic Facility, Cibi, Cancer Research Center in Marseille, Paoli-Calmettes Institute, Marseille, France; [4]Aix-Marseille Univ, Department of Obstetrics and Gynecology, Hôpital Nord, APHM, Marseille, France; [5]Department of Surgical Oncology, Institut Paoli-Calmettes, CRCM, Marseille, France; [6]Institut Paoli-Calmettes, Department of Medical Oncology, Marseille, France

Keywords: Tertiary Lymphoid structures, Cervical Cancer, Tumor infiltrating Lymphocytes, Tumor microenvironment, Prognosis

Cervical Cancer represents the 4th cause of women cancer-related death worldwide. It is mainly caused by HPV infection. Cervical cancer development is a very dynamic process involving viral replication cycle, cervical epithelium differentiation and local inflammation driven by the immune system. Unfortunately, the cervical tumor microenvironment and its interaction with the immune system are poorly understood. Tertiary lymphoid structures (TLS) are lymphnode-like structures forming in tissues in response to inflammation. Their formation requires higher immune cell infiltrate along with cytokines and chemokines,

and overall vascularization. Usually, TLS are associated with better prognosis. In this study, we study cervical tumor TLS and their incidence on patient outcome by multiplex IHC, soluble factor measurements by luminex, mass cytometry and transcriptomics. First, we quantified B, T and dendritic cells, assessed their density by IHC to identify TLS+ and TLS- tumors. TLS+ tumors were associated with increased chemokine, inflammatory cytokine and IgG, M and A secretion compared to TLS- or healthy control. Using CyTOF, we assessed the immune cell composition and phenotype in TLS+ and TLS- tumors. We showed that the presence of cDC2, B cells, CD8+CD69+ T cells and CD4+ TEMRA cells were associated with the presence of TLS. Overall, immune checkpoint markers were enriched on TLS+ tumors. Indeed, CD40 was expressed on cDC2, PD1 on CD8+Tcells, DNAM-1 on NK cells in TLS+ tumors compared to TLS-. Using these data we could define a phenotypic signature of TLS+ vs TLS- tumors by using the BGA algorithm. Finally we demonstrated that the presence of TLS and the enrichment in cDC2 and Bcells are almarks of patient survival in our cohort and in Immune cell enrichment analysis on cervical cancer TCGA dataset. Taken together, our results show that TLS can be considered as a marker for improved prognosis and immunotherapy response in cervical cancer.

P **03** - 038

ANTIBODY THERAPY NP137 TARGETS TUMORAL PMNS

Anna Rita Redavid[2], Ruxanda Chira[1], David Goldschneider[1], Benjamin Ducarouge[1], David Neves[1], Benjamin Gibert[2], Maeva Hervieu[2], Alicia Demonti[1], Agnès Bernet[1], Patrick Mehlen[2]

[1]Netris Pharma, Lyon, France; [2]Cancer Research Center of Lyon , Lyon, France

Keywords: Netrin-1, Therapy, Immuno-oncology, Cancerology

Netrin-1 is a multifunctional protein initially identified for its key roles in neuronal navigation and angiogenesis during development. Most of its activity is mediated by its main receptors, Deleted in Colorectal Carcinoma (DCC), UNC5-Homolog (UNC5A, UNC5B, UNC5C, UNC5D), and Neogenin.

More recently, deregulation of Netrin-1 expression has been described to be involved in various pathological processes such as diabetes, cardiovascular disease, immune cell infiltration during inflammation, and cancer. This led our group to develop a novel monoclonal IgG1 anti-netrin-1 antibody, NP137 which has proved its efficacy in phase 1 clinical trials in patients with various types of advanced solid cancers. Here we propose that Netrin-1 could play a role in the regulation of the immune function in tumors.

The activity of NP137 has been tested in different mouse models of spontaneous cancer or syngeneic tumor grafts. We identified a potentiating anti-tumor effect of the NP137 on anti-CTLA-4 immune checkpoint inhibitor. Consequently, by analyzing their tumor microenvironment, we realized that NP137 treatment decreased an immune population: the polymorphonuclear cells (PMN) that are commonly associated with poor prognosis, tumoral stemness, and spreading. Thus, the results presented here will clarify the role of Netrin-1 in controlling PMNs.

P **03** - 039

IMMUNE AND GENETIC SIGNATURE OF HER2-DRIVEN BREAST CARCINOMAS TRIGGERING YO PARANEOPLASTIC CEREBELLAR DEGENERATION

Elise Peter[1,2,3], Isabelle Treilleux[4], Valentin Wucher[1,2], Emma Jougla[3,5], Alberto Vogrig[1,2,3], Daniel Pissaloux[4,6], Sandrine Paindavoine[4,6], Justine Berthet[3,5,8], Géraldine Picard[2], Véronique Rogemond[1,2], Marine Villard[2], Clémentine Vincent[1,2], Laurie Tonon[7], Alain Viari[7], Jérôme Honnorat[1,2,3], Bertrand Dubois[5,8], Virginie Desestret[1,2,3]

[1]NeuroMyogene Institute, SynatAc Team, INSERM U1217/UMR CNRS 5310, Lyon, France; [2]French Reference Center on Paraneoplastic Neurological Syndrome, Hospices Civils de Lyon, Lyon, France; [3]University of Lyon, Université Claude Bernard Lyon 1, Lyon, France; [4]Department of Biopathology, Centre Leon Berard, Lyon, France; [5]INSERM 1052, CNRS 5286, Centre Leon Berard, Centre de Recherche en Cancérologie de Lyon, Lyon, France; [6]Cancer Genomics Platform, Department of Translational Research, Centre Leon Berard, Lyon, France; [7]Synergie Lyon Cancer-Bioinformatics Platform-Gilles Thomas, Centre de Recherche en Cancérologie de Lyon, Lyon, France; [8]Laboratoire d'Immunothérapie des Cancers de Lyon (LICL), Lyon, France

Keywords: paraneoplastic cerebellar degeneration, breast cancer, anti-tumour immunity

Background: Paraneoplastic cerebellar degeneration (PCD) with anti-Yo antibodies are cancer-related auto-immune diseases directed against neural antigens expressed by tumour cells. A putative trigger of the immune tolerance breakdown is genetic alteration of Yo antigens. We aimed to identify the genetic and immune tumour specificities involved in Yo-PCD pathogenesis.

Methods: Using clinicopathological data, immunofluorescence imaging and whole-transcriptome analysis, 22 breast cancers (BC) associated with Yo-PCD were characterised in terms of oncological characteristics, genetic alteration of Yo antigens, differential gene expression profiles compared to matched control BC and morpho-functional specificities of their in situ anti-tumour immunity.

Results: Yo-PCD BC were invasive carcinoma of no special type, which early metastasise to lymph nodes. They overexpressed human epidermal growth factor receptor 2 (HER2) but were hormone receptor-negative. All Yo-PCD BC carried at least one genetic alteration (mutation or gain in copy number) on CDR2L, encoding the main Yo antigen aberrantly overexpressed in Yo BC. Analysis of the differentially expressed genes found 615 upregulated and 54 downregulated genes in Yo-PCD BC compared to control HER2-driven BC without associated PCD. Pathway enrichment analysis found significantly upregulated adaptive immune response pathways in Yo-PCD BC. Immunofluorescence imaging confirmed an intense immune infiltration with overwhelming predominance of IgG-producing plasma cells.

Conclusion: These data confirm the role of genetic alterations of Yo antigens in triggering the immune tolerance breakdown but also outlines a specific biomolecular profile in Yo-BC suggesting a cancer-specific pathogenesis.

P **03** - 040

NOVEL GENETIC IMMUNIZATION FOR THE DEVELOPMENT OF ANTIBODIES TARGETING CONFORMATIONAL AND NATIVE ANTIGENS FOR DIAGNOSTIC AND THERAPY

Meddy El Alaoui[1]

[1]COVALAB, Bron, France

Keywords: COVID-19, neutralizing antibodies, DNA immunization, conformational antigen, therapeutic antibodies

The recent COVID-19 pandemic shows the limitation of the conventional vaccination which uses an antigen such as peptide, recombinant protein or attenuated virus… The genetic immunization based on in vivo transfection of RNA or DNA gave a strong and consistent response. These simple and efficient approaches share a common point, the antigen is displayed conformationally to the immune system due to the in-vivo expression of native antigens. Since few years, Covalab has developed its own technology for DNA immunization which was applied to tumor antigens for a proof of concept, and then to SARS-CoV-2 proteins (S, RBD, N, M and E). The easy-to-use method do not require any specific material such as gene gun or electroporator allowing its application to various animals (mouse, rat, rabbit, ship, goat, lama, …). In Different comparative immunization studies using DNA versus recombinant protein (standard method), the DNA immunization process showed a faster immune response, a better ratio IgG/IgM and high affinity and specificity of the antibodies.

The developed method of "DNA-designed antibodies" is time and cost effective, does not require protein purification and enables generation of antibodies targeting membrane-anchored and glycosylated proteins. Sufficient expertise for immunization procedure and novel optimized vectors, enhancing Ab quality and vector immunogenicity via the increased intracellular trafficking of plasmid DNA exists now at Covalab. In the current pandemic context of SARS-Cov-2, there is a great demand for effective therapies for the prevention and treatment of COVID-19. The advances of antibody technologies have greatly accelerated the discovery of SARS-Cov-2 neutralizing antibodies (nAb) and some of which are now in the clinic under the Emergency

Use Authorization. Thanks to its novel and validate DNA-immunization technology Covalab has now one potent nAb which is currently in preclinical validation against all the variants.

P **03** - 041

IMAGING MASS CYTOMETRY OF HUMAN CUTANEOUS SQUAMOUS CELL CARCINOMA REVEALS A SPECIFIC SIGNATURE ASSOCIATED WITH RELAPSE

Roxane Elaldi[1,2], Aida Meghraoui-Kheddar[1], Axel Elaldi[3], Patrice Hemon[4], Jacques-Olivier Pers[4], Luciana Petti[1], Gilles Poissonnet[2], Anne Sudaka[2], Veronique M Braud[1], Fabienne Anjuère[1]

[1]IPMC CNRS UMR7275, Valbonne, France; [2]Centre Antoine Lacassagne, Nice, France; [3]NYU Tandon school of Engineering, New-York, United States; [4]LBAI, Brest, France

Keywords: skin cancer, Imaging mass cytomery, tumor microenvironment, pronostic marker, relapse

Background: Cutaneous squamous cell carcinomas (cSCC) are the second deadliest skin cancer. They are currently treated by excisional surgery but can reach, for some patients, a non-operable stage associated with rapid local and nodal relapses and a very poor prognosis. However, no consensus has been reached on the clinical or molecular factors predicting these recurrences. It is therefore crucial to improve the identification of patients that would relapse by the characterization of prognostic biomarkers. An integrative spatial characterization of cSCC immune microenvironment and the interactions of their components are required to identify such biomarkers. The objective of our study is to obtain an exhaustive characterization of the immune microenvironment (TiME) of recurrent and non-recurrent cSCC, including the interaction maps between the main actors described to be associated to tumor progression. The comparison of the specific signature of these two cSCC groups will allow the identification of prognostic biomarker candidates that could help predict relapses.

Methods: A cohort of cSCC with different prognosis comprising one group of non-relapsing tumors (n=10) and one group of primary tumors having a local relapse at 2 years (n=10) were used. An imaging mass cytometry (IMC) panel of 39 antibodies targeting components of the cSCC TiME (tumor cells, immune subtypes, fibroblasts, blood and lymphatic vessels, extracellular matrix and nerves fibers) was designed (Elaldi et al, Front Immunol 2021) and used to stain a section of each formalin fixed and paraffin-embedded (FFPE) tumor of the cohort. Each section was analyzed by IMC and the 40-dimensional images obtained were processed using an in-house developed analysis pipeline.

Results: IMC image preprocessing and computational analysis of the single cell phenotypes extracted from the images allowed (1) the identification of each targeted TiME component subset, from tumor cells and activated fibroblasts to both myeloid and lymphoid immune cells, (2) in addition to their functional status (proliferation, apoptosis, exhaustion) and (3) their localization within tumor structures. This analysis also led to the identification of specific spatial features characterizing the TiME of each tumor group. The comparison of these specific signatures uncovered a predictive signature associated with relapse risk after surgical excision of cSCC tumors that will be confirmed in an independent validation cohort.

P **03** - 042

INDUCTION OF A STRONG AND LONG-LASTING NEUTRALIZING IMMUNE RESPONSE BY A BI-SPECIFIC SUB UNIT NANOPARTICULATE VACCINE AGAINST HEPATITIS B VIRUS

Fanny Charriaud[1], Myriam Lamrayah[1], Manon Desmares[2], Celine Coiffier[1], Evelyne Colomb[1], Julie Lucifora[2], Simon Megy[3], Raphaël Terreux[3], David Durantel[2], Bernard Verrier[1]

[1]Colloidal vectors and targeted therapeutic engineering, Tissue Biology and Therapeutic Engineering Laboratory (LBTI), CNRS UMR5305 - UCBL, Lyon, France; [2]HepVir team, International Center for Infectiology Research (CIRI), INSERM U1111 - CNRS UMR5308 - UCBL, Lyon, France; [3]PRABI Gerland, Tissue Biology and Therapeutic Engineering Laboratory (LBTI), CNRS UMR5305 - UCBL, Lyon, France

Keywords: biotechnology, nanovaccine, hepatitis B virus, TLR2 ligand, neutralizing antibodies

Chronic hepatitis B, caused by persistent hepatitis B virus (HBV) infection, significantly increases the risk of developing severe liver disease (cirrhosis and hepatocellular carcinoma). Despite the successful implementation of a prophylactic vaccine since 1982, HBV infection is still pandemic in Western Pacific and African regions, where 6% of the adult population is chronically ill. Among the most exciting antiviral strategies under investigation nowadays are the drug delivery systems, particularly nanoparticle platforms, which have been gaining interest since the COVID19 crisis. Nanoparticles (NP) aim at overcoming the limitations of conventional delivery systems by improving the stability and solubility of molecules in addition to facilitating their transport across membranes via their nanosize. In the vaccine field, NP allow the engagement of key immune pathways and provide strategies for modulating the trafficking and the delivery of vaccine components throughout the lymphatic tissues.

Here, we develop bi-specific biodegradable NP as a new vaccine alternative to eradicate HBV. Our approach is unique in that it uses the PreS1 domain of the large HBV surface protein, as opposed to the HBsAg-based conventional vaccine, together with a Toll-Like-Receptor 1/2 ligand (TLR-L) for adjuvanticity. The PreS1 domain is the major viral attachment site for entry of the virus in the liver, thus offering a great prophylactic target; but its poor immunogenicity limits clinical translation. In this way, the NP are functionalized by both the coating of myristoylated (2-48)PreS1 and the entrapment of Pam3CSK4 (TLR-L), whose role is to finely tune the immune response of the vaccine candidate. We show that the NP mimic HBV infection with an efficient peptide recognition in vivo, exemplified by the elicitation of potent antibody responses in naive mice during one year after immunization. This NP platform exhibits significant lymph node targeting ability, leading to prolonged retention time in draining lymph nodes after s.c injection. Interestingly, the most efficient viral neutralization was observed with NP-Pam3CSK4-PreS1 on in vitro assays, hinting that the immunogenicity of the HBV-derived antigen is improved by the synergistic action of both the vector and the adjuvant.

Substantially, this work offers a promising strategy to achieve a functional cure as it induces a high level and persistent anti-PreS1 response that could result in viral clearance in HBV-infected models.

P **03** - 043

VACCINATION INDUCES STRONGER SPIKE IMMUNITY AGAINST SARS-COV-2 INFECTION IN COVID-19 RECOVERED SUBJECTS THAN IN NAÏVE INDIVIDUALS

Carla Saade[1], Bruno Pozzetto[1,5], Christelle Compagnon[2], Véronique Barateau[1], Julie Chaix[6], Sophia Djebali[1], Loïc Peyrot[1], Study group Lyon-Covid[2,7], Bruno Lina[1,7], Jacqueline Marvel[1], Manuel Rosa-Calatrava[1], Andrès Pizzorno[1], Delphine Maucort-Boulch[3,4], Philippe Vanhems[1,8], Karen Brengel-Pesce[2], Stéphane Paul[1,5], Thierry Defrance[1], Thierry Walzer[1], Sophie Trouillet-Assant[1,2]

[1]CIRI - Centre International de Recherche en Infectiologie, Univ Lyon, Université Claude Bernard Lyon 1, Inserm, U1111, CNRS, UMR5308, ENS Lyon, Université Jean Monnet de Saint-Etienne, Lyon, France; [2]Joint Research Unit Civils Hospices of Lyon-bioMérieux, Civils Hospices of Lyon, Lyon Sud Hospital, Pierre-Bénite, France; [3]Hospices Civils de Lyon, Pôle Santé Publique, Service de Biostatistique et Bioinformatique, Lyon, France; [4]CNRS, UMR 5558, Laboratoire de Biométrie et Biologie Évolutive, Équipe Biostatistique-Santé, Villeurbanne, France; [5]Immunology laboratory, CIC1408, CHU Saint-Etienne, Saint-Etienne, France; [6]BIOASTER, 40 Avenue Tony Garnier, Lyon, France; [7]Virology laboratory, Institute of Infectious Agents, Laboratory associated with the National Reference Centre for Respiratory Viruses, Hospices Civils de Lyon, Lyon, France; [8]Service D'Hygiène, Épidémiologie, Infectiovigilance et Prévention, Hôpital Édouard Herriot, Hospices Civils de Lyon, Lyon, France

Keywords: Hybrid immunity, Long-term response, SARS-CoV-2 vaccination

Background

As the Covid-19 pandemic is still ongoing despite the introduction of multiple vaccines, it is essential to identify populations with the highest risk of being infected or re-infected as well as the effectiveness of long-term immune response following vaccination or infection.

Methods

To address this question, Spike-specific humoral and cellular immunity were compared in several groups of 613 subjects from different cohorts, 6 months post-infection or post-vaccination in naïve or previously

infected individuals (convalescents).
Blood sampling was performed from a) 386 unvaccinated convalescent patients (after severe COVID-19 (n=183) or mild COVID-19 (n=203)), b)116 vaccinated convalescent patients receiving i) two doses of Pfizer BNT162b2 (BNT, n=50), ii) one dose of BNT (n=34) and iii) one dose of ChAdOx1-S-nCoV-19 (ChAd, n=32) (conferring so-called hybrid immunity) and c) 111 fully vaccinated COVID-19-naïve subjects (vaccinated with 2 doses of BNT (n=76) or one dose of ChAd followed by one dose of BNT (n=35)). Written informed consent was obtained from all included patients (ClinicalTrials.gov identifier: NCT04637867 and NCT04341142).
Humoral investigation was assessed by ELISA assays detecting anti-RBD IgG as well as anti-S1 IgA, live virus neutralization assays (using Vero E6 models or reconstructed human airway epithelium) and bio-layer interferometry assay to evaluate anti-RBD IgG avidity. As for cellular investigation, an interferon-γ release assay was performed to estimate T cell response following RBD peptide stimulation.
Findings
Here, we report that, in comparison to naïve vaccinated subjects or unvaccinated convalescent patients, individuals with hybrid immunity have significantly higher i) anti-RBD IgG levels with higher antibody avidity, ii) serum neutralization capacity against 3 different SARS-CoV-2 strains (19A, Delta, Omicron), iii) anti-S1 IgA levels and iv) IFN-γ release following T-cell stimulation with RBD peptides
This advantage is independent of the vaccination regimen post infection (ChAdOx1, BNT162b2 one dose, BNT162b2 two doses).
Interpretation
Hybrid immunity conferred by vaccination following infection is greatly superior to all other forms of immunity. These results suggest that vaccinated individuals with no history of prior infection should be prioritized for booster doses 6 months post initial vaccination and that monitoring of spike-specific immunity is required for vaccines follow-up.

P **03** - 044

AUTOLOGOUS T CELL RESPONSES TO PRIMARY HUMAN COLORECTAL CANCER SPHEROIDS ARE ENHANCED BY ECTONUCLEOTIDASE INHIBITION

Julie Bonnereau[1,2], Tristan Courau[1], Nicolas Asesio[3], Delphine Salfati[3], Fatiha Bouhidel[4], Hélène Corte[5], Sarah Hamoudi[1,2], Nassim Hammoudi[1,2,3], Julie Lavolé[1,2], Justine Vivier-Chicoteau[3], Victor Chardiny[1], Mathieu Blery[6], Cécile Bonnafous[6], Purnima Bhat[7], Pierre Cattan[5], Antoine Toubert[1], Matthieu Allez[1,2,3], Thomas Aparicio[1,2,3], Lionel Le Bourhis[1]

[1]INSERM U1160, Institut de Recherche Saint-Louis, Saint Louis Hospital, Paris, France; [2]Université de Paris, Paris, France; [3]Department of Hepato-Gastroenterology , APHP, Hôpital Saint-Louis, Paris, France; [4]Anatomopathology Department, APHP, Hôpital Saint-Louis, Paris, France; [5]Department of Surgery, APHP, Hôpital Saint-Louis, Paris, France; [6]Innate Pharma, Marseille, France; [7]ANU Medical School/Canberra Hospital - Gastroenterology, Garran, Australia

Keywords: Colorectal T cells, Anti-tumor T cell response, CD39, Autologous spheroids, Tumor

T cells are major effectors of the anti-tumoral immune response. Their activation by tumor-associated antigens can unleash their proliferation and cytotoxic functions, leading to tumor cell elimination. However, immunosuppressive mechanisms including the overexpression of immune checkpoints, like PD-1, are also engaged, promoting tumor immune escape. Immunotherapies targeting these pathways have been developed but demonstrated a weak efficacy in colorectal cancer (CRC) highlighting the need to find new therapeutic targets, the main objective of this project.
To do so, we first studied in a prospective cohort of CRC patients the phenotype of tumor and non-tumoral matched mucosa infiltrating T cells. In the tumor, we showed the increased expression of CD39, and its co-expression with PD-1, on CD4 T cells. CD39 expression was higher in the right colon and early stage tumors, thus defining a subset of patients potentially responsive to CD39 blockade. Then, to assess the effectiveness of targeting CD39, we set up an innovative autologous coculture model between primary spheroids and lymphocytes isolated from the same tumor. We developed two main readouts to assess the activation of T cells (live imaging of the ten first hours of coculture), their infiltration and the subsequent spheroid death (confocal microscopy on fixed cocultures). We showed that CD39 blockade promotes T cell infiltration and spheroid destruction, highlighting the potential of this new target for immunotherapy in CRC.

P **03** - 045

REPRESSION OF PROTEIN MATURATION INHIBITS PD-1 EXPRESSION AND ENHANCES TUMOR CLEARANCE AND TILS: VIRTUAL LIGAND SCREEN-ING AND DRUG REPURPOSING APPROACH

Alexia François[1], Bruno Villoutreix[2], Serge Evrard[1,3], Simon Pernot[1,3], Abdel-Majid Khatib[1,3]

[1]INSERM , Bordeaux , France; [2]INSERM, Paris, France; [3]Institut Bergonié, Bordeaux , France

Keywords: PD-1, cancer , Drugs, T cell, PCs

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.

P **03** - 046

ZEB1 TRANSCRIPTION FACTOR PROMOTES IMMUNE ESCAPE IN MELANOMA

Maud Plaschka[1], Valentin Benboubker[1], Maxime Grimont[1], Justine Berthet[2,3], Laurie Tonon[4], Jonathan Lopez[1], Myrtille Le-Bouar[5], Brigitte Balme[5], Garance Tondeur[5], Arnaud De La Fouchardiere[1], Lionel Larue[6], Alain Puisieux[6], Yenkel Grinberg-Bleyer[7], Nathalie Bendriss-Vermare[2,3], Bertrand Dubois[2,3], Christophe Caux[2,3], Stephane Dalle[1,5], Julie Caramel[1]

[1]Cancer Research Center of Lyon, Université de Lyon, Université Claude Bernard Lyon 1, INSERM 1052, CNRS 5286, Centre Léon Bérard, "Cancer cell Plasticity in Melanoma" team., Lyon, France; [2]Cancer Research Center of Lyon, Université de Lyon, Université Claude Bernard Lyon 1, INSERM 1052, CNRS 5286, Centre Léon Bérard, "Cancer immune surveillance and therapeutic targeting" team., Lyon, France; [3]Laboratory of Immunotherapy of Cancer of Lyon (LICL), Lyon, France; [4]Fondation Synergie Lyon Cancer, Plateforme de bio-informatique Gilles Thomas, Centre Léon Bérard,, Lyon, France; [5]Dermatology Unit, Hospices Civils de Lyon, CH Lyon Sud, Lyon, France; [6]Institut Curie, PSL Research University, Paris, France; [7]Cancer Research Center of Lyon, Université de Lyon, Université Claude Bernard Lyon 1, INSERM 1052, CNRS 5286, Centre Léon Bérard, "Molecular regulation of immunity in cancer", Lyon, France

Keywords: Melanoma, Immune microenvironment, EMT-TF, Plasticity, Resistance to immunotherapy

The efficacy of immunotherapies in metastatic melanoma depends on a robust T cell infiltration. Therefore, defining cancer cell intrinsic mechanisms mediating T cell exclusion and immune resistance is crucial. The EMT inducing transcription factor ZEB1 is a major regulator of melanoma cell plasticity, driving resistance to MAPK targeted therapies. Here, we demonstrate the major role of melanoma-cell-

intrinsic ZEB1 signaling in preventing T-cell infiltration in melanoma. Spatial analysis of the immune infiltrates in human melanoma samples revealed a correlation between high ZEB1 expression in tumor cells and decreased CD8+ T lymphocyte infiltration, independently of b-catenin pathway activation. Moreover, gain- or loss-of-function experiments in melanoma mouse models showed that ZEB1 impairs CD8+T cell recruitment by suppressing the production of T cell attracting chemokines, resulting in tumor immune evasion and resistance to immune checkpoint blockade. Finally, ZEB1 targeting improves the efficacy of anti-PD-1 immunotherapy, pointing to a new therapeutic target in metastatic melanoma.

P **03** - 047

TL-532 SPECIFIC TLR3-AGONIST INDUCES LIFE-LONG ANTI-TUMOR AUTOVACCINATION, CROSS-IMMUNITY AGAINST UNRELATED CANCER AND REVERSES RESISTANCE TO IMMUNE CHECKPOINT INHIBITORS

Marc Bonnin[1], Saïd Ourfali[1,2], Mathilde Boucard-Jourdin[1], Clémence Perret[1], Chloé Renoux[1], Nelly Vey[1], Marc Colombel[2], Bettina Werlé[1], Sylvain Thierry[1]

[1]TOLLYS, Lyon, France; [2]Service d'Urologie et Chirurgie de la Transplantation, Hospices Civils de Lyon, Lyon, France

Keywords: TLR3, Autovaccination, TME switch, cross-immunity

Toll-Like Receptor 3 (TLR3) is an innate immunity receptor that triggers inflammation by recognizing double stranded RNAs. In the past decades, TLR3-agonists have been used to fight cancer as inducer of both type-I interferons and apoptosis specifically in tumor cells but not in normal cells through the TLR3 Death-Receptor activity. To date, no TLR3-agonist has reached the market due to lack of manufacturing reproducibility, specificity and/or due to toxicity. In this context, a new family of synthetic, specific and fully defined TLR3-agonist has been rationally designed (WO2019211492).

Here we report the preclinical proof-of-concept of TL-532 in non-muscular invasive bladder cancer models ex vivo and in vivo.

TL-532 induces cell death by apoptosis specifically in tumor cells, while it is well tolerated by normal cells, demonstrating a broad therapeutic window both ex vivo and in vivo. This tumor specific apoptosis is associated with a tumor microenvironment switch, evidenced by increased antitumor biomarker secretion (IFN-α, IFN-l1, IFN-g, CCL5, CXCL9, CXCL10), and decreased protumor biomarkers CCL22, sFAS. Tumor microenvironment (TME) switch is associated in vivo with recruitment and activation of conventional dendritic cells (cDCs) and cytotoxic T-lymphocytes (CTLs), together with a drastic decrease of M2 and tumor associated macrophages (TAMs). In vivo activity of TL-532 leads to substantial tumor growth inhibition (88%) and delay (370%), translating in 35% complete response (CR) rate and 5.3-fold median survival benefit. Interestingly, among these CRs, 62% (13/21) show life-long tumor auto-vaccination after 3 consecutive rechallenges at 3, 10 and up to 32 months. Remarkably, 54% (13/24) of the autovaccinated mice also demonstrate cross-immunity against an unrelated and poorly immunogenic, syngeneic osteosarcoma cancer cell model (LM8). Strikingly, TL-532 treatment is able to reverse the anti-PD-L1 tumor-resistance when combined with the ICI, leading to an increase of the CR-rate up to 50%.

Conclusion: We identified TL-532 as spearhead of a new rationally designed TLR3-agonist family. In monotherapy, it demonstrates substantial tolerance and promising anti-cancer and autovaccinal activity, including unrelated cancers. TL-532 also demonstrates its remarkable ability to overcome ICI tumor-resistance, thus increasing the clinical landscape for ICI combination treatment.

P **03** - 048

INTERFERON-GAMMA REGULATES ANTI-TUMOUR RESPONSE BY RESTRICTING CYTOTOXIC T CELL POTENTIAL IN MELANOMA

Julie Mazet[1], Jagdish Mahale[1], Orion Tong[2], Vivian Lau[1], Moustafa Attar[1], Lada Koneva[1], Stephen Sansom[1], Benjamin Fairfax[2], Audrey Gerard[1]

[1]The Kennedy Institute of Rheumatology - University of Oxford, OXFORD, United Kingdom; [2]Oncology department - University of Oxford, OXFORD, United Kingdom

Keywords: Cancer, immunity, exhaustion, stem-like T cells, IFN gamma

T-cell exhaustion is a T-cell state whereby T-cells are becoming dysfunctional over time, with the upregulation of inhibitory molecules known as immune checkpoints. Immunotherapies targeting those immune checkpoints, or checkpoint blockade, in late-stage melanomas are only successful in a fraction of patients. Therefore, there is a critical need to decipher other mechanisms involved in T-cell dysfunction in cancer. Interferon-gamma (IFNγ) is a major immune-modulatory cytokine that has concomitant pro- and anti-tumour functions. Because IFNγ is inherently linked to the efficacy of tumour immunity, it is critical to dissect its pleiotropic effects during carcinogenesis. Here, we explore the hypothesis that IFNγ sensing by the main producers, the cytotoxic T cells (CTLs), is a negative regulator of T cell anti-tumour responses. Using RNA-sequencing, we characterized the relationship between IFNγ-receptor (IFNγR) expression in circulating CTLs from metastatic melanoma patients and their response to checkpoint blockade. Importantly, expression of the beta chain of the IFNγR in CTLs negatively correlates with response to checkpoint blockade, suggesting that CTLs have to down-regulate their IFNγR for successful anti-tumour immunity. To address whether IFNγ signalling in T-cells directly impacts tumour immunity, we used a mouse model where the IFNγR is specifically ablated in mature CD8+ T-cells (IFNγR1KO) and engrafted with B16F10 melanoma cells. Depleting the IFNγR1 in CD8+ T-cells is sufficient to restore immunity towards melanoma, although it does not substantially affect CTL function. Rather, inhibition of IFNγ sensing by CD8+ T cells promotes their proliferation and infiltration, resulting in an increased number of tumour-infiltrating CTLs. Importantly, IFNγ targets a specific T cell subset called stem-like T cells. Stem-like T cells have self-renewal properties and represent a pool of resource cells constantly giving rise to more differentiated exhausted T cells. Stem-like T cells are depleted over time at the tumour site, and we found that IFNγ participates in this by limiting their maintenance and expansion. As a consequence, IFNγ restricts the rate of generation of early differentiated CTLs at the tumour site. Altogether, our data show that IFNγ drives a negative feedback loop in CD8+ T cells restricting their anti-tumour potential.

P **03** - 049

TARGETING CISH ENHANCES NATURAL CYTOTOXICITY RECEPTOR SIGNALING AND REDUCES NK CELL EXHAUSTION TO IMPROVE SOLID TUMOR IMMUNITY

Pierre-Louis Bernard[1], Rebecca Delconte[2], Sonia Pastor[1], Vladimir Laletin[1], Costa Da Silva Cathy[1], Armelle Goubard[1], Emmanuelle Josselin[1], Rémy Castellano[1], Adrien Krug[5], Julien Vernerey[1], Raynier Devillier[1], Daniel Olive[1], Els Verhoeyen[5], Eric Vivier[4], Nicholas Huntington[3], Jacques Nunes[1], Geoffrey Guittard[1]

[1]Cancer Research Center of Marseille , Marseille , France; [2]Memorial Sloan Kettering Cancer Center, New York, United States; [3]Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University; oNKo-Innate Pty Ltd., Melbourne, Australia; [4]Innate Pharma Research Labs, Innate Pharma; Aix Marseille Université, Inserm, CNRS, Centre d'Immunologie de Marseille-Luminy, CIML, Marseille, France; Service d'Immunologie, Marseille Immunopole, Hôpital de la Timone, Assistance Publique-Hôpitaux de Marse, Marseille, France; [5]Université Côte d'Azur, INSERM, Nice, France., NICE, France

Keywords: NK cells, immunotherapy , signalling

Cytokine inducible SH2-containing protein (CISH) is a natural killer (NK) cell negative regulator of cytokine signaling pathway. To further understand CISH functions in NK cells, we developed a conditional Cish-deficient mouse model in NK cells (Cishfl/flNcr1Ki/+). We detected no developmental or homeostatic difference in NK cells.

However, global gene expression of Cishfl/flNcr1Ki/+ NK cells

compared to Cish+/+Ncr1Ki/+ NK cells revealed upregulation of pathways and genes associated with NK cell cycling and activation. We show that CISH does not only regulate interleukin-15 (IL-15) signaling pathways but also natural cytotoxicity receptors (NCR) pathways. Indeed, CISH protein expression level increases upon NCR triggering. Primed Cishfl/flNcr1Ki/+ NK cells display increased activation upon NCR stimulation. Cishfl/flNcr1Ki/+ NK cells display lower activation thresholds and Cishfl/flNcr1Ki/+ mice are more resistant to tumor metastasis. Remarkably, we found that Cishfl/flNcr1Ki/+ mice were also more resistant to primary breast cancer growth in addition to superior control of spontaneous tumor metastasis. CISH deletion favors NK cell accumulation to the primary tumor, optimizes NK cell killing properties and decreases TIGIT immune checkpoint receptor expression, limiting NK cell exhaustion. Finally, we argue that specifically enhancing NK cell function is sufficient to boost anti-tumor response to both primary and secondary tumor models. Using CRISPRi, we then targeted CISH in human NK-92 or primary NK cells. According to the results in our mouse model, CISH deletion favors NCR signaling and anti-tumor functions in human NK cells. Our results validate CISH as an emerging therapeutic target to enhance NK cell immunotherapy.

P **03** - 050

CLONAL EXPANSION OF INTRA-EPITHELIAL T CELLS IN BREAST CANCER REVEALED BY SPATIAL TRANSCRIPTOMICS

Lou Romanens[1], Prasad Chaskar[1,2], Rachel Marcone[3], Stephan Ryser[1], Jean-Christophe Tille[4], Raphael Genolet[5], Ketty Hu-Heimgartner[1], Killian Heimgartner[1], Jonathan S. Moore[1], Nicolas Liaudet[6], Gurkan Kaya[7], Mikael J. Pittet[1,2,5,8], Pierre-Yves Dietrich[1,2], Mauro Delorenzi[3,5], Daniel E. Speiser[5], Alexandre Harari[5], Petros Tsantoulis[1,2], S. Intidhar Labidi-Galy[1,2]

[1]Department of Medicine and Center of Translational Research in Onco-Hematology, Faculty of Medicine, University of Geneva, Geneva, Switzerland; [2]Department of Oncology, Hôpitaux Universitaires de Genève, Geneva, Switzerland; [3]SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland; [4]Department of Diagnosis, Division of Clinical Pathology, Hôpitaux Universitaires de Genève, Geneva, Switzerland; [5]Ludwig Institute for Cancer Research and Department of Oncology, University of Lausanne, Lausanne, Switzerland; [6]Bioimaging Core Facility, Faculty of Medicine, University of Geneva, Geneva, Switzerland; [7]Department of Medicine, Division of Dermatology, Hôpitaux Universitaires de Genève, Geneva, Switzerland; [8]Department of pathology and immunology, Faculty of Medicine, University of Geneva, Geneva, Switzerland

Keywords: spatial transcriptomics, RNA-sequencing, laser-capture microdissection (LCM), tumor microenvironment, T-cell receptor (TCR)

The presence of tumor-infiltrating lymphocytes (TIL) correlates with a better outcome in patients with breast cancer. Importantly, their spatial distribution is also a prognostic marker for the outcome of breast cancer patients and their response to systemic therapy, highlighting the importance of an intact tissue structure for the characterization of patients' tumors. Here, we developed a robust method for RNA extraction and exome-capture RNA-sequencing of laser-capture microdissected tumor compartments from formalin-fixed paraffin-embedded (FFPE) samples. We applied this spatial transcriptomics approach to study immune cell infiltration within different tumor compartments (stromal versus intraepithelial) of triple-negative breast cancer. We found a highly variable spatial distribution of immune cell subsets among tumors. This transcriptomic analysis revealed that the immune repertoires of intra-epithelial T and B cells were consistently less diverse than those of stromal T and B cells. T-cell receptor (TCR) sequencing confirmed a reduced diversity and an increased clonality of intra-epithelial T cells as compared to matched stromal T cells. Most clonotypes were present in both intra-epithelial and stromal T cell repertoires, however the cumulative frequency of shared clonotypes was higher in intra-epithelial versus stromal TIL, likely reflecting the expansion of tumor antigen-specific T cells capable of migrating into tumor cell nests. Our method is widely applicable and can elucidate intratumoral immune cell dynamics that may lead to improved anti-cancer immunotherapy.

P **03** - 051

DEVELOPMENT OF AN INNOVATIVE APPROACH FOR MELANOMA TREATMENT USING NEOSPORA CANINUM

Arthur Battistoni[1], Louis Lantier[1,2], Stephanie Germon[1], Isabelle Dimier-Poisson[1]

[1]UMR 1282 ISP Université de Tours - INRAE, Tours, France; [2]Kymeris santé, Tours, France

Keywords: Immunotherapy, melanoma, protozoan, Neospora caninum

Immunotherapy is an innovative cancer treatment based on the stimulation of the immune system of the patient to fight specifically the tumor. This revolutionary therapy includes therapeutic antibodies, CAR-T cells and live microorganisms. These latter show promising results thanks to both their lytic activity and their capacity to reverse the immunosuppressive tumor microenvironment and to elicit both innate and adaptive immune responses. Among oncolytic microorganisms, viruses are mostly explored, but intracellular bacteria or protozoa are also investigated. Neospora caninum – an obligate intracellular protozoan not infectious to humans – is one of those candidates studied by the BioMAP research team.

In a preclinical mouse model of solid tumor (thymic lymphoma EG7), we showed that remote or intratumoral alive N. caninum injection inhibits tumor development, promotes recruitment of immune cells and reprograms immunosuppressed immune cells within the tumor. Moreover N. caninum did not persist more than a week, underlining its safety.

After this proof of concept of N. caninum efficiency against solid tumor, we studied N. caninum antitumoral properties in a metastatic model such as lung metastasis melanoma B16F10. In this model, three administration routes (subcutaneous, intravenous and intranasal) of N. caninum were tested. With each of them, N. caninum was able to control the metastasis development in the lung, but with an improved efficacy via the intranasal and intravenous routes. This control of tumor development was associated within the lungs with a recruitment of NK cells and activated macrophages characterized by an up-regulation expression of MHC-II and a polarization towards a M1 phenotype.

To optimize the effectiveness of N. caninum, an engineered strain able to secrete human IL-15 was designed. An even better antitumoral protection was obtained and mechanisms underlying this improved efficiency are currently analyzed.

In order to validate the antitumoral properties of N. caninum to human cancers, we developed explant and spheroid technology on human melanoma metastasis explants. Promising results were obtained after N. caninum treatment with induction of antitumoral cytokines production and control spheroid growth.

All these results highlight the great potential of N. caninum as a novel and promising cancer immunotherapeutic agent.

P **03** - 052

ANTI-PD-1 REINVIGORATE VG9VD2+ T CELLS IN HUMAN BREAST CANCER

Stephane Fattori[1,2], Laurent Gorvel[1,2], Samuel Granjeaud[3], Amira Ben Amara[1,2], Nicolas Boucherit[1,2], Marie-Sarah Rouvière[1,2], Philippe Rochinieux[1,2,4], Anthony Goncalves[4,8], Carole Tarpin[4], Jihane Pakradouni[5], Eric Lambaudie[6,7], Gilles Houvenaeghel[6,7], François Bertucci[4,8], Jacques A. Nunès[1], Anne-Sophie Chrétien[1,2,7], Daniel Olive[1,2,7]

[1]Team Immunity and Cancer, Centre de Recherche en Cancérologie de Marseille (CRCM), Inserm, U1068, CNRS, UMR7258, Institut Paoli-Calmettes, Aix-Marseille University, UM105, Marseille, France; [2]Cancer Immunomonitoring Platform, Centre de Recherche en Cancérologie de Marseille (CRCM), Inserm, U1068, CNRS, UMR7258, Institut Paoli-Calmettes, Marseille, France; [3]Systems Biology Platform, Centre de Recherche en Cancérologie de Marseille (CRCM), Inserm, U1068, CNRS, UMR7258, Institut Paoli-Calmettes, Aix-Marseille University, UM105, Marseille, France; [4]Department of Medical Oncology, Institut Paoli-Calmettes, Marseille, France; [5]Department of Clinical Research and Innovations, Institut Paoli-Calmettes, Marseille, France; [6]Department of Surgical Oncology, Institut Paoli-Calmettes, Marseille, France; [7]Faculty of Medical and Paramedic Sciences, Aix Marseille University, UM105, Marseille, France; [8]Team Predictive Oncology, Centre de Recherche en Cancérologie de Marseille (CRCM), Inserm, U1068, CNRS, UMR7258, Institut Paoli-Calmettes, Aix-Marseille University, UM105, Marseille, France

Keywords: single-cell RNA sequencing , Vg9Vd2+ T cell , breast cancer, cancer immunotherapy, pembrolizumab

Vγ9Vδ2+ γδ (Vγ9Vδ2+) T cells are promising therapeutic targets with efficient antitumor properties both in vitro and in preclinical models of breast cancers. Yet, little information has been reported regarding their functional state in human breast cancers, owing to scarce Vγ9Vδ2+ T cell infiltrates. Here, we employed single-cell transcriptomic and proteomic analytic tools to provide a comprehensive landscape of Vγ9Vδ2+ T cells from untreated and anti-PD-1-treated human breast cancers. We report skewed Vγ9Vδ2+ T cell differentiation profiles trending towards early memory Tc1 phenotypes, along with no detectable terminally differentiated phenotypes, both in untreated and pembrolizumab-treated breast tumors as opposed to normal tissues. Importantly, pembrolizumab monotherapy increased activating hallmarks in breast tumor-infiltrating Vγ9Vδ2+ T cells. Our data may provide central information for the development of Vγ9Vδ2+ T cell-based immunotherapies in breast cancer.

P **03** - 053

TERTIARY LYMPHOID STRUCTURES REQUIRE A SPECIFIC IMMUNE MICROENVIRONMENT AND IMPROVED PROGNOSIS IN CERVICAL CANCER

Laurent Gorvel[1], Stephane Fattori[1], Marylou Panouillot[2], Jumaporn Sonongbua[1], Nicolas Boucherit[1], Marie-Sarah Rouvière[1], Samuel Granjeaud[3], Clara Degos[1], Amira Ben Amara[1], Xavier Carcopino[4], Eric Lambaudie[5], Renaud Sabatier[6], Jacques Nunes[1], Anne-Sophie Chretien[1], Marie-Caroline Dieu-Nosjean[2], Daniel Olive[1]

[1]Tumor Immunology laboratory, INSERM U1068, Immunomonitoring platform, Cancer Research Center in Marseille, Paoli-Calmettes Institute, Marseille, France; [2]Centre d'Immunologie et des Maladies Infectieuses, Cimi-Paris, INSERM U1135, Paris, France; [3]CRCM Integrative Bioinformatic Facility, Cibi, Cancer Research Center in Marseille, Paoli-Calmettes Institute, Marseille, France, Marseille, France; [4]Aix-Marseille Univ, Department of Obstetrics and Gynecology, Hôpital Nord, APHM, Marseille, France; [5]Department of Surgical Oncology, Institut Paoli-Calmettes, CRCM, Marseille, France, Marseille, France; [6]Institut Paoli-Calmettes, Department of Medical Oncology, Marseille, France, Marseille, France

Keywords: Tertiary Lymphoid Structures, Cervical cancer, Tumor microenvironment, Tumor Inifltrating Lymphocytes, Pronostic

Cervical Cancer represents the 4th cause of women cancer-related death worldwide. It is mainly caused by HPV infection. Cervical cancer development is a very dynamic process involving viral replication cycle, cervical epithelium differentiation and local inflammation driven by the immune system. Unfortunately, the cervical tumor microenvironment and its interaction with the immune system are poorly understood. Tertiary lymphoid structures (TLS) are lymphnode-like structures forming in tissues in response to inflammation. Their formation requires higher immune cell infiltrate along with cytokines and chemokines, and overall vascularization. Usually, TLS are associated with better prognosis. In this study, we study cervical tumor TLS and their incidence on patient outcome by multiplex IHC, soluble factor measurements by luminex, mass cytometry and transcriptomics. First, we quantified B, T and dendritic cells, assessed their density by IHC to identify TLS+ and TLS- tumors. TLS+ tumors were associated with increased chemokine, inflammatory cytokine and IgG, M and A secretion compared to TLS- or healthy control. Using CyTOF, we assessed the immune cell composition and phenotype in TLS+ and TLS- tumors. We showed that the presence of cDC2, B cells, CD8+CD69+ T cells and CD4+ TEMRA cells were associated with the presence of TLS. Overall, immune checkpoint markers were enriched on TLS+ tumors. Indeed, CD40 was expressed on cDC2, PD1 on CD8+Tcells, DNAM-1 on NK cells in TLS+ tumors compared to TLS-. Using these data we could define a phenotypic signature of TLS+ vs TLS- tumors by using the BGA algorithm. Finally we demonstrated that the presence of TLS and the enrichment in cDC2 and Bcells are almarks of patient survival in our cohort and in Immune cell enrichment analysis on cervical cancer TCGA dataset. Taken together, our results show that TLS can be considered as a marker of improved prognosis and immunotherapy response in cervical cancer.

P **04** - 001

INTRATUMORAL MICROBIOTA REMODELS TUMOR IMMUNE MICROENVIRONMENT AND INFLUENCES CHEMO-IMMUNOTHERAPY RESPONSE IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA

Wu Hong[1], Mao Tianqing[1], Liu Qianshi[1], Liu Yiqiang[1], Liang Renchuan[1], Xu Chuan[1]

[1]University of Electronic Science and Technology of China, Cheng Du, China

Keywords: Intratumoral Microbiota, Chemo-immunotherapy, Immune Microenvironment, ESCC

Background: Intratumoral microbiota have been identified in various tumors and influences survival and immunoactivation in pancreatic cancer and melanoma. Whether intratumoral microbiota exist and impact the immune microenvironment, and subsequently affects the response to neoadjuvant chemo-immunotherapy (NACI) in esophageal squamous cell carcinoma (ESCC) remain unknown.

Aim: To comprehensively delineate the intratumoral microbiota signature including composition and diversity, and investigate its impact on NACI efficacy, we performed a comprehensive analysis of the intratumoral microbiota in tumor tissues, paired adjacent normal tissues and peripheral blood from locally advanced ESCC patients.

Methods: Using 16S rRNA gene sequencing, we analyzed the intratumoral microbiota signatures including composition and diversity in tumor tissues and adjacent normal tissues of 27 patients with locally advanced ESCC who underwent NACI. The complex cellular and microenvironmental composition of ESCC were obtained through Immunohistochemical staining (IHC). The impact of intestinal microbiota on intratumoral microbiota and NACI efficiency were assessed by FMT from NACI response and no response patients.

Results: We found significantly distinct intratumoral microbiota signatures between NACI responders and no-responders. Intratumoral microbiota signatures were associated with the anti-tumor immune cells infiltration, suggesting responsive intratumoral microbiota signatures could have enhanced anti-tumor immunity. In vivo, we confirmed that fecal transplants from responders potentiated immune checkpoint inhibitors response.

Conclusions: Collectively, our study reveals that intratumoral microbiota signatures could predict NACI response via shaping tumor immune microenvironment in ESCC, and thus provide insights into future application of intratumral microbiota in this clinical scenario.

P **04** - 002

TGF-BETA SIGNALLING IN T CELLS PREVENTS TRANSFORMATION OF THE ANORECTAL JUNCTION EPITHELIUM

Valentin Thevin[1], Hector Hernandez-vargas[1], Nicolas Benech[1], David Bauché[1], Géraldine Guash[2], Julien Marie[1]

[1]Department Tumor Escape Resistance Immunity, Centre de Recherche en Cancérologie de Lyon (CRCL), Lyon, France; [2]Aix-Marseille University, CNRS, INSERM, Institut Paoli-Calmettes, CRCM, Epithelial Stem Cells and Cancer Team, Marseille, France

Keywords: Microbiota, Inflammation, stem cells

The intestinal mucosa is highly enriched in Transforming Growth Factor Beta (TGF-b), a cytokinic polypeptide known to play a critical role in the repression of numerous T lymphocyte functions. SMAD4 and TRIM-33 are the two main actors by which TGF-b signals. Here, we report that the selective deletion of Smad4 and Trim33 in T lymphocytes led to chronic inflammation in the distal part of the colon in association with the transformation of the anorectal junction in 100% of the animals. This localized transformation was supported by the exacerbated permeability of the epithelial cells and penetrance of bacteria into the lamina propria. Interestingly the tumor at the anorectal junction exhibit a double origin squamous and glandular, and scRNAseq analysis demonstrated a hypersensitivity of stem cells present at the anorectal junction to chronic inflammation. Spatial analysis allowed us to establish an interaction map between immune cells and stem cells. In sum, this study reveals an unexpected cross-talk between T lymphocytes and epithelial stem cells at the anorectal junction that is controlled by both TGF-b signalling in T lymphocytes and the microbiota and prevents from anorectal transformation.

P **04** - 003

SKIN MICROBIOME LIMITS CIS-UROCANIC ACID INDUCED IMMUNE SUPPRESSION

Vijaykumar Patra[1,2], Slave Trajanoski[3], Chloé Goyet[1], Meghana Somlapura[4], Harald Köefeler[6], Camille Braun[1], Jean-Francois Nicolas[5], Peter Wolf[2], Marc Vocanson[1]

[1]Centre International De Recherche En Infectiologie, Lyon, France; [2]Department of Dermatology, Medical University of Graz, Graz, Austria; [3]Computational bioanalytics, Center for Medical Research, Medical University of Graz, Graz, Austria; [4]Diagnostic and Research Institute of Pathology, Medical University of Graz, Graz, Austria; [5]Allergy and Clinical Immunology Department, Lyon Sud University Hospital, Lyon, France; [6]Core Facility for Mass Spectrometry, Medical University of Graz, Graz, Austria

Keywords: skin microbiome, immune suppression, metabolism, urocanic acid, ultraviolet radiation

Urocanic acid (UCA) is one of the key mediators of ultraviolet radiation (UVR)- induced immune suppression in the skin. Upon UV exposure trans-UCA which is localized in upper layers of skin is isomerized to its immunosuppressive cis isoform. Skin resident microbes are known to degrade/metabolize trans- and cis-UCA and hypothesized to potentially modulate the subsequent immune response. In the present study by using 16s rRNA sequencing, we show that cis-UCA application results in significant changes in microbial population, where certain microbes could thrive by utilizing UCA. In vitro experiments indeed revealed that these microbes degrade/metabolize UCA for their growth and survival. Finally, using murine models and immune assays, we show that presence of skin microbiome limits immunosuppressive capacity of cis-UCA, by depleting its bioavailability. Taken together, our results demonstrates that the skin microbiome can regulate cis-UCA mediated immune suppression.

P **04** - 004

EXPRESSION OF KRAS MUTATION AND LOSS OF A C-TYPE LECTIN COOPERATES TO SUSTAIN A BACTERIAL-INDUCED IMMUNE-SUPPRESSIVE MICROENVIRONMENT IN RIGHT-SIDED COLON CANCER

Nilmara de Oliveira Alves[1], Amaury Vaysse, Guillaume Dalmasso, Olivier Boulard, Thomas Paz Del Socorro, Pierre Sauvanet, Lionel Poulin, Iradj Sobhani, Richard Bonnet, Mathias Chamaillard[1]

[1]University of Lille, Inserm, U1003, F-59000 Lille, France.

Key-words: Kras mutation, microbiota, lipid metabolic and colibactin.

Mutational activation of KRAS have been associated with poorer survival and tumor resistance, but whether such mutations induce gut microbiota dysbiosis that contributes to the development of right-sided colon cancer (RCC), remains unknown. We hypothesized that gut dysbiosis in patients with KRAS mutation status may induce changes in the the metabolome and in the biosynthesis of bacterial genotoxins. Herein, we found that the regenerating family member 3 alpha (Reg3A), a gene encodes a C-type lectin that demonstrates bactericidal activity, has significant negative correlation with lipid metabolic pathway in patients with KRAS-mutant tumors (r=-0.89 and p=0.006). Interestingly, when analyzing wild type KRAS patients, there was no significant correlation (r=-0.37 and p=0.07). Furthermore, this RCC patients' group with poorer progression free-survival showed a greater colonization with colibactin-producing bacteria, that probably modulates genes involved in the lipid metabolic pathway. In Reg3b-knockout mice, tumors have similarities to human tumors, including differential regulation of defensin expression, neutrophils and macrophages signatures. Of particular note, in a subset of patients, including KRAS mutation status, characterized by CMS2 (canonical) and CMS3 (metabolic cancer phenotype); we observed that these tumors are associated with an increase in Reg3A and PDL1 (r=0.42 and p=0.01) and inversely correlated with Arginase1 (r=-0.38 and p=0.03). These data reveal that Reg3A expression play a critical role in the gut dysbiosis, which may be influenced by RCC-associated genetic alterations (KRAS mutation) and sensitivity to immunotherapy.

P **04** - 005

ANTIBIOTICS DISRUPT THE ILEAL MADCAM-1/A4B7 AXIS, COMPROMISING TUMOR IMMUNOSURVEILLANCE DURING PD-1 BLOCKADE

Marine Fidelle[1,2], Conrad Rauber[1,2], Gladys Ferrere[1,2], Romain Daillère[4], Meriem Messaoudene[5], François-Xavier Danlos[1,2], Benjamin Besse[2], Aurélien Marabelle[1,2,10], Bertrand Routy[5,11], Lisa Derosa[1,2,3], Guido Kroemer[2,6,7,8,9], Laurence Zitvogel[1,2,3,10]

[1]U1015 INSERM, Paris Saclay University; Gustave Roussy Campus Cancer, Villejuif, France; [2]Gustave Roussy Campus Cancer, 114, rue Édouard Vaillant, Villejuif, France; [3]Clinicobiome, Programme médico-scientifique Gustave Roussy, Villejuif, France; [4]EverImmune, Gustave Roussy Cancer Campus, Villejuif, France; [5]University of Montreal Research Center, CRCHUM, Montreal, Canada; [6]Centre de Recherche des Cordeliers, Inserm U1138, Université de Paris, Sorbonne Université, Paris, France; [7]Team «Metabolism, Cancer & Immunity» labellisée par la Ligue contre le Cancer, Paris, France; [8]Cell Biology and Metabolomics platforms, Gustave Roussy Cancer Campus, Villejuif, France; [9]Pôle de Biologie, Hôpital Européen Georges Pompidou, AP-HP, Paris, France; [10]Center of Clinical Investigations in Biotherapies of Cancer (BIOTHERIS), Villejuif, France; [11]Hematology-Oncology Division, Department of Medicine, Centre Hospitalier de l'Université de Montréal (CHUM), Montreal, Canada

Keywords: Microbiota, Antibiotics, MAdCAM-1, Immunotherapy, Cancer

Introduction

Numerous studies showed that antibiotics (ATB) compromise the outcome of immune checkpoint inhibitors (ICI) in cancer patients. Mechanisms underlying their immunosuppressive effects remain unknown. Given the impact of gut microbiota on ICI efficacy, we hypothesized that gut immune cells might interfere with immune responses in cancer. Gut-specific homing relies on leukocyte expression of the integrin α4β7 and its binding to the addressin MAdCAM-1, which is expressed in gut-associated lymphoid tissue (GALT). Our goal was to understand the role of the intestinal MAdCAM-1/α4β7 axis in PD-1 blockade efficacy and its relationship with microbiota.

Methods

The effect of ATB-induced dysbiosis on MAdCAM-1 levels was assessed in mouse and human. In mice, dysbiosis was induced by broad-spectrum ATB and microbial recolonization following ATB cessation was studied. Oral gavages with bacteria or feces from Non/Responder (NR/R) patients to ICI were performed in mice. The dynamics of intestinal cell migration to the tumor microenvironment (TME) were assessed using in vivo cell tracing models and blocking MAdCAM-1 with a specific antibody. Finally, the soluble form of MAdCAM-1 in plasma (sMAdCAM-1), a mirror of its intestinal level, was measured in two cohorts of lung cancer patients treated with ICI.

Results

We showed that ATB decreased ileal and soluble MAdCAM-1 levels in mice and patients. Recolonization after ATB was accompanied by an overrepresentation of Enterocloster genus in mice, as in NR patients. The ileal MAdCAM-1 level depends on the gut microbiota composition: increased by bacteria found in R patient feces (Akkermansia muciniphila) and decreased by Enterocloster spp after oral gavage. We also discovered that PD-1 blockade efficacy relies on ileal MAdCAM-1. Disruption of MAdCAM-1 with ATB or antibody led to the loss of anti-PD-1 efficacy in mice. This mechanism relied on the emigration of immunosuppressive gut tropic α4β7 cells to the TME. Finally, sMAdCAM-1 levels in lung cancer patients predict resistance to ICI.

Conclusion

Our findings uncover the pivotal role of the small intestine and gut microbiota in hosts facing carcinogenesis, in which GALT, involving the expression of MAdCAM-1, controls the emigration of immunosuppressive gut tropic cells. Remarkably, sMAdCAM-1 levels could be a novel in-clinic biomarker to predict ICI resistance in patients with lung cancer treated with ICI.

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Session 5

Genetics and epigenetics

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P **05** - 001

FUNCTION OF ATM AND MSH2 IN B CELL CLASS SWITCH RECOMBINATION

Emily Sible[1], Mary Attaway[1], Nyima Sherpa[1], Alec Wishnie[1], Tzippora Chwat-Edelstein[1], Giuseppe Fiorica[1], Bao Q. Vuong[1]

[1]City College of New York (CUNY), New York, United States

Keywords: B cell, CSR, ATM, MSH2, DNA repair

Class switch recombination (CSR) produces secondary immunoglobulin isotypes and requires AID-dependent DNA deamination of intronic switch (S) regions within the immunoglobulin heavy chain gene locus. Non-canonical repair of deaminated DNA by mismatch repair (MMR) or base excision repair (BER) creates DNA breaks that permit recombination between distal S regions. ATM-dependent phosphorylation of AID at serine-38 (pS38-AID) promotes its interaction with APE1, a BER protein, suggesting that ATM regulates CSR through BER. However, pS38-AID may also play a role in MMR during CSR, although the mechanism remains unknown. To examine whether ATM modulates BER- and/or MMR-dependent CSR, ATM-/- mice were bred to mice deficient for the MMR gene MSH2. Surprisingly, the predicted Mendelian frequencies of ATM-/-MSH2-/- adult mice were not obtained. To obtain ATM- and MSH2-deficient B cells, ATM was conditionally deleted on an MSH2-/- background using a floxed ATM allele [ATMF] and B cell-specific cre recombinase expression (CD23-cre) to generate a deleted ATM allele (ATMD). As compared to the ATMD/D and MSH2-/- mice and B cells, the ATMD/DMSH2-/- mice and B cells display a reduced CSR phenotype. Interestingly, Sμ-Sγ1 junctions from ATMD/DMSH2-/- B cells that were induced to switch to IgG1 in vitro revealed a significant loss of blunt end joins and an increase in insertions as compared to wildtype, ATMD/D, or MSH2-/- B cells. This data suggests that the absence of both ATM and MSH2 blocks nonhomologous end joining (NHEJ) and leads to inefficient end joining and the reduced CSR. We identify complementary roles for ATM and MSH2 in NHEJ and alternative end joining (A-EJ) during CSR and propose a model whereby ATM and MSH2 function cooperatively to regulate end-joining during CSR through pS38-AID.

P **05** - 002

KALLIKREIN-8 EXPRESSION IN MELANOMA AND ITS INFLUENCE ON ACTIVIN-A PROCESSING AND SIGNALING

Manon Bulliard[1], Katarina Pinjusic[1], Laura Iacobucci[1], Pierpaolo Ginefra[1,2], Daniel Constam[1]

[1]EPFL, Lausanne, Switzerland; [2]UNIL, Lausanne, Switzerland

Keywords: Melanoma, Activin-A, precursor cleavage, Kallikrein-8

Activin-A is upregulated in multiple cancer types, including melanoma, where a shift from autocrine to paracrine signaling promotes primary and metastatic tumor growth by facilitating immune evasion. To enable receptor binding, Activin-A must mature from a precursor dimer of two βA chains, but how this endoproteolytic cleavage is regulated is unknown. Recently, we reported that one βA chain is cleaved at the expected RRRRR motif independently of known proprotein convertases (PCs) such as Furin. Here, RNAi screening in B16-F1 melanoma cells reveals that PC-independent hemicleavage of proActivin-A involves Kallikrein (Klk)-8. In line with a role in Activin signaling, KLK8 expression in melanoma correlates with INHBA mRNA. In addition, we show that while PC-independent cleavage of one βA chain is sufficient for receptor binding and SMAD3 signaling in vitro, strategies that selectively block cleavage by Klk8 or by Furin, respectively, each suppressed the tumor-promoting activity of Activin-A in vivo. Together, these findings suggest that interfering with cleavage of either of the two βA chains of Activin-A holds promise as therapeutic strategies to boost anti-tumor immunity in melanoma.

P **05** - 003

SELECTIVE CONTROL OF CD4+ T-CELL FUNCTION IN CANCER AND AUTOIMMUNUNITY BY CANONICAL NF-KB SUBUNITS

Guilhem Lalle[1], Raphaëlle Lautraite[1], Allison Voisin[1], Maud Ligier[1], Khaled Bouherrou[1], Julie Caramel[1], Christophe Caux[1], Ulf Klein[2], Sankar Ghosh[3]

[1]Cancer Research Center of Lyon, UMR INSERM 1052, CNRS 5286, Université Claude Bernard Lyon 1, Centre Léon Bérard, 69008, Lyon, France; [2]Division of Haematology & Immunology, Leeds Institute of Medical Research at St. James's, University of Leeds, Leeds, United Kingdom; [3]Department of Microbiology & Immunology, College of Physicians & Surgeons, Columbia University, New York, United States

Keywords: NF-kB, Conventional CD4+ T cells, Autoimmunity, Cancer

Conventional CD4+ T cells (Tconv cells) play multifaceted roles in immune responses. Whereas they are critical to shape the tumor immune microenvironment and limit cancer progression, their uncontrolled activation can also lead to autoimmunity. The identification of intracellular pathways and transcription factors orchestrating their biology is thus of utmost importance for the discovery of novel biomarkers and immunotherapeutic targets. The NF-kappaB (NF-κB) family of transcription factors has been largely associated with the regulation of immune responses; however, the contribution of each subunit of the family is to date lacking due to the absence of adequate study models.

Here we explored the specific contributions of the RelA and C-Rel subunits of the canonical NF-kB pathway in Tconv biology, using conditional knock-out mouse models and CRISPR-edited primary human Tconv cells.

Transcriptome analyses of in vitro stimulated murine Tconv cells isolated from mice carrying conditional ablation of each gene in T cells, demonstrated a dramatic effect of RelA ablation on the expression of cytokines and activation markers, while C-Rel ablation led to more subtle effects. ChIP-Seq analyses revealed that RelA modulated Tconv gene expression patterns both by directly binding to important genes and through the regulation of other master transcription factors. In in vitro assays, RelA was required for the optimal proliferation and effector function of Tconv as well as THelper (TH)1 and TH17 polarization. Consistent with this, RelA-deficient mice were fully protected against autoimmune symptoms in murine models of multiple sclerosis and inflammatory bowel disease.

Surprisingly, in the context of cancer, acute ablation of C-Rel, but not RelA, in Tconv cells, dramatically increased the growth of transplanted tumors. This was associated with reduced production of inflammatory cytokines in the tumor microenvironment and highlighted a major role for C-Rel in orchestrating cancer immunity.

Finally, in an effort of translational research, we showed that CRISPR-Cas9-mediated ablation of RELA and C-REL in human primary Tconv, led to defective proliferation and cytokine secretion, associated with impaired gene expression profiles comparable to those observed in mouse cells.

Together, our data demonstrate a division of labor between the different subunits of the NF-kB pathway, paving the way for subunit-targeted immunotherapies.

P **05** - 004

EVOLUTIONARY AND FUNCTIONAL DIVERSIFICATION OF A BROAD-SPECTRUM ANTIVIRAL IMMUNE EFFECTOR IN BATS

Stéphanie Jacquet[1,2,3], Michelle Culbertson[4], Chi Zang[5], Adil El Filali[1], Clément de La Myre Mory[2], Jean-Baptiste Pons[3], Ondine Filippi[3], Jeanne Duhayer[1], Barthélémy Ngoubangoye[6], Chorong Park[5], Clayton Carey[4], Greg Brennan[5], Andrea Cimarelli[2], Stefan Rothenburg[5], Nels Elde[4], Dominique Pontier[1,3], Lucie Etienne[2]

[1]Laboratoire de Biométrie et Biologie Evolutive (LBBE), UMR 5558, UCBL1, CNRS, Lyon, France; [2]Centre International de Recherche en Infectiologie (CIRI), CNRS UMR5308, INSERM U1111,Université Lyon 1, ENS de Lyon, Lyon, France; [3]LabEx ECOFECT (ANR-11-LABX-0048) de l'Université de Lyon, Lyon, France; [4]Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, United States; [5]School of Medicine, University of California Davis, Department of Medical and Immunology, Davis, United States; [6]International Center of Medical Research of Franceville, Primatology Centre, Franceville, Gabon

Keywords: bats, PKR, gene duplication, innate immunity, evolution

Bats host several high-profile zoonotic viruses but they appear

asymptomatic to most of them. One hypothesis is that bats possess a balance between immune resistance and viral tolerance protecting them from pathogenesis.
Here, we combined novel genetic data, genomic / phylogenetic analyses, and functional assays to decipher the mechanisms underlying the interplay between viruses and bat immunity.
We characterized the diversification of a major innate immune protein with broad antiviral spectrum, the Protein kinase RNA (PKR), in 33 bat species, including 14 newly sampled ones. Our evolutionary analyses reveal that the gene has been under recurrent positive selection in bats, and has undergone repeated genomic duplications - an intriguing finding since a single copy is conserved in all studied mammals.
To assess the functional impacts and drivers of adaptation, we ectopically expressed the bat PKRs and assayed for cellular functions, and sensitivity to antagonist proteins from primate and bat viruses. We showed that bat orthologs and paralogs encode functional proteins that differ in their ability to resist viral antagonists in a species-specific manner. In particular, the host-virus determinants overlap with the adaptive sites, and swapping these sites in natural variants drives the virus antagonist-host specificity. Finally, we show that the paralogs have differential capacity to restrict poxviral replication, indicating functional diversification of their antiviral activity.
Altogether, our findings suggest that ancient conflicts with pathogenic viruses have driven the adaptation of a broad antiviral immune protein in bats. These changes impact modern virus-bat interactions and may account for bat specific immunity.

P **05** - 005

REITERATIVE MODELLING OF COMBINED TRANSCRIPTOMIC AND PROTEOMIC FEATURES REFINES AND IMPROVES THE PREDICTION OF EARLY RECURRENCE IN SQUAMOUS CELL CARCINOMA OF HEAD AND NECK

Amir Salehi[1], Lixiao Wang[1]

[1]Umeå university, Umeå, Sweden

Keywords: artificial intelligenc, machine learning, XGBoost, SCCHN, Recurrence

Introduction: Patients with squamous cell carcinoma of the head and neck (SCCHN) have a high-risk of recurrence. We aimed to develop machine learning methods to identify transcriptomic and proteomic features that provide accurate classification models for predicting risk of early recurrence in SCCHN patients.
Objectives: The objective was to build an artificial intelligence model by implementing a comprehensive analysis of SCCHN early recurrence risk using clinical data, high-throughput genomic, transcriptomic and reverse phase protein array (RPPA) proteomic data derived from TCGA datasets.
Methods: Clinical, genomic, transcriptomic and proteomic features distinguishing recurrence risk in SCCHN patients from The Cancer Genome Atlas (TCGA) were examined. Recurrence within one year after treatment was regarded high-risk and no recurrence low-risk.
Results: Using conventional statistical analysis no significant differences in individual clinical characteristics, mutation profiles or mRNA expression patterns were seen between the groups.
Using the machine learning algorithm extreme gradient boosting (XGBoost) ten proteins (RAD50, 4E-BP1, MYH11, MAP2K1, BECN1, NF2, RAB25, ERRFI1, KDR, SERPINE1) and five mRNAs (PLAUR, DKK1, AXIN2, ANG and VEGFA) made the greatest contribution to classification. These features were used to build improved models to predict recurrence based on XGBoost, achieving the best discrimination performance when combining transcriptomic and proteomic data.
Conclusion: This study highlights machine learning to identify transcriptomic and proteomic factors that play important roles in predicting risk of recurrence in patients with SCCHN and develop such models by iterative cycles to enhance their accuracy, thereby aiding the introduction of personalized treatment regimens.

P **05** - 006

DIFFERENTIAL GENE REGULATION THROUGH TYPE I IFN DEPENDENT PATHWAYS IN PRIMARY MOUSE MYELOID CELLS

Marisol Alvarez-Martinez[1], Xuemei Wu[1], Anne O'Garra[1,2]

[1]Immunoregulation and Infection Laboratory, The Francis Crick Institute, London, United Kingdom; [2]National Heart and Lung Institute, Imperial College London, London, United Kingdom

Keywords: Monocyte, Neutrophil, Type I IFN, LPS, Multi-omics

Monocytes and neutrophils reside in the bone marrow and can be found in the circulation at the steady state. Upon immune challenge, they are promptly recruited to sites of injury, infection and inflammation where they play a key role in protective immune responses and pathogen clearance, largely by their production of proinflammatory cytokines. However, if such responses are not properly regulated this may result in pathology and chronic inflammation. Although both monocytes and neutrophils can respond to pathogen derived products, such as LPS, a component of gram-negative bacteria, each have different roles during infection. My research aims to investigate how responses to LPS are regulated in primary monocytes and neutrophils using in vitro and in vivo models of inflammation.
Herein, I show that primary murine bone marrow monocytes and neutrophils show differential transcriptional and epigenetic responses in vitro to LPS, which signals through the receptor TLR4. Furthermore, monocytes and neutrophils showed different requirements to the autocrine signals resulting from type I IFN, which signals through the type I IFN receptor (IFNAR). Neutrophils showed a potent transcriptomic signature in response to LPS, which was partially reduced upon deletion of Ifnar1. On the contrary, monocytes showed a modest response to LPS, which was mostly dependent on the presence of type I IFN signalling. Additionally, complementary ATAC-seq data on these in vitro stimulated primary cells, revealed minimal chromatin remodelling upon LPS treatment only in neutrophils, but a considerable change in the accessibility of chromatin upon deletion of Ifnar1 in primary monocytes and to a lesser degree in neutrophils.
To verify the physiological relevance of these findings, I am assessing the dependency of type I IFN in shaping responses of monocytes and neutrophils to LPS in vivo in a peritoneal cavity LPS immune challenge model. I show that monocyte and neutrophil recruitment to the site of inflammation is partially decreased in the absence of IFNAR signalling. Integration of the "omic" datasets produced by my research will be key to define the gene regulatory networks underlying TLR4 and type I IFN responses in myeloid cells, forming the basis of their response during in vivo immune challenges.
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P **05** - 007

A CGAS-DEPENDENT RELA RHEOSTAT TUNES INNATE-LIKE INTERFERON I/III RESPONSES IN HUMAN T CELLS

Nadia Jeremiah[1], Kevin De Azevedo[1], Hermine Ferran[1], Jovan Nikolic[1], Mathieu Maurin[1], Philippe Benaroch[1], Nicolas Manel[1]

[1]Institute Curie, Paris, France

Keywords: RELA, IFN, CGAS, STING, IFN-I/III

The classic paradigm of immunology describes a division of labor between the innate and adaptive immune cell types. Innate immune cells sense the presence of danger and respond by producing cytokines, upregulating co-stimulatory molecules and presenting antigens to adaptive immune cells. Adaptive immune cells require antigenic peptide presentation to be activated, expand and mediate helper and effector functions. However, intracellular innate sensors are also expressed in adaptive cells, indicating their potential to mediate innate functions. T cells are not generally recognized for having the capacity to efficiently detect and signal pathogens or danger the way innate immune cells do. The mechanisms that differentiate innate and adaptive cells downstream of PRR are elusive. Here, we identify a transcriptional rheostat orchestrated by RELA in human CD4+ T cells

that confers upon them innate-like capabilities to produce interferon I/III, resulting in enhanced antiviral protection and anti-tumor activity. The cGAS-STING pathway recognizes dsDNA in both infectious and non-infectious contexts leading to type I and III interferon (IFN-I/III) responses. We found that while cGAS-STING signaling is intact in CD4+T cells, IFN-I/III responses are restricted compared to dendritic cells or macrophages. We identify RELA and functional mutants of RELA that tune the IFN-I/III response, at baseline and in response to STING stimulation. We further show that IRF7 is an essential positive regulator of the IFN-I /III responses mediated by RELA. Tonic cGAS signaling is required to activate the IFN-I feedback. A DNA demethylating drug, 5-azacytidine, and increased IRF3 levels additionally enhance STING mediated IFN-I/III responses in CD4+ T cells. By combining RELA, IRF3 and DNA demethylation, IFN-I/III production is unlocked in CD4+T cells to levels observed in dendritic cells. We show that this intrinsic IFN production arms CD4+ T cells against HIV infection. We also show that RELA enhances the ability of CAR T cells to eliminate solid spheroid tumors. These results demonstrate that innate-like functions can be tuned and leveraged in human T cells for therapeutic purposes. We propose that the constraint on IFN-I/III expression in T cells is a mechanism to avoid pathogenic levels of a toxic cytokine from a cell type that constitutes a large proportion of blood cells.

P **06** - 001

EARLY HEPATITIS B VIRUS EXPOSURE IMPACTS IMMUNE CELL GENE EXPRESSION IN HUMAN AND MONKEY MODELS

Armando Andres Roca Suarez[1], Bao Vuong[2], Donna Bedasee[2], Uzma Hasan[3], Isabelle Chemin[1]

[1]INSERM U1052, LYON, France; [2]Dept of Biology Graduate Center, New York, United States; [3]Centre International de Recherche en Infectiologie (CIRI, Inserm U1111, ENS, UCBL, CNRS), Lyon, France

Keywords: Hepatitis B, Immune cells, Human, Monkey, RNASeq

The evasion of both innate and adaptive immune responses by microbial pathogens is a well documented series of mechanisms aimed to favour pathogen replication, survival and long-term persistence. A clear example of this is Hepatitis B virus (HBV) infection, as chronic persistence of the virus is the primary consequence of infection. Both infection control and damage to hepatocytes are strictly dependent on protective immune responses, since damage to hepatocytes is the price the host pays for shedding intracellular viruses. Indeed, resolution of acute hepatitis B is associated with a functionally potent multi-specific antiviral T cell response, followed by a weak induction of intracellular innate responses during the early stages of infection. However, a cell-specific characterization of the transcriptional profiles associated with this early responses still requires exploration. The development of potential HBV therapeutics has been hampered by the lack of a suitable infection/efficacy model. Non Human Primate models such as cynomolgus macaques are widely used in virological studies. It is believed that HBV cure will be a multi-layered combination approach of anti-viral and immune-boosting strategies. Thus we wanted to evaluate and compare the PBMC's response to HBV exposure in both human and Macaque PBMCs.

In this project, we studied the early gene expression changes of human and macaque peripheral blood mononuclear cells (PBMCs) exposed to HBV during a two-hour period. PBMCs were then sorted into T cells, B cells, myeloid dendritic cells (mDCs), and plasmacytoid dendritic cells (pDCs) populations. For each cell type we compared control vs HBV-stimulated cells, employing six samples per species, which were then used to perform bulk RNA-sequencing. Our initial differential expression analysis revealed a wide variety of transcriptional changes, providing a detailed characterization of the very early immune response associated with HBV. Moreover, our data represents a useful resource that allows not only comparison between immune cell types, but also the gene expression changes that are shared between both species. Currently ongoing analyses are focused on the catergoryzation of these transcriptomic modifications into common signaling pathways and evaluating if these alterations persist at later stages of the disease.

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P **06** - 002

INTEGRATIVE SYSTEMS IMMUNOLOGY ANALYSIS REVEALS NEW IMMUNOMODULATORY SIGNATURES IN PATIENTS WITH DENGUE VIRUS INFECTIONS

Desirée Plaça[1,2], Gabriela Baiocchi[2], Paula Freire[2], Dennyson Fonseca[1,2], Shahab Pour[3], Lena Schimke[2], Yehuda Shoenfeld[4,5], Otávio Cabral-Marques[2]

[1]Department of Clinical and Toxicological Analyses, School of Pharmaceutical Sciences, São Paulo, Brazil; [2]Departament of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil; [3]Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil; [4]Ariel University, Ariel, Israel; [5]Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer, Israel

Keywords: immunomodulation, dengue, T cells, transcriptomics

Viral infections such as COVID-19, Zika, Measles, and Dengue activate several immunological mechanisms that can evolve into autoimmune phenomena during severe disease. Particularly, the infection caused by the dengue virus (DENV) is a global health problem that affects approximately 400 million people yearly and is highly associated with the risk to develop autoimmune diseases. Nevertheless, the signatures and molecular pathways by which DENV can trigger autoimmunity remain unknown. Here, we performed a multi-study analysis of publicly available RNA sequencing data of T cell subsets isolated from peripheral blood leukocytes of Dengue patients (n = 40) to investigate these mechanisms. We assessed the immunotranscriptomic profile of T effector and memory cells, finding patterns according to cell type and disease severity. We identified 108 differentially expressed genes (DEGs) that enrich negative regulation of T-helper 17 type immune response (GO:2000317) as well as the regulation of T-helper 17 cells differentiation (GO:2000319) and/or regulation of regulatory T cell differentiation. Furthermore, while these processes and pathways appear upregulated in CD4+ TCM, TEM, and TEMRA cells, they are downregulated in CD8+ effector cells. Of note, these DEGs are mostly dysregulated in patients with severe Dengue. Among them, IRF4, VDAC1, UQCRQ, CTLA4, NDUFC2, RARA, and BCL6, are the most consistently dysregulated genes. Thus, this study suggests new immunomodulatory signatures which might be involved in the development of autoimmunity in patients with DENV infections.

P **06** - 003

ROLE OF B2-INTEGRINS CD11B AND CD11C IN THE PROGRESSION OF PYELONEPHRITIS

Olena Babyak[1], Katarzyna Jobin[1], Cristina Martin-Higueras[1], Christian Kurts[1]

[1]Institute of Molecular Medicine and Experimental Immunology, University Clinic of Bonn, Bonn, Germany

Keywords: E. coli, pyelonephritis, innate immune system, integrins, macrophages

Urinary tract infections (UTIs) belong to one of the most common bacterial infections, and affect approximately 150 million people each year. UTIs can result in asymptomatic, symptomatic cystitis, pyelonephritis and in some cases – urosepsis. The pathogen responsible for most cases of UTIs is uropathogenic Escherichia coli (UPEC). Previous studies have shown that among the receptors that mediate uptake of UPEC are β2-integrins: CD11b and CD11c, which are expressed on macrophages and conventional dendritic cells type 2 (cDC2s).

The aim of the study was to evaluate the influence of CD11b and CD11c on the progression of pyelonephritis using a newly generated ItgamItgax-/- mouse line, which lacks the genes encoding both CD11b (Itgam) and CD11c (Itgax). Our results showed that mice without β2-integrins had attenuated symptoms of pyelonephritis, as evident by lower bacterial load in kidneys. Flow cytometry analysis revealed increased numbers of neutrophils and inflammatory macrophages in kidneys of ItgamItgax-/- mice compared to WT mice, but no differences in the numbers of adaptive immune cells. Also, titers of anti-E. coli antibodies on day 24 post-infection were not changed between the groups, suggesting that innate immune system is mainly responsible for the attenuation of the disease in ItgamItgax-/- mice. The number of viable intracellular bacteria inside bone marrow-derived macrophages (BMDMs) without CD11b/c was slightly decreased compared to WT cells, which could potentially result in faster recovery of the mice lacking β2-integrins due to restricted ability of E. coli to enter macrophages and use them as a niche for survival and replication inside cells.

These findings suggest that blocking of CD11b and CD11c may be a therapeutic strategy in the treatment of pyelonephritis.

P **06** - 004

THE ROLE OF IKKB / NF-KB IN THE IMMUNE SURVEILLANCE OF MELANOMAS

Clara Poncet[1], Grégory Verdeil[2], Pauline Moussard[1], Toby Lawrence[1], Nathalie Auuphan-Anezin[1]

[1]CIML, Marseille, France; [2]Université de Lausanne, Lausanne, Switzerland

Keywords: IKKB / NF-KB, immune surveillance, melanoma

The initial description of the hallmarks of cancer are tumour intrinsic. Indeed, oncogene-induced the activation of the transcription NF-κB leading to the expression of proliferation, survival and invasion genes. However, little is known about the induction of deregulated genes by NF-κB that can impact the immune surveillance.

In addition, NF-κB can affects tumour extrinsic properties. Inflammation induced by NF-κB downstream oncogenic mutations drives various signalling pathways and induces cytokine expression such as IL-6, IL-10, VEGF favouring the recruitment of immunosuppressive myeloid cells (IMC), tumour-associated macrophages (TAMs) and other immune cells but not only.

We previously developed a HRAS-driven murine melanoma model with or without genetic inactivation of IKKβ in melanoma cells. We showed that intrinsic NF-κB activation in melanoma promotes the recruitment of TAMs with a pro-tumour ("M2-like") phenotype and accumulation of "exhausted" CD8+ T cells, thereby impairing tumour immune-surveillance.

Given that activation of the BRAF oncogene is prevalent in human melanoma, my thesis project aims to evaluate the role of IKKβ/NF-κB signalling in BRAFV600E-driven melanomas.

We showed that IKKβ-deficient BRAF-driven melanomas grow normally in immunodeficient mice, however, in immunocompetent mice IKKβ-deficient melanomas are rapidly rejected. These results highlight a crucial role for IKKb/NF-kB activation in melanomas for evasion of anti-tumour immunity. Further analyses are ongoing to define more precisely the role of tumour-specific CD8+ T cells for immune-surveillance of IKKβ-deficient melanomas and the contribution of type 1 conventional dendritic cells (cDC1s).

Finally, to better understand the intrinsic mechanisms of immune-suppression regulated by NF-κB in melanoma, bulk RNAseq analyses are being conducted on IKKβ-sufficient and IKKβ-deficient HRAS- and BRAF- driven melanoma cells. This analysis should reveal NF-κB-regulated genes in melanoma that affect immune-surveillance in these distinct oncogenic contexts.

P **06** - 005

RECONCILING THE ROLE OF INNATE AND ADAPTIVE IMMUNITY TO STAPHYLOCOCCUS AUREUS IN THE DEVELOPMENT OF ATOPIC DERMATITIS

Camille Braun[1,2], Cédric Badiou[1], Vijaykumar Patra[1], Willem van Wamel[3], Saeko Nakajima[4], Thomas Henry[1], François Vandenesh[1,5,6], Jean-François Nicolas[1,7], Gerard Lina[1,5,6], Audrey Nosbaum[1,7], Marc Vocanson[1]

[1]Centre International de Recherche en Infectiologie, Lyon, France; [2]Service de Pneumologie et Allergologie pédiatriques, Hôpital Femme Mère Enfant, Hospices Civils de Lyon, Bron, France; [3]Department of Medical Microbiology and Infectious Diseases, Erasmus Medical Center, Rotterdam, Netherlands; [4]Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, Japan; [5]Service de Microbiologie Clinique, Groupement Hospitalier Nord, Hospices Civils de Lyon, Lyon, France; [6]Centre National de Référence des Staphylocoques, Hospices Civils de Lyon, Lyon, France; [7]Service d'Allergologie et Immunologie Clinique, Centre Hospitalier Lyon Sud, Hospices Civils de Lyon, Pierre-Bénite, France

Keywords: Staphylococcus aureus, atopic dermatitis, skin hypersensitivity, immunology, resident memory T cells

Context. Atopic dermatitis (AD) is a chronic inflammatory dermatosis associated with Staphylococcus aureus (Sa) dysbiosis. The immunological mechanisms by which Sa contributes to this pathology remain poorly characterized.

Methods. We developed a mouse model of AD-like skin inflammation induced by repeated applications of different clinical strains of Sa to explore the role of innate and adaptive immunity in this inflammation.

Results. The intensity of the inflammation was found to be variable depending on the clinical strain applied, and dependent on the activation of the Sa quorum sensing. In addition, the response was significantly inhibited in inflammasome- or monocyte/macrophage-deficient animals, but not in T-cell-deficient mice, suggesting a major role of innate immunity. However, a robust memory T and B response against Sa toxins was detected in the weeks following application, with an accumulation of memory resident T cells (Trm) at the sites of previous injury. Sa-induced Trm, nevertheless, failed to exacerbate local responses upon re-exposure. Interestingly, when Sa isolates were applied at the time of immunization on inflamed skin instead of normal skin, the number of Sa-specific Trm that accumulated to the site of previous skin injury dramatically increased, and Sa-induced Trm, this time, mediated potent AD flares.

Conclusion. This study demonstrates the complementarity of the mechanisms of innate and adaptive immunity in the development of SA-induced inflammation. It also suggests that there is a major interest in rapidly curing AD inflammation to avoid future Sa-induced Trm-mediated relapses.

P **06** - 006

IMPACT OF « PATHOGENS EXPOSOME » ON IMMUNE SYSTEM PROPERTIES

Sophia Djebali[1,6], Antonio Peixoto[2], Guillaume Pavlovic[3], Loic Lindner[3], Claire Ciancia[4], Rick Maizels[4], Daphne Laubreton[1], Maxence Dubois[1], Margaux Prieux[1], Cyrille Mionnet[5], Olivier Neyrolle[2], Thierry Walzer[1], Jacqueline Marvel[1,6]

[1]Centre International de Recherche en Infectiologie, INSERM U1111, Ecole Normale Supérieure de Lyon, Université Lyon 1, CNRS, UMR 5308, Lyon, France; [2]Institut de Pharmacologie et Biologie Structurale (IPBS), Université de Toulouse, CNRS, UPS, Toulouse, France; [3]Université de Strasbourg, CNRS, INSERM, CELPHEDIA, PHENOMIN, Institut Clinique de la Souris, Illkirch, France; [4]Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, United Kingdom; [5]Aix Marseille Univ, CNRS, Inserm, Centre d'immunologie de Marseille-Luminy (CIML), Parc Scientifique de Luminy, Marseille, France; [6]AniRA ImmOs, SFR BioSciences, CNRS UAR 3444, Inserm US8, UCBL, ENS de Lyon, Celphedia, Lyon, France

Keywords: exposome, pathogens, immune system, SPF

Introduction

The "exposome" can be define as the sum of exposures an individual experienced from birth to death (diet, exercise, environmental exposure, vaccination, etc…). This exposome is a highly variable and a dynamic entity. However, among plethora of environmental exposures, infections (i.e. encounter with pathogens) represent a constant threat for organisms and can lead to substantial changes in immune system properties. Surprisingly, most of our immune system understanding comes from studies of mouse models with specific pathogen free health profile (SPF). More, recently some studies showed that environmental changes, on SPF laboratory mice influences steady-state immune function and matures mouse immune transcriptome. These datas have clearly highlighted the impact of "natural exposure" to environmental pathogens on the immune system phenotype and responsiveness. Today, this "pathogens exposome" concept represents a new challenge for immunologists and highlights the need for new mouse models that integrate environmental variables.

Objective

With the aim to identify the role of "life-history" on immune system fitness, our project propose to expose naïve animals to several pathogens that will trigger different immune responses. Our goal was to explore how infections/vaccination history shapes the immune system.

Methods

A cohort of mice have been sequentially exposed to a parasite (N.brasiliensis), a virus (Influenza) and a bacteria (L.monocytogenes). A longitudinal follow-up of this cohort was performed on blood by flow cytometry and multiplex cytokines dosages on sera. More, in order to analyze the impact on microbiota, feces have been collected and microbiota analysis was performed (RNA16s). Next, a group of exposed mice has been challenged with dextran sulfate sodium (DSS) in order to induce a colitis and to evaluate inflammatory response in both groups. A second group of exposed mice has been challenge with Vaccinia Virus and the antigen-specific response has been analysed in several organs. Finally, a third group of exposed mice was vaccinated against BCG. Twelve weeks after vaccination, antigen-specific response has been analyzed and histopathological studies of lungs have been performed.

Results

Our preliminary data show that "exposed" mice seems to have a better antigen-specific response to a new viral challenge and are less susceptible to colitis challenge. More, sequential acute infections does not modify drastically microbiota.

P **06** - 007

DEFINITION OF TWO LY49-EXPRESSING CD8 T CELLS SUBSETS WITH DISTINCT PROPERTIES

Daphné Laubreton[1], Margaux Moindrot-Prieux[1], Sophia Djebali[1], Thierry Walzer[1], Jacqueline Marvel[1]

[1]CIRI, Centre International de Recherche en Infectiologie, Univ Lyon, Inserm, U1111, Universite? Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, , F-69007 Lyon, France

Keywords: Ly49, CD8, thymus, virus, regulatory

Ly49+ CD8 T cells are memory-phenotype CD8 T cells characterized by the expression of Ly49A, Ly49F and Ly49G2 inhibitory receptors and high levels of IL-15 receptor CD122 and Helios expression. These cells are best known for their regulatory role in autoimmune diseases targeting CD4 TFH cells1. Moreover, they play a role in the control of LCMV acute infection2,3. However, little is known about their origin and controversy about their phenotype still remains.

We dissected the thymic origin of those cells and showed that Ly49 receptors are essentially expressed by mature CD4 and TCRβ + double negative (DN) cells. We demonstrated that the generation of mature Ly49+ CD8 T cells is controlled by the Zeb1 transcription factor which regulates the expression of multiple genes involved in the cell cycle and TCR signaling. Zeb1 also participates in the control of the agonist selection process and double positive (DP) thymocytes of Cellophane mice bearing Zeb1 hypomorphic mutations display stronger signaling upon TCR engagement4. Altogether, our results reinforced the hypothesis of thymic origin of those cells.

Furthermore, we performed a complete characterization of Ly49+ CD8 T cells and defined two populations based on surface receptor expression and functions that are not represented equivalently over age in mice and differs in Ly49 receptors repertoire and Helios expression. Indeed, we showed that Ly49+ CD8 T cells are able to proliferate in response to vaccinia virus infection, however, only a part of those cells was able to produce IFN? in a TCR-dependent manner while the other part proliferates following bystander activation driven by cytokines such as IL-2, IL-15 and IL12/18. Finally, we evaluate their ability to inhibited CD4T cells proliferation in vitro and showed that this function is associated with the antigen specific Ly49+ CD8 T cells subset.

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P **06** - 008

INTERCELLULAR COMMUNICATION WITHIN TUMOR MICROENVIRONMENT AFFECTS T CELL FUNCTION

Daniel Ryan[1], Leonid Pobezinsky[1], Elena Pobezinskaya[1]

[1]UMASS, Amherst, United States

Keywords: Intercellular, transfer, cancer, T cells, Tregs

Intercellular transfer of molecules is a phenomenon that has been described both within immunological and non-immunologic settings. T cells acquire molecules from other cells through various mechanisms including: TCR-dependent membrane acquisition (trogocytosis), extracellular vesicle uptake and through membrane nanotubes. The in vivo functional significance of these processes remains largely unexplored. Here, using a tumor mouse model, where tumor cells express ZsGreen, we observed that tumor infiltrating T lymphocytes (TILs) acquire tumor-derived material, with half of the cells having homogeneous cytoplasmic distribution of ZsGreen while the other half demonstrating punctate pattern. Interestingly, majority of ZsGreen-positive CD4 TILs appear to be Tregs. We found that transfer is not dependent on TCR/MHC interaction or the presence of antigen, however cell-cell contact is required. Moreover, we showed that the degree of transfer increases with T cell differentiation. Both CD4 and CD8 TILs that acquire ZsGreen from tumor cells have more profound exhaustion phenotype, characterized by co-expression of inhibitory receptors PD-1 and TIM-3. Furthermore, our preliminary data suggest that such transfer has impact on functionality of cells. Thus, we discovered a new mechanism that modulates T cell function within tumor microenvironment.

P **06** - 009

SEVERE COVID-19 PATIENTS HAVE IMPAIRED PLASMACYTOID DENDRITIC CELL-MEDIATED CONTROL OF SARS-COV-2-INFECTED CELLS

Manon Venet[1]*, Margarida Sa Ribeiro[1]*, Elodie Décembre[1]*, Alicia Bellomo[1]*, Garima Joshi[1], Marine Villard[1], David Cluet[2], Magali Perret[1], Rémi Pescamona[1], Helena Paidassi[1], Thierry Walzer[1], Omran Allatif[1], Alexandre Belot[1], Sophie Assant[1], Emiliano Ricci[2], Marlène Dreux[1]

[1]CIRI, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, École Normale Supérieure de Lyon, Univ Lyon, F-69007, LYON, France; [2]Laboratory of Biology and Modeling of the Cell, Université de Lyon, ENS de Lyon, Université Claude Bernard, CNRS UMR 5239, Inserm U1293, Lyon, France.

* Equal contributions as first co-authors.

Aim/Objective: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) emerged in December 2019 and is responsible for the still-ongoing coronavirus disease 2019 pandemic. The type I and III interferons (IFN-I/λ) is now thought to be a critical host response against SARS-CoV-2 infection and its pathogenesis. Especially, early reports on SARS-CoV-2 suggest that severe COVID-19 featured low level of IFN-I/λ but overproduction of other pro-inflammatory cytokines. Accordingly, genetic deficiency, neutralization by autoantibodies directed against the IFN-I system, or viral-mediated inhibition of the IFN-I/λ response aggravates SARS-CoV-2 pathogenesis. It is therefore critical to understand the regulation of the optimal production and activity of IFN-I/λ.

Design: Here, we explored the molecular mechanisms underlying the IFN-I/λ response against SARS-CoV-2 infection. We also performed a functional analysis of the host response against SARS-CoV-2 infected cells by the immune cells issued from infected patients in a longitudinal study and across various COVID-19 severity.

Results: We demonstrate that plasmacytoid dendritic cells (pDCs) are the predominant IFN-I/λ source following their sensing of SARS-CoV-2-infected cells. Mechanistically, this short-range sensing by pDCs requires sustained integrin-mediated cell adhesion with infected cells. In turn, pDCs restrict viral spread by an IFN-I/λ response directed toward SARS-CoV-2-infected cells. This specialized function enables pDCs to efficiently turn-off viral replication, likely *via* a local response at the contact site with infected cells. By exploring the pDC response in SARS-CoV-2 patients, we further demonstrate that pDC responsiveness inversely correlates with the severity of the disease. The pDC response is particularly impaired in severe COVID-19 patients.

Conclusion: Overall, we propose that pDC activation is essential to control SARS-CoV-2-infection. Failure to unfold this response could be key to understand severe cases of COVID-19.

What is already known about this subject?

Since the beginning of COVID-19 pandemic, it has represented an explosive area of research. This contributed to the rapid design of vaccines and growing knowledge on SARS-CoV-2. The viral escape mechanism from the antiviral host response, and especially the inhibition of IFN-I/λ pathways by SARS-CoV-2 within infected cells have been deeply studied, and more recently characterized across emerging variants. Nonetheless our knowledge on the molecular basis of activation and functions of specialized immune cells such as pDCs and their impact on disease progression were still elusive. This likely owing to the difficulties for ex vivo study on these cells (e.g., low frequency and limited ex vivo life span).

What are the new findings?

Our results uncovered that pDCs establish cell contact with SARS-CoV-2 infected cells via $a_L\beta_2$ integrin/ICAM-1 adhesion complex and regulators of actin network. This physical contact between pDCs and infected cells is required for the pDC-mediated antiviral response by TLR7 recognition.

Capitalizing on our findings that pDCs strongly respond by physical sensing of SARS-CoV-2-infected cells, we then showed that impaired pDC IFN-I/λ response associates with COVID-19 severity.

It is now increasingly recognized that pDCs differentiate into different subsets with distinct phenotypes and functionalities. Here, we showed that the differentiation of pDCs into subsets is altered when stimulated by contact with SARS-CoV-2-infected cells as compared to other activation. Especially, when in contact with SARS-CoV-2-infected cells, pDCs preferentially differentiate into a subset, which efficiently produces IFN-I/λ, then leading to a robust antiviral control directed towards the infected cells.

How might it impact on immunological research in the foreseeable future?

SARS-CoV-2 is a still-ongoing worldwide health threat, currently causing a significant human and economic burden, being exacerbated by divergence into more severe variants. Here, we provide compelling evidence that pDCs are a key cell type in the initiation of antiviral responses against SARS-CoV-2. Further, this study identified the failure of pDC response as critical in COVID-19 severity. Moving forward our finding shall provide guidelines for predictive biomarkers, as associated with pDC responsiveness to SARS-CoV-2 infections and background IFN-I/λ response at different stages of disease. Furthermore, strategies to boost the pDC response, and especially their recruitment to the lung, can lead to the development of potential therapeutics against pulmonary viral infections.

Whether the work is submitted, in revision or in press in peered reviewed journals

The manuscript is currently under review (=invitation to revise the manuscript) for publication in Nature Communication (IF= 15.805).

P **06** - 010

PROTECTIVE EFFICACY OF A LASSA FEVER VACCINE IN NON-HUMAN PRIMATES

Mathieu Mateo[1,2], Stéphanie Reynard[1,2], Alexandra Journeaux[1,2], Kodie Noy[1,2], Clara Germain[1,2], Xavier Carnec[1,2], Caroline Picard[1,2], Virginie Borges-Cardoso[1,2], Jimmy Hortion[1,2], Audrey Vallve[3], Stéphane Barron[3], Ophélie Jourjon[3], Orianne Lacroix[3], Aurélie Duthey[3], Manon Dirheimer[4], Caroline Carbonnelle[3], Hervé Raoul[3], Frédéric Tangy[5], Sylvain Baize[1,2]

[1]Unité de Biologie des Infections Virales Emergentes, Institut Pasteur, 69007, Lyon, France; [2]Centre International de Recherche en Infectiologie (CIRI), Université de Lyon, INSERM U1111, Ecole Normale Supérieure de Lyon, Université Lyon 1, CNRS UMR5308, 69007, Lyon, France; [3]Laboratoire P4 INSERM – Jean Mérieux, INSERM US003, 69007, Lyon, France; [4]INSERM, Délégation Régionale Auvergne Rhône-Alpes, 69500, Bron, France; [5]Viral Genomics and Vaccination, Institut Pasteur, CNRS UMR-3569, 75015, Paris, France

Lassa virus (LASV) is endemic to West Africa where it causes Lassa fever, a viral hemorrhagic fever for which there is no approved treatment or vaccine. Lassa fever is in the WHO top list of priority pathogens for which vaccine and treatments are urgently needed. According to the WHO, a good Lassa fever vaccine should be a single shot prophylactic vaccine, safe for all age groups, inducing a cross-protective immunity against several LASV lineages and a long-term immunity.

We have developed MV-LASV, a measles recombinant vaccine expressing two major antigens of LASV strain Josiah, the prototypic LASV strain. We then tested its immunogenicity and efficacy *in vivo* in cynomolgus monkeys, the only good animal model for Lassa fever. We demonstrated that MV-LASV protects monkeys against Lassa fever after a single MV-LASV injection one month prior to a LASV challenge and that protection was correlated to a potent T cell response but not to neutralizing antibodies. We also showed that MV-LASV induces a cross-protective immune response against highly divergent LASV strain from different lineages. Monkeys immunized with a single shot of MV-LASV one year prior to challenge were also protected against LASV Josiah and neutralizing antibodies, in addition to T cell responses, may have participated in protection. We recently demonstrated that reducing the time between vaccination and challenge down to 8 days was still sufficient to induce protective immunity linked to a potent CD8 T cell response, even in presence of pre-existing immunity against Measles. MV-LASV is therefore a very good vaccine candidate against Lassa fever that could ultimately be used in endemic countries. With the support of the CEPI, MV-LASV has already entered phase I clinical trials.

P **06** - 011

FINE-TUNING THE GERMINAL CENTRE REACTION VIA ?V?8-MEDIATED ACTIVATION OF TGF?

Sébastien This[1], Véronique Barateau[1], Helena Paidassi[1]

[1]CIRI, Lyon, France

Keywords: Germinal Center, Follicular T cell, TGF?, Auto-immunity, B cell

Objective. In T-dependent B cell responses, germinal centres (GC) are the sites of production of high-affinity antibodies required for pathogen clearance and formation of long-lasting humoral protection. Transforming Growth Factor beta (TGFβ) is a known modulator of antibody responses but appears to have multiple and sometimes opposing effects on GC B cell responses. TGFβ is a peculiar cytokine: ubiquitously expressed but produced in a latent form. In the gut, αvβ8 integrin plays a key role in regulating intestinal responses via activation of TGFβ.

Design. Using a new reporter mouse model for β8 gene expression as well as conditional β8-KO, we focused on the mechanisms that control TGFβ availability during the GC reaction via regulation of αvβ8 expression on follicular T cells, which might help resolve the seemingly contradictory effects of TGFβ on B cell responses.

Results. Here, we show that αvβ8 integrin is expressed by follicular T cells (Tfh and Tfr). Surprisingly, while β8 deficiency does not seem to affect the frequencies of GC B cells nor the proportion of memory B cells induced upon immunization, β8-KO mice display a decreased proportion of Ag-specific plasma cells at day 60 and upon recall.

Conclusion. Altogether, these data suggest that αvβ8-mediated activation of TGFβ regulates the outcome of the GC reaction. We are now exploring its involvement in pathological B cell responses in order to determine whether αvβ8 could represent an interesting therapeutic target to improve protective B cell response and/or to develop new strategies of immunotherapy.

P **06** - 012

IL-10 AND PD1/PD-L1 IMMUNOREGULATORY PLASMA CELLS : A NOVEL MECHANISM OF SEPSIS-INDUCED IMMUNOSUPPRESSION

Morgane Gossez[1,2], Alexandra Vandermoeten[3], Remy Coudereau[1,3], Olivier Thaunat[1,2], Thierry Defrance[2], Jonathan Lopez[1], Laurent Jallades[1], Anne-Claire Lukaszewicz[1,3], Thomas Rimmele[1,3], Laurent Argaud[1], Martin Cour[1], Guillaume Monneret[1,3], Fabienne Venet[1,2]

[1]Hospices Civils de Lyon, Lyon, France; [2]Centre International de Recherche en Infectiologie - INSERM, LYON, France; [3]EA 7426 PI3 - UCBL1/HCL/bioMérieux, LYON, France

Keywords: sepsis, immunosuppression, regulatory B cells, translationnal research

Aim/objective. Sepsis (life-threatening organ dysfunction caused by a dysregulated host response to infection) is the first cause of death in ICU. Septic patients develop immune dysfunctions associated with mortality. We previously observed that septic patients present with reactive plasmacytosis. We investigated the role of these cells as a novel mechanism of sepsis-induced immunosuppression.

Design. In a murine model of sepsis-induced immune alterations (reanimated caecal ligation and puncture CLP), we explored the phenotype and immunoregulatory properties on T cell proliferation of

plasma cells. We confirmed these data in clinical samples from viral (COVID-19) and bacterial septic patients.
Results. Reactive plasmacytosis was observed in spleen of CLP mice. Those plasma cells had features of extrafollicular plasmablasts (CD138+IgM+Blimp1lowIrf4lowCxcr5-). Plasma cells purified from CLP mice but not Sham animals inhibited T cell proliferation ex vivo. In vivo depletion of plasma cells by bortezomib injection after CLP improved splenocyte proliferation. Both cell-cell contact and production of soluble mediator were involved in plasma cell immunoregulatory functions (Transwell experiments). Sepsis-induced plasma cells produced increased IL-10 levels compared with plasma cells from Sham mice. PD-1/PD-L1 pathway was also upregulated in these cells. Blockade of PD-L1 pathway in co-culture improved T cell proliferation. In two prospective cohorts of bacterial and viral (COVID-19) septic patients, sepsis-induced plasma cells overexpressed PD1 and PDL1 and possessed immunoregulatory functions on T cell proliferation ex vivo.
Conclusion. We describe a novel mechanism of sepsis-induced immunosuppression through the induction of IL-10 and PD1/PD-L1 immunoregulatory plasma cells. These cells could represent innovative therapeutic targets in sepsis.

P **06** - 013

APOPTOSIS-INDUCED CLEAVAGE OF CD43 ON T CELLS PROMOTES EFFEROCYTOSIS

Shengpan Zhang[1], Linnea Drexhage[1], Maeva Dupont[1], Quentin Sattentau[1]

[1]Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom

Keywords: CD43 , Mucin, T cells, Apoptosis, Efferocytosis

Efferocytosis is the clearance of dead and dying cells by phagocytes, and is essential for organism development, tissue homeostasis and pathogen control. Efferocytic recognition by phagocytes is regulated by a series of 'eat-me' and 'don't eat-me' signals on target cells. Identifying these signals would enable us to modulate the efferocytic pathway, with implications for regulating inflammation, infection and cancer. In this project, I have focussed on apoptosis in T lymphocytes as these cells need to be silently removed after initial clonal expansion following infection, by highly active efferocytic mechanisms. I have confirmed that human T cell apoptosis activates CD43 ectodomain cleavage by the sheddase 'A Disintegrin And Metalloproteinase domain-containing protein 10' (ADAM10). Loss of CD43 ectodomain enhanced T cell uptake by macrophages, whereas overexpression of CD43 and pharmacologic inhibition or genetic elimination of ADAM10 decreased T cell uptake. Since CD43 is a large, negatively charged mucin-like molecule, we hypothesise that it acts as a "don't eat me" signal, the shedding of which promotes the T cell engulfment by macrophages. Mechanistic understanding of CD43 shedding is critical because it has important implications in a fundamental mechanism regulating efferocytic recognition of dying cells, and the role of ADAM10 in several inflammatory and autoimmune diseases that may implicate defective efferocytosis.

P **06** - 014

INTEGRATIVE TRANSCRIPTOMIC ANALYSIS OF EXHAUSTION-ASSOCIATED GENES AND PREDICTION OF MIRNA-MRNA INTERACTIONS IN COVID-19

Gabriela Baiocchi[1], Desirée Plaça[2], Dennyson Fonseca[2], Igor Filgueiras[1], Shahab Pour[3], Niels Camara[1], Vera Calich[1], Otavio Cabral-Marques[1]

[1]Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil; [2]Department of Clinical and Toxicological Analyses, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil; [3]Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

Keywords: COVID-19, T cell exhaustion, Immunoregulation

Several studies have highlighted functional impairment, reduced numbers, and increased expression of exhaustion markers by T cells in patients with coronavirus disease 2019 (COVID-19). Nevertheless, the landscape of exhaustion-associated genes induced by SARS-CoV-2 infection and the factors that post-transcriptionally regulate the expression of these genes during COVID-19 remains uncharted territory. Here, we performed an integrative analysis (1278 subjects) of publicly available RNA sequencing (RNAseq) data of peripheral blood leukocytes (PBLs) and nasopharyngeal swabs to define the global exhaustion gene signature in COVID-19 and predicted functionally validated microRNA (miRNA)-mRNA interactions to identify potential regulators of EAGs in COVID-19. We retrieved the previously defined consensus exhaustion signature, inferred regulatory networks, and compared transcription patterns according to sample type and disease severity. This approach identified 64 exhaustion-associated genes (EAGs) meta-differentially expressed in upper respiratory airways such as PDCD1, CTLA4, LAG3, TIGIT, and 17 EAGs in PBLs. Machine learning modeling (Random Forest) indicated some EAGs such as CCR5, BUB1, TIGIT, and LAG3 as important predictors of disease severity. Enrichment analysis using these meta-differentially expressed genes revealed pathways associated with negative regulation of regulatory T cell differentiation and chemokine signaling pathway. Moreover, EAGs presented increased correlation patterns in COVID-19 and were found as hubs in co-expression networks. Single-cell RNAseq analysis revealed 37 EAGs differentially expressed across distinct leukocyte populations (B cells, dendritic cells, NK cells, and monocytes). In silico prediction of functional validated miRNA-mRNA interactions using the MiRTarBase tool revealed 1077 and 496 potential miRNAs that regulate EAGs differentially expressed in upper respiratory airways and PBLs, respectively. Of note, miR-124-3p, miR-26b-5p, miR-195-5p and miR-16-5p presented the higher number of target-mRNAs, targeting EAGs such as TOX, PDCD1 and TCF3. Thus, our work provides a global exhaustion gene signature in COVID-19, suggesting that the modulation of EAGs is not a restricted T cell-related event and reveals new potential miRNAs-mRNAs interactions associated with immunoregulation and exhaustion during SARS-CoV-2 infection.

P **06** - 015

SINGLE-CELL PROFILING IDENTIFIES ACE+ GRANULOMA MACROPHAGES AS A NON-PERMISSIVE NICHE FOR INTRACELLULAR BACTERIA DURING PERSISTENT SALMONELLA INFECTION

Pham Trung[1]

[1]Stanford University School of Medicine, Palo Alto, United States

Keywords: macrophage, Salmonella, granuloma, angiotensin converting enzyme, single-cell

Macrophages mediate key antibacterial immune responses against intracellular bacterial pathogens, such as Salmonella enterica. Yet, they can also act as a cellular niche for these pathogens to persist in infected tissues within granulomas, which are immunological structures comprised of macrophages and other immune cells. Modulating macrophage functions presents a promising host-directed therapeutic approach; however, we currently lack a complete understanding of the functional diversity that underlies the capacity of macrophages to permit or limit bacterial persistence. Here, we apply single-cell transcriptomics to investigate macrophage functional diversity during persistent Salmonella enterica serovar Typhimurium (STm) infection in mice. We identify determinants of macrophage heterogeneity in infected spleens and describe populations of distinct phenotypes, functional programming, and spatial localization. Using a STm mutant with impaired intra-macrophage persistence, we delineate macrophage phenotypes that contribute to limiting infection. We find that angiotensin converting enzyme (ACE)+ granuloma macrophage are non-permissive for intracellular bacteria and their abundance anticorrelates with tissue bacterial burden. Ace+ macrophages are differentially enriched in transcripts of genes involved in tumor necrosis factor (TNF) signaling. Disruption of pathogen control by neutralizing TNF preferentially depletes ACE+ macrophage niche in infected tissues. Our study thus reveals ACE+ macrophages as a functionally distinct macrophage phenotype that could be modulated to limit intracellular-bacteria persistence in infected tissues.

P **06** - 016

SARS-COV-2 INFECTION INDUCES A BROAD LOSS OF SELF-TOLERANCE THAT ASSOCIATES WITH COVID-19 SEVERITY

Gabriela Baiocchi[1], Aristo Vojdani[2], Avi Rosenberg[3], Elroy Vojdani[4], Gilad Halpert[5], Yuri Ostrinski[5], Israel Zyskind[6], Igor Filgueiras[1], Yael Lavi[8], Jonathan Silverberg[9], Jason Zimmerman[11], Dennyson Fonseca[14], Desirée Plaça[14], Paula Freire[1], Niels Camara[1], Carmen Scheibenbogen[15], Hans Ochs[18], Howard Amital[20], Otavio Cabral-Marques[1], Yehuda Shoenfeld[21]

[1] Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil; [2]Department of Immunology, Immunosciences Laboratory, Los Angeles, United States; [3]Department of Pathology, Johns Hopkins University, Maryland, United States; [4]Regenera Medical , Los Angeles, United States; [5]Ariel University, Israel, Israel; [6]Department of Pediatrics, NYU Langone Medical Center, New York, United States; [7]Department of Internal Medicine, Haraldsplass Deaconess Hospital, Bergen, Norway; [8]Department of Chemistry Ben Gurion University Beer-Sheva, Israel, Israel; [9]Department of Dermatology, George Washington University School of Medicine and Health Sciences, Washington, DC, United States; [10]ResourcePath, Sterling, United States; [11]Maimonides Medical Center, New York, United States; [12]Data Science Initiative at Brown University, Providence, United States; [13]Department of Biostatistics and the Data Science Initiative at Brown University, Providence, United States; [14]Department of Clinical and Toxicological Analyses, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil; [15]Institute for Medical Immunology, Charité – Universitätsmedizin Berlin, Berlin, Germany; [16]CellTrend Gesellschaft mit beschränkter Haftung (GmbH), Luckenwalde, Germany; [17]Department of Biology, Yeshiva University, New York, United States; [18]Department of Pediatrics, University of Washington School of Medicine, and Seattle Children's Research Institute, Seattle, United States; [19]Department of Rheumatology, University Medical Center Schleswig-Holstein Campus Lübeck, Lübeck, Germany; [20]Department of Medicine B, Sheba Medical Center, Tel Hashomer, Israel; [21]Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel

Keywords: COVID-19, autoantibodies , autoimmune diseases

The SARS-CoV-2 infection is associated with increased levels of autoantibodies targeting immunological proteins such as cytokines and chemokines. Reports further indicate that COVID-19 patients may develop a wide spectrum of autoimmune diseases due to reasons not fully understood. Even so, the landscape of autoantibodies induced by SARS-CoV-2 infection remains uncharted territory. To gain more insight, we carried out a comprehensive assessment of autoantibodies known to be linked to diverse autoimmune diseases observed in COVID-19 patients, in a cohort of 248 individuals, of which 171 were COVID-19 patients (74 with mild, 65 moderate, and 32 with severe disease) and 77 were healthy controls. Dysregulated autoantibody serum levels, characterized mainly by elevated concentrations, occurred mostly in patients with moderate or severe COVID-19 infection and was accompanied by progressive disruption of physiologic IgG and IgA autoantibody signatures. A similar perturbation was found in patients with anosmia. Notably, autoantibody levels often accompanied anti-SARS-CoV-2 antibody concentrations, being both indicated by random forest classification as strong predictors of COVID-19 outcome, together with age. Moreover, higher levels of autoantibodies (mainly IgGs) were seen in the elderly with severe disease compared with young COVID-19 patients with severe disease. These findings suggest that the SARS-CoV-2 infection induces a broader loss of self-tolerance than previously thought, providing new ideas for therapeutic interventions.

P **06** - 017

THE TRANSCRIPTIONAL OVERLAP BETWEEN ANTI-DENGUE VACCINE AND NATURAL IMMUNE RESPONSES ASSOCIATES WITH HOST PROTECTION AND DISEASE SEVERITY

Desirée Plaça[1,2], Gabriela Baiocchi[2], Dennyson Fonseca[1,2], Shahab Pour[3], Alexandre Marques[2], Ranieri Salgado[2], Caroline Prado[1], Igor Filgueiras[2], Júlia Usuda[4], Igor Jurisica[5,6,7], Niels Camara[2], Lena Schimke[2], P 06 - 017[2], Hans Ochs[8], Paula Freire[2], Otavio Cabral-Marques[2,9]

[1]Department of Clinical and Toxicological Analyses, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil; [2]Departament of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil; [3]Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil; [4]School of Pharmaceutical Sciences, University of São Paulo, São Paulo, São Paulo, Brazil; [5]Osteoarthritis Research Program, Division of Orthopedic Surgery, Schroeder Arthritis Institute and Data Science Discovery Centre for Chronic Diseases, Krembil Research Institute, University Health Network, Toronto, Canada; [6]Departments of Medical Biophysics and Computer Science, University of Toronto, Toronto, Canada; [7]Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia; [8]Department of Pediatrics, University of Washington School of Medicine, and Seattle Children's Research Institute, Seattle, United States; [9]Network of Immunity in Infection, Malignancy, and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), São Paulo, Brazil

Keywords: Dengue, Vaccine, Transcriptomics, Immune response, disease severity

Dengue disease is a global health problem that affects approximately 400 million people yearly but no specific therapy or vaccine is currently available to effectively combat dengue virus (DENV) infection. While most individuals infected by DENV are asymptomatic, others can present from mild (Dengue Fever [DF]) to severe and life-threatening disease manifestations (Dengue Hemorrhagic Fever [DHF]) due to mechanisms still poorly understood. Here, we performed a comprehensive analysis of publicly available transcriptome data of 273 individuals with natural DENV infection (NDI) and DENV vaccine trials (DVT1 and 2). We identified transcriptional and pathways signatures according to time of infection and severity in NDI and DVT1 overlap. DVT1 presented 237 common differentially expressed genes (DEGs) with NDI. Of note, 28 genes were validated in DVT2 and are marked by the enrichment of type I and II interferon signaling and regulation of cytokines. Correlation and Random Forest prediction analysis ranked 10 DEGs as the most important genes determining disease severity. Among these, IFI27, ISG15, GBP1, and HERC5 are associated with host protection against DENV and showed important dysregulation in severity (DHF) when compared with mild disease (DF). Hence, this work provides the landscape of NDI and vaccine-challenge-induced immune response, suggesting biomarkers to monitor the effectiveness of vaccinations and the development of specific therapies against DENV.

P **06** - 018

INTRATUMOURAL ACCUMULATION OF IFNG DUE TO LOSS OF IFNGR1 IN CANCER CELLS ENHANCES ANTI-TUMOUR RESPONSES IN VIVO

Vivian Lau[1], Julie Mazet[1], Gerard Audrey[1]

[1]University of Oxford, Oxford, United Kingdom

Keywords: cancer, antigen, cytokines, antigen presentation, microenvironment

Inhibition of IFNγ-dependent signaling in tumour cells is key to evading T cell-dependent killing, as cancers lacking IFNγ sensitivity demonstrate clinical resistance to checkpoint immunotherapy. IFNγ plays pleiotropic roles within the tumour microenvironment, and insufficient signaling in tumour cells results in impairment of MHC-I upregulation, prohibiting tumour recognition by T cells. Consequently, we hypothesized that IFNGR1 deletion in tumour cells would lead to decreased apoptosis and thereby diminish the anti-tumour response via direct and cross-presentation pathways.

To test this hypothesis, we deleted IFNGR1 in the B16F10 murine melanoma cell line and analysed key features of the tumour microenvironment. We unexpectedly found an enhanced rate of tumour rejection and increased immune infiltration of tumour-specific CD8+ T cells in IFNGR1 KO tumours compared to WT. Furthermore, KO tumours showed accumulation of intratumoural IFNγ which may trigger active re-modeling of the tumour microenvironment to enhance anti-tumour immunity. This indicates a likely adaptation of the immune response against IFNγ-resistant tumour cells, and suggests that other anti-tumour mechanisms may be at play.

We further analyzed tumour-infiltrating CD45+ cells within WT and IFNGR1 KO tumours via single-cell RNA sequencing. Interestingly, CD8+ T cells from WT tumours showed higher levels of IFNγ RNA, whereas similar T cells from KO tumours showed greater activation of IFN-dependent signaling pathways. NK cells were also more metabolically active in KO tumours than WT, indicating a possible compensatory mechanism whereby anti-tumour functions of NK cells supersede cytotoxic T cells in KO tumours. Pathway analysis showed significantly greater responses to IFNγ-signaling among multiple immune cell subsets including dendritic cells. Based on these observations, future experiments aim to elucidate the main cellular populations which dominate the anti-tumour response in KO tumours, beginning with NK cell depletion studies and immune-activating capabilities of myeloid populations.

Whereas IFNγ-signaling deficient tumours in human melanomas are

linked to poor prognosis, we hope to understand how the immune microenvironment can be modulated to exploit MHC-independent pathways which are absent following loss of IFNγ sensitivity.

P **06** - 019

LATENT TUBERCULOSIS DIAGNOSIS IN SOLDIERS BY USING QUANTIFERON-TB GOLD IN TUBE ASSAY AND QUANTIFERON-TB PLUS ASSAY IN SOUTH KOREA

Youn Jung Jun[1], Jong Wook Hong[1], Hwi-Jun Kim[1], Kim Hee-Jin[1], Kwang-Hyeok Seok[1], Seungmo Kim[1], Cheon-Tae Kim[1], Hee Joo Lee[1]

[1]Korean National Tuberculosis Association, Seoul, Korea (Republic of)

Keywords: Latent tuberculosis infection, Mycobacterium tuberculosis, QuantiFeron-TB Gold In tube assay, QuantiFeron-TB plus assay, , , Mycobacterium tuberculosis

Background

QuantiFeron-TB Gold In tube assay and QuantiFeron-TB plus assay were used for the diagnosis of latent tuberculosis infection.

In this study, we retrospectively analyzed the positive rates of Interferon gamma release assay in young Korean Soldiers by using QuantiFeron-TB Gold In tube assay and QuantiFeron-TB plus assay.

Methods

Blood samples were collected from 990,106 young Korean soldiers in South Korea. Majority of the soldiers (94.4% in 2018 and 94.8% in 2019) are under 19 years old.

We have tested the samples by QuantiFeron-TB Gold In tube assay from 2018 to Feb 2019 and QuantiFeron-TB plus assay from Mar 2019 to Dec 2019.

Results

The positive rates was 2.9% (9,732/333,739) in 2017, 2.58% (8,356/323,800) in 2018 and 1.31% (4,364/332,567) in 2019.

The positive rate decreased every year.

Conclusions

The positive rates are 2.3% (22,452/980,106) and the positive rate decreased every year in Korea.

This is the first and largest IGRA study in young Korean Soldiers.

P **06** - 020

CROSSTALK OF MCPYV-INDUCED IL17-A-INFLAMMATION AND AUTOPHAGY

Junchao Miao[1,2], David Rouillon[1,2], Rabia Masud[1,2], Romain Crespo[2,3], Hélène Valentin[2], Tarik Gheit[4], Massimo Tommasino[4], Jérôme Nigou[5], Antoine Touze[6], Isabelle Vergne[5], Christine Delprat[1,2]

[1]UnivLyon, Université Claude Bernard Lyon 1, Villeurbanne, France; [2]Centre de Recherche en Cancérologie de Lyon (CRCL), Centre Léon Bérard, INSERM U1052, CNRS UMR5286, Lyon, France; [3]Ecole Pratique des Hautes Etudes (EPHE), Paris, France; [4]International Agency for Research on Cancer (IARC), World Health Organization, Lyon, France; [5]Institut de Pharmacologie et de Biologie Structurale, UMR 5089 CNRS, Université de Toulouse, CNRS, Université Paul Sabatier, Toulouse, France; [6]Infectiologie et santé publique «Biologie des infections à Polyomavirus» team, UMR INRA 1282, Uni-versité de Tours, Tours, France

Keywords: Merkel cell carcinoma, Merkel cell Polyomavirus, MCPyV early region, IL-17A, Autophagy

Merkel cell carcinoma (MCC) is a rare aggressive skin malignancy affecting aged or immunosuppressed patients. Merkel cell polyomavirus (MCPyV) is the causative agent associated to 80% of MCC. C-terminal truncation of the MCPyV large T antigen (LT) gives rise to onco-type large T antigens (otLT), which is a hallmark of cancer cells in MCPyV+ MCC. To investigate the role of MCPyV antigens, we built five plasmids encoding the early region (ER) sequence, and four sub-products: small T antigen (sT), 57kT protein (57kT), wild-type LT (wtLT) or otLT. We studied IL-17A-induction in transfected 293T cells. Transfection efficiency and IL-17A induction were assessed by western-blot and flow cytometry analysis. This is the first demonstration that ER products strongly induce IL-17A expression. sT alone did not induce IL-17A contrary to 57kT, wtLT or otLT. Interestingly, C-terminal truncation of LT in otLT lowered IL-17A induction, compared to ER or wtLT. Autophagy is known to occur during innate antiviral inflammation. Beyond its antiviral role, autophagy may also remove transduction compounds from the cytoplasm thereby affecting inflammation. We first investigated whether MCPyV antigens induced autophagy using the autophagy flux assay, and then studied the crosstalk between autophagy and IL-17A-mediated inflammation. Autophagy increased after LT transfection but not after otLT transfection, suggesting a critical role of the C-term of LT in autophagy induction. Using engineered HEK-Blue cells expressing IL-17R, we observed that NF-κB-dependent IL-17R-signaling induced autophagy. Conversely, autophagy activation by rapamycin inhibited transduction downstream of IL-17R. Thus, autophagy may downregulate wtLT-induced acute inflammation. In the meantime, the C-term truncation of LT, as observed in otLT, may promote the transition from high-grade acute to low-grade chronic IL-17A-mediated inflammation in the skin due to a defective feedback when autophagy is missing. Thus, the crosstalk between autophagy and IL-17R-signaling may foster MCC.

P **06** - 021

MERKEL CELL POLYOMAVIRUS SMALL TUMOR ANTIGEN CONTRIBUTES TO IMMUNE EVASION BY INTERFERING WITH TYPE I INTERFERON SIGNALING

Denise Ohnezeit[1], Jiabin Huang[1], Manja Czech-Sioli[1], Thomas Günther[2], Adam Grundhoff[2], Nicole Fischer[1]

[1]UKE, Hamburg, Germany; [2]HPI, Hamburg, Germany

Keywords: Innate immunity, Tumorigenesis, Gene regulation

Introduction: Merkel cell polyomavirus (MCPyV) is the etiological agent of the majority (60-80%) of Merkel cell carcinomas (MCCs), an aggressive skin cancer in elderly and immunosuppressed patients. While MCC is extremely rare, MCPyV infection is a frequent, persistent infection within the human population. MCPyV encodes for a few genes only, including the early genes large T antigen (LT) and small T antigen (sT). LT is expressed in its full-length form during infection, however in MCC, the integrated viral genome expresses only a truncated form of LT (LTtr). In contrast, sT is expressed both during infection and tumorigenesis and is being suggested the main driver of tumorigenesis. Intriguingly, both sT and LTtr are necessary for the survival of tumor cells.

Objectives: Using genome-wide RNA-Seq and ChIP-Seq analysis we aimed at identifying mechanisms how sT alone or in combination with LT or LTtr contributes to MCPyV pathogenesis.

Material and Methods: Overexpression experiments reflecting the viral gene expression profile observed in infection or transformation were performed in primary human dermal fibroblasts. Subsequently, genome-wide transcriptome analysis and chromatin immunoprecipitation experiments were conducted.

Results: We show that sT, LT and LTtr regulate the transcription of many genes involved in cell proliferation, as well as inflammatory cytokine and chemokine signaling. In addition, we identify a novel function of sT: sT overexpression leads to downregulation of genes involved in innate immune recognition and regulation. Gene ontology analysis reveals the ISGF3 complex, consisting of STAT1, STAT2 and IRF9, as a major target of sT, resulting in a negative regulation of interferon-stimulated genes. To elucidate whether this function of sT plays a significant role during MCPyV infection or MCC pathogenesis, gene expression experiments were conducted in cells expressing sT in combination with LT and LTtr.

Conclusion: We identify a novel function of MCPyV, impairment of the innate immune response via the Jak-STAT pathway. Furthermore, we give some first mechanistic insights and investigate the consequences of this novel function in MCPyV infection and pathogenesis.

P **06** - 022

ANTIGEN SPECIFIC ACTIVATION OF CYTOTOXIC T CELLS BY S. AUREUS INFECTED DENDRITIC CELLS

Adèle Friot[1], Sophia Djebali[1], Séverine Valsesia[1], Peggy Parroche[2], Julie Chaix[2], Maxence Dubois[1], Jessica Baude[1], François Vandenesch[1], Jacqueline Marvel[1], Yann Leverrier[1]

[1]Centre International de Recherche en Infectiologie, Inserm U1111, CNRS UMR5308, École Normale Supérieure de Lyon, Université de Lyon, Université Claude Bernard Lyon 1, Lyon, France; [2]BIOASTER Technology Research Institute, Lyon, France

Keywords: Staphylococcus aureus, Dendritic cells, Antigen-specific activation, Cytotoxic T cells

Staphylococcus aureus is a pathogen associated with a wide variety of diseases, from minor to nosocomial life-threatening infections. As with other bacteria, antibiotic-resistant strains have emerged leading to a growing concern about the control of S. aureus infections. The development of vaccines could be a way to overcome these resistant strains. However, S. aureus ability to internalize into cells – and consequently to constitute a reservoir escaping to humoral immunity - is a challenge to develop a vaccine. A better comprehension of cellular immunity is therefore needed, especially as cytotoxic T cells play a major role in the clearance of other intracellular bacteria.

A first step was to decipher if dendritic cells having captured S. aureus antigens or infected by S. aureus can activate antigen-specific CD8 T cells. In order to address this question, we used NP68 specific TCR transgenic CD8 T cells (F5 T cells) as a reporter model and S. aureus 6850 strain expressing NP68 epitope.

We evidenced in vitro that both primary mouse dendritic cells and DC2.4 cell line can be infected by S. aureus that is internalized through phagocytosis. Contact with S. aureus induces DC maturation and presentation of S. aureus epitopes on major histocompatibility complex class I. Moreover, infected DC induce antigen-specific CD8 T cell activation, that can then kill S. aureus infected cells.

Altogether these results show that intracellular S. aureus can be recognized by CD8 T cells, leading to infected cells killing.

P **06** - 023

CROSSTALK OF ENDOTHELIUM AND IMMUNE CELLS IN LIPOPOLYSACCHARIDE-INDUCED ACUTE LIVER DISEASE

Sophia Papaioannou[1,2], Jia-Xiang See[1,2], Mingeum Jeong[1], Ankita Batra[3], Reiners-Koch Philipp-Sebastian[2,4], Michael Platten[2,3,5], Ana Stojanovic[1,2], Adelheid Cerwenka[1,2]

[1]Immunobiochemisty, Mannheim Institute for Innate Immunoscience, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany; [2]European Center for Angioscience (ECAS), Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany; [3]DKTK CCU Neuroimmunology and Brain Tumor Immunology, DKFZ, Heidelberg, Germany; [4]Department of Dermatology, Venereology, and Allergology, University Medical Center and Medical Faculty Mannheim, Heidelberg University, and Center of Excellence in Dermatology, Mannheim, Germany; [5]Department of Neurology, Medical Faculty Mannheim and Mannheim Center of Translational Neurosciences (MCTN), Heidelberg University, Mannheim, Germany

Keywords: NK cells, Liver, LPS, CXCL10, Endothelial Cells

The liver is a highly vascularized organ with dual blood supply via the hepatic artery and the portal vein. Liver sinusoidal endothelial cells (LSECs) form capillary vessels without a basement membrane, with high permeability and low blood flow rate. LSECs have both physiological and immunological functions, including antigen presentation, leukocyte recruitment and the induction of immune tolerance in the liver. The liver immune system has evolved to cease responses to microbial and food antigens, which are continuously supplied from the gut via the portal circulation, while enabling efficient and balanced responses in case of infections.

Here, we show that Lipopolysaccharide (LPS)-induced endotoxemia in the liver resulted in both LSEC and immune cell activation. Innate immune cells, including inflammatory monocytes and NK cells, accumulated in the inflamed liver tissue. LSECs were the major producers of the monocyte- and NK cell-attracting chemokines, CCL2 and CXCL10. While CCL2 was induced by direct exposure of LSECs to LPS, CXCL10 expression required LSEC stimulation with IFN-γ. Moreover, LSECs affected the ability of activated NK cells to produce IFN-γ in response to pro-inflammatory cytokines derived by the myeloid compartment. Therefore, LSECs not only represent the central component orchestrating immune tolerance, but also have an active role shaping immune cell reactivity in the context of liver inflammation.

P **06** - 024

PEPTIDES RESULTING FROM UPSTREAM OPEN READING FRAME TRANSLATION IN HIV-1 TRANSCRIPTS ELICITS SPECIFIC T CELL IMMUNE RESPONSES IN INFECTED INDIVIDUALS

Emmanuel Labaronne[1], Didier Décimo[2], Lisa Bertrand[3], Laura Guiguettaz[1], Thibault Sohier[1], David Cluet[1], Valérie Vivet-Boubou[4], Clara Dahoui[2], Pauline François[3], Lucie Etienne[2], Caroline Goujon[7], Jean-Christophe Paillart[4], Olivier Namy[3], Berta-Cecilia Ramirez[3], Théophile Ohlmann[2], Arnaud Moris[3], Emiliano Ricci[1]

[1]Laboratoire de Biologie et Modélisation de la Cellule - ENS de Lyon, Lyon, France; [2]Centre International de Recherche en Infectiologie - ENS de Lyon, Lyon, France; [3]Institute for Integrative Biology of the Cell - Université Paris-Saclay, Gif-sur-Yvette, France; [4]Institut de Biologie Moléculaire et Cellulaire - Université de Strasbourg, Strasbourg, France; [5]Université Paris-Saclay, Le Kremin Bicêtre, France; [6]Center for Immunology of Viral Infections and Autoimmune Diseases - Inserm UMR-1184, Le Kremlin Bicêtre, France; [7]Institut de Recherche en Infectiologie de Montpellier, Montpellier, France

Keywords: RNA, translation, MHC, HIV, T cell

Human immunodeficiency virus type-1 (HIV-1) is a complex retrovirus which relies on alternative splicing, translational and post-translational mechanisms to produce more than 15 functional proteins from its single ~10kb transcriptional unit. Here, we have applied ribosome profiling and nascent protein labeling at different time points during infection of CD4+ T lymphocytes to characterize the translational landscape of cellular and viral transcripts during the course of infection. Our results indicate a strong impact of viral infection on host cellular transcript levels but a modest impact on global translation rates. Analysis of ribosome profiling reads from viral transcripts further reveals extensive and productive non-AUG translation from multiple upstream open reading-frames located in the 5' long terminal repeat, resulting in the expression of small peptides. Using IFN-lambda ELISPOT assays in PBMCs from HIV-1 infected individuals, we are able to detect specific T cell responses directed against uORF-derived peptides, thus indicating that they encode MHC-ligands and highlighting their potential relevance in mediating an immune response against infected cells in vivo. uORF translation from viral transcripts also occurs in the closely related lentivirus HIV-2 and in the more phylogenetically distant retrovirus HTLV-1 (Human T Lymphotropic Virus 1), suggesting that it could be a conserved feature among retroviruses.

P **06** - 025

CD8 MEMORY PRECURSOR CELLS GENERATION IS A CONTINUOUS PROCESS UPON AN ACUTE INFECTION

Helena Todorov[2], Margaux Prieux[1], Daphné Laubreton[1], Matteo Bouvier[3], Shaoying Wang[1], Simon De Bernard[5], Christophe Arpin[1], Robrecht Cannoodt[2], Wouter Saelens[2], Arnaud Bonnaffoux[4], Olivier Gandrillon[3], Fabien Crauste[6], Yvan Saeys[2], Jacqueline Marvel[1]

[1]Centre International de recherche en Infectiologie, Université de Lyon, INSERM U1111, Lyon, France; [2]Data Mining and Modeling for Biomedicine, VIB Center for Inflammation Research, Ghent, Belgium; [3]Laboratoire de Biologie et de Modélisation de la cellule, Université de Lyon, ENS de Lyon, CNRS UMR 5239, Lyon, France; [4]Vidium, Lyon, France; [5]Altrabio, Lyon, France; [6]Laboratoire MAP5, Université de Paris, Paris, France

Keywords: CD8 T cells, Memory precursors, single-cell RNA-seq, Differentiation trajectory, RNA velocity

Upon an acute infection, naive CD8 T cells proliferate and differentiate into antigen-specific effector T cells that eradicate the pathogen. The majority then die by apoptosis; however, a small fraction persists and give rise to memory cells conferring an effective long-lasting protection to their host. Many theories have been advanced in the attempt to describe different subsets of CD8 T cells that are being produced in response to a viral infection. The lineage relationships between these different cell subsets and the stage at which activated CD8 T cells diverge from the effector fate to commit to the memory lineage remains debated, with functional studies suggesting that this engagement may occur at multiple stages of the effector response.

In our work, we used qualitative and quantitative approaches to study the generation of memory precursor cells following an acute infection by analyzing single-cell RNA-seq data that contained CD8 T cells collected during the post-infection expansion phase. By applying trajectory inference methods, we reconstruct the developmental trajectory that CD8 T cells followed after activation. The direction followed by the cells within this trajectory was then determined by a new approach, scVelo, based on the measurement of RNA splicing rates. These tools have made it possible to show that differentiation is driven by cell cycle and immune function genes. Then, cells that exhibited a memory precursor signature were identified and positioned on this trajectory. We found that memory precursors are generated continuously, from early stages short after activation, to fully differentiated effector cells. Using differential expression analysis and mathematical modeling, we found that the generation of the total pool of memory cells can only be generated based on this model. The ability of cells that become quiescent during the effector phase to differentiate into memory cells was confirmed by BrdU pulse-chase experiment in vivo. Analysis of cell counts indicates that the vast majority of memory cells are generated at later time points, and derive from cells that have extensively divided. This work, by adding this numerical understanding, thus reconciles previously proposed conflicting models of the generation of memory CD8 T cells.

P **06** - 026

PROOF OF PRINCIPLE THAT LOSS OF MISMATCH REPAIR PROTEIN REDUCES TUMOUR BURDEN IN MOUSE MODEL OF GASTRIC CANCER

Anne Huber[1], Christine Dijkstra[1], Vicki Whitehall[2], Matthias Ernst[1], Moritz Eissmann[1]

[1]Olivia Newton-John Cancer Research Institute, Melbourne, Australia; [2]QIMR Berghofer Medical Research Institute, Brisbane, Australia

Keywords: Gastric Cancer, DNA mismatch repair, MSI, mouse model, Immunotherapy

Aim

Gastric cancer (GC) remains the third leading cause of cancer-related death worldwide. Unfortunately, only a subset of GC patients, characterised by tumours with high high microsatellite instability (MSI), responds to immune checkpoint (ICI) therapy. In the context of GC, the mechanisms of how MSI improves ICI therapy responses remain poorly understood. Here we are undertaking a proof of principle study to demonstrate in novel GC mouse models that loss of the mismatch repair protein MLH1 confers MSI phenotype and impairs tumour growth via altered anti-tumour immune responses.

Methods

To study the functional and mechanistic effects of loss of MLH1 protein, we established MLH1-deficient (Kras-, Pi3kca-, Tp53-mutant) murine tumour organoids via CRISPR/Cas9 technology. These organoids were subcutaneously allografted into immunocompetent C57BL/6 mice to study tumour progression, immune surveillance, and responses to immunotherapy in vivo.

Results

We successfully generated MLH1-deficient (Kras-, Pi3kca-, Tp53-mutant) murine tumour organoids. MSI testing confirmed that MLH1-proficient parental organoids are MSI low, whereas MLH1-deficient organoids are MSI high. Low passage MLH1-deficient organoid tumours grew similarly to MLH1-proficient tumours. However, after culturing of organoids in vitro for a prolonged time prior to injection to allow accumulation of mutations, subcutaneous allograft MLH1-deficient tumours were considerably smaller compared to MLH1-proficient tumours. In addition, MLH1-deficient tumours showed a significantly higher number of CD8+ T cells. In addition, MLH1-deficient tumours grew similar to MLH1-proficient tumours when allografted subcutaneously into Rag1-/- mice. However, treatment with the ICI anti-PD-1 only marginally reduced tumour mass further. We are currently investigating the underlying mechanism for the impaired growth of MLH1-deficient organoid allograft tumours via tumour mutational burden analysis, neoantigen testing and in vivo T cell depletion experiments.

Conclusion

Taken together, we provide evidence that loss of MLH1 leads to high MSI in gastric tumours, reduces tumour growth after prolonged in vitro culture but does not increase anti-PD-1 therapy. Our findings encourage further studies to investigate the mechanisms of impaired tumour growth after MLH1 loss in GC and may provide insights leading to improve ICI therapy responses for GC patient.

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