ANR-11-LABX-0016

LABEX IGO REPORT

2016-2018

SCIENTIFIC ADVISORY BOARD MEETING

19 & 20 of April 2018

LABEX IGO REPORT 2016-2018

GENERAL DESCRIPTION

Labex IGO « Immunotherapy Graft Oncology » (http://www.labexigo.univ-nantes.fr/) has been selected by the French Ministry of Research and Education in the framework of the very competitive scientific clusters of excellence called Labex for "Laboratoire d'Excellence", part of the governmental initiatives "Investissements d'Avenir" (2nd session: 2011). These initiatives aim to distribute significant resources to French laboratories to increase their international visibility, and attractivity, to develop research, to support training initiatives and to favour to promote research findings.

Labex IGO will receive 5,5 million euros for a 8-year period starting from March 2012. The period of use of the funds has been extended until 31 December 2022.
It aims at fostering transdisciplinary projects between teams with complementary expertise in transplantation and tumor immunology, autoimmunity, and radio-immunotherapy.

Currently Labex IGO gathers about 350 people (scientists, students, technical staff) from 13 research teams in immunology, oncology and transplantation, located in 4 academic research units (UMR1232, UMR1064, UMR1236, UMR1227) of the French large-western region (Nantes, Angers, Rennes, Brest).

PROGRAM DESCRIPTION

Mammals have developed immune mechanisms that protect them against external aggressions (eg from infectious agents), and ensure early detection and control of cell dysregulations. Immunotherapy aims at exploiting such mechanisms, in order to either boost immune-mediated clearance of infected or tumor cells, or to dampen inappropriate or unwanted activation of the immune system. This can be achieved by either passive transfer of immune cells or antibodies, or by the injection of therapeutic vaccines and/or immune adjuvants.
The clinical indications for immunotherapy are many, and include chronic infectious diseases with unmet medical needs (eg tuberculosis, hepatis C, HIV...), cancers resistant to radio-, chemo- or targeted therapies, allogeneic solid and hematopoietic transplantation, as well as most autoimmune diseases (eg rheumatoid arthritis or lupus).
Although immunotherapies have revolutionized the therapeutic handling of several chronic diseases (eg rheumatoid arthritis with the TNF inhibitors), hematopoietic or solid tumors (with tumor-specific vaccines, ACT or anti-check point antibodies), there is still a crucial need to improve the clinical efficacy of Immunotherapeutic strategies, in terms of patient selection, immune monitoring and analysis of immune mechanisms. Indeed, the design of new immunotherapies is based on insufficient knowledge of the targeted mechanisms, frequently involves reagents that are not fully standardized or characterized, and such approaches are not sufficiently supported by strong preclinical proofs-of-concept. Moreover, we still lack monitoring tools allowing proper selection of the right clinical indication and eligible patient subsets for new approaches, and proper assessment of their immunotherapeutic efficacy. More importantly because of the redundancy of the immune mechanisms contributing to tumor clearance, allograft rejection or autoimmune responses, therapeutic efficacy can sometimes be achieved only through concomitant targeting of distinct mechanisms. Unfortunately, regulatory and intellectual property constraints currently hamper assessment of combination approaches involving immunotherapeutic molecules that have not been validated yet as monotherapy.

The Labex IGO proposal aims at addressing each of the above challenges and changing the current paradigms of pharmaceutical development, in order to design **innovative immunotherapies in transplantation, oncology and autoimmunity**.

To this end, we built a transdisciplinary network initially involving 15 teams (16 since December 2013) with strong expertise in human immunobiology and setting of immunotherapeutic trials in the above medical fields, as well as specialists in radioimmunotherapy and tumor cell survival pathways, in order to:

1. **Accelerate and improve obtention of preclinical and clinical proofs of concept for new immunotherapies**, (i) through in depth and comparative analysis of tolerogenic mechanisms in **allotransplantation, oncological situations and autoimmunity**, (ii) through implementation and regional structuration of facilities providing relevant animal models for early obtention of preclinical proofs of concept (POC) for new immunotherapies and (iii) through the development of new immunomonitoring tools and approaches, tighter coordination of existing immune monitoring platforms, and creation of an open data base compiling standardized operating procedures for monitoring of a broad set of innate and adaptive immune markers
2. **Design and assess cellular immunotherapy approaches with enhanced efficacy in oncology and transplantation**, through (i) implementation of new clinical grade versatile protocols for generation of homogeneous T, B or dendritic cell preparations with desired specificity and functional features and (ii) design of innovative approaches for in vivo follow up of adoptively transferred immune cells using radiolabelled antibodies.
3. **Improve selection of clinical indications and patient subsets eligible for existing or novel immunomodulating therapies,** through (i) improved patient immune monitoring, (ii) preclinical cross-assessment of their immunomodulating efficacy in cancer, allotransplantation, or autoimmune diseases, and (iii) preclinical assessment of new combination of immunomodulating therapies

To reach these goals, two kinds of actions have been proposed:

- to provide starting grants for ambitious and transdisciplinary basic and translational projects integrated within **three main workpackages**, that will address each of the above objectives. IGO does not merely provide additional funding for existing projects already run by individual teams, but instead supports new collaborative proposals yielding conceptual, technical or clinical breakthroughs that could only be reached through synergistic interactions between the participating teams.
- to provide specific support for implementation, standardization and dissemination of innovative monitoring approaches and animal models that could accelerate obtention of preclinical POC and new biomarkers for better selection of eligible patients and assessment of immunotherapeutic efficacy.

Furthermore, Labex IGO supports education and various communication and animation activities to stimulate internal and external collaborations, and enhance the visibility of the consortium and also carries on valorization of scientific results.

CHANGES IN THE ORGANISATION

➢ RENEWAL OF RESEARCH UNITS PARTNERS OF LABEX IGO

At the end of 2016, research units partners of Labex IGO were evaluated by the High Council for Evaluation of Research and Higher Education (HCERES). 4 of the 5 initial partner units of Labex IGO were very positively evaluated and renewed (UMR1064, UMR892, UMR917 and EA2216/ERI29). 3 changed their name at the beginning of 2017:

- UMR892, Nantes, became UMR1232
- UMR917, Rennes, became UMR1236
- EA2216/ERI29, Brest, became UMR1227

The UMR1102 research unit was not renewed in 2017.

➢ WP GOVERNANCE

Three management levels allow to pilot project progression. Some changes have occurred within these committees (*cf. below*).

- **The institutional committee**

It is composed with representatives of the different Universities, Inserm and CNRS, and the 3 hospitals. This committee is in charge to validate the strategy and budget of Labex IGO on the basis of progress reports. It meets 1 time per year: 5 meetings have been held so far (14/03/2013, 22/04/2014, 30/04/2015, 06/06/2016, 06/06/2017).

- **The Scientific Advisory Board**

It is composed of seven scientists internationally renowned, Paolo Dellabona (Italy), Barbara Seliger (Germany) (*who replaced T. Gajewski in may 2017*), Pierre Coulie (Belgium), Megan Sykes (USA) Jonathan Bromberg (USA) Hans-Dieter Volk (Germany), Marc Bonneville (France), chaired by Professor P.Dellabona. This SAB is in charge to study and validate the scientific strategy proposed by the consortium and identify priority projects to be funded. The SAB meets once every two years (1st meeting was held on 14th - 15th /04/2014 and 2nd meeting was held on 21st – 22nd /04/2016).

- **The steering committee**

It includes:

- the scientific manager of Labex IGO (initially Mr. Bonneville, replaced since October 2013 by N. Labarrière, and I. Anegon as deputy director, then, from April 2016 until the end of the program, by I. Anegon, and N. Labarriere as deputy director) and the deputy director,
- the coordinators of scientific projects (I. Anegon, P. Amé-Thomas (*who replaced K. Tarte in January 2018*), F. Haspot, R. Josien, F. Lang, J.F. Fonteneau (*who replaced Y. Delneste in November 2016*), P.F. Cartron and M. Cherel,
- the coordinators for training program (L. Gautreau and N. Degauque)

Other participants are:

- the representative of the team UMR1227 (J.O. Pers)
- the coordinator for valorisation (G. Duisit) representing Atlanpole Biotherapies.
- the general delegate of Labex IGO (*L. Wolff who replaced L. Salaün in August 2016*) and V. Pecqueret in charge of communication.

Meetings are held once every two months to study project progression, discuss about valorisation strategies and co-funding of selected projects, and about general organization and animation (congress and meetings).

In addition to these committees, 3 operating committees “research” have also been established (one per workpackage) and a technical committee "training", who meet whenever necessary.

- **The technical committee « training »** is composed with professors and assistant professors from the 4 Universities: L. Gautreau, N. Degauque, M. Chérel, N. Gervois, Y. Guilloux, R. Josien, A. Moreau-Aubry, X. Saulquin (Nantes), C. Beauvillain (Angers), C. Jamin (Brest), P. Amé-Thomas, Cédric Ménard and Karin Tarte (Rennes). This committee is responsible for implementing Labex IGO training program. It is coordinated by L. Gautreau and N. Degauque and has developed an action plan throughout the duration of Labex IGO.

RESEARCH

Labex IGO research program is organized around 3 Workpackages according to a rationale starting from target and biomarkers identification and leading to innovative therapeutic strategies (WP.1: New immune markers and therapeutic targets for immunomodulating or depleting therapies, WP.2: Innovative cellular immunotherapies and WP.3: Combined therapies).

- "Call 2012"

In spring 2012, research projects have been selected for funding by the steering committee of Labex IGO for the first 3 years. From October 2012 to January 2013, Labex IGO has gradually started these first **8 research projects** (NB: some are divided into subprojects). These research projects have received 60k€ for operating cost and 96k€ for personnel cost for 3 year
In summer 2013, the **2 research facilities** of Labex IGO ("humanized rodents" and "immunomonitoring") have started their activities. These research facilities will receive about 200k€ for operating cost and 200k€ for personnel cost for 7 years (2013-2019).

24 people have been recruited on those first research projects and facilities: 9 PhD students, 4 post-doctoral research assistants, 7 research assistants and 4 technical assistants.

- "Call 2014"

In December 2013, Labex IGO has launched a call for proposal for new research projects: **6 projects** have been selected in April 2014 by the steering committee following recommendations of the Scientific Advisory Board (3 new projects in WP1, 2 in WP2 and 1 in WP3)
4 of these new projects have started in autumn 2014 and 2 of them have started in spring 2015. Each of the selected projects has received 75k€ for operating cost and 96k€ for personnel cost for 3 years.

10 people have been recruited on those projects: 2 PhD students, 3 post-doctoral research assistants and 5 technical assistants.

- "Call 2016"

In December 2015, Labex IGO has launched its 2nd call for proposal. **5 projects** have been selected by the Scientific Advisory Board in April 2016 (2 new projects in WP1, 2 in WP2 and 1 in WP3). This call focus on two axes: immunoregulation (including more cognitive aspects on macrophages, DC…) and immuno-intervention (translational programs based on antibodies or genetic engeneering).
The context of this last call for projects has been modified to improve the scientific strategy of the program and to favor collaborative programs involving several IGO teams.
These new projects have started in autumn 2016. Selected projects have received funding for 3 years according to the number of teams from Labex IGO involved in the project. For project led by 2 teams from Labex IGO: 171k€ (75k€ for operating cost and 96k€ for personnel cost); for project led by 3 teams from Labex IGO: 256.5k€ (112.5k€ for operating cost and 144k€ for personnel cost); for project led by 4 teams from Labex IGO: 342k€ (150k€ for operating cost and 192k€ for personnel cost).

8 people have been recruited on those projects: 3 PhD students, 3 research assistants and 2 technical assistants.

Labex IGO involves many interactions between the different teams. At least two scientific partners are involved in the majority of projects and each call bor bids is open to all members of the network.

A scientific report for each of these funded projects is included in this document (p.20).

- Call on “projects with industrial and clinical value”

In 2017 we launched a call with the Region Pays de la Loire in which two projects were funded with 50 k€ each focusing on projects with strong potential for industrial or clinical applications. Eleven projects were submitted, 7 were found eligible and were evaluated by the SAB for selection of 2 of them. Funding begun in June 2017.

- “Call 2018” (last call)

In December 2017, Labex IGO has launched its last call for proposal. 336 k€ will be allocated to new research projects. We received 25 projects and 4 projects will be awarded with 84 k€. **These 4 new projects** will be selected by the Scientific Advisory Board in April 2018; they will start in spring 2018 and end up in December 2021.

The aim of this call is to bring out new projects within the 13 partner teams of LabEx IGO. These **emerging projects** should be entirely new or correspond to new aspects of existing projects but not already funded and/or already published.

EDUCATION

Coordinators: Laetitia Gautreau-Rolland and Nicolas Degauque

And their colleagues: Patricia Amé-Thomas, Cédric Ménard, Céline Beauvillain, Michel Chérel, Nadine Gervois, Yannick Guilloux, Christophe Jamin, Régis Josien, Agnès Moreau Aubry, Xavier Saulquin & Karin Tarte

➢ MAIN OBJECTIVES

1. Enroll excellent students Master and PhD in expert fields of IGO laboratories
2. Become experts in innovative technologies, confront the practices across IGO laboratories
3. Prepare our students to be actors of their future

➢ ACADEMIC TRAINING

To promote high quality training, the Labex IGO provides funds to support the organization of hands-on training (purchase of reagent and small lab equipment). For instance, a bench-size flow cytometer has been purchased and can be easily moved to the classroom. The training of M1/M2 students takes place at the various universities belonging to the Labex IGO. External scientific speakers are invited to give lectures during the initial training of M2 students. As an example, in 2017, Emmanuel Donnadieu and Bruno Lucas from Paris, Julien Marie from Lyon and Marina Bousquet from Toulouse have given lectures in addition of 10 local researchers in the course Immunology/Oncology. The Labex IGO also provides a financial and logistic support for invited speakers, lectures that are opened to all the scientific community of the Labex IGO. Thereby, 46 speakers have been invited by the Labex IGO to come and present their work between 2012 and 2018. Finally, the Labex IGO supports the Roscoff workshop dedicated to immuno-oncology and co-organized by the universities of Nantes, Angers and Rennes.

➢ ATTRACTIVENESS

Excellent master 1 student has the unique opportunity to perform a 3-months training in one of the Labex IGO teams. Selected students will be funded, including two months financially covered by the Labex IGO. In the last six years the LabEx has selected 39 students (2013:1, 2014:6, 2015:7, 2016:11, 2017:7, 2018: 7) for such training in LabEx teams.

Since 2017, PhD students from Labex IGO teams can receive a short-term grant (1-3 months) to support the end of their thesis and/or to ease the transition toward a post-doctoral position. For this first year, 7 PhD students benefited from this grant. Being able to attend an international meeting is key in the development of young scientists (networking, presentation of the scientific knowledge gather during a Labex IGO grant). The travel expenses can be paid by the WP Dissemination of Labex IGO (amount may vary according the location of the meeting). Since 2012: 33 PhD students benefited from this help.

- **THE LABEX IGO NETWORK EXCELLENCE AS A FIRST STEP IN THE SCIENTIFIC CAREER DEVELOPMENT OF FUTURE SCIENTISTS**

English journal club gathering a broad audience from Master 2 and PhD students to established researchers to extend the scientific knowledge, to develop fair and accurate appraisal of scientific literature and to promote scientific discussion among members of the Labex IGO. Since 2017, the established journal club of CRTI – INSERM UMR1064 has been opened to the CRCINA, and CRCINA contribute to one third of Journal club members (12 out 32).

Intra-Labex workshop. Scientific and social networking during a 2-days meeting to share knowledge obtained thanks to Labex IGO grants. A special attention will be paid to offer the opportunity of Labex IGO youngest members to present their work.

Extensive hands-on training. Throughout the first year, PhD students will be trained in the core facilities available within the IGO consortium (flow cytometry, microscopy, production of recombinant protein, genetic analysis, animal handling, gene cloning …). Our objective is to implement each core facility with high-tech equipment that would be dedicated in part to educational training. The IGO network will cover access fees, consumables and co-funding of some devices. Training will be dispensed by the teaching-personal of the Universities (Medicine, Pharmacy and Science) in close collaboration with technical supervisors of the various core facilities. This extensive hands-on training should yield highly motivated students selected from the M1 and M2.

Labex IGO meeting. An international Labex IGO meeting (every two years: the first in 2014 and the second in 2016) is organized aiming to gather international and national excellent scientific speakers during a 2 days meeting. Students from Labex IGO teams will have a unique opportunity to join the local organization committee and to present their work to a broad scientific audience. The third IGO meeting will be held in Nantes on April 17th & 18th 2018 (see Website for scientific program).

In 2016 Labex IGO students participated to the organization of the 2nd IGO meeting which was held in Nantes on April 21st & 22nd. To alternate with this IGO meeting, which is organized every two years, an Intra-Labex workshop was held in April 2017. During these days, Labex IGO students presented their ongoing projects. The meeting was opened to the whole scientific community.

Overall, PhD thesis initiated in 2016-2017 were 5.

DISSEMINATION AND COMMUNICATION

Between 2016 and 2018:

- Several partnerships with academic institutions were developped.
- Labex IGO was the co-sponsor of 11 conferences.
- 25 PhD students and post-doctoral assistants obtained a funding from Labex IGO to attend to conferences.
- 2 PhD students obtained a fellowship from Labex IGO for training purpose.
- 3 foreign researchers were invited by Labex IGO.

PARTNERSHIP WITH ACADEMIC INSTITUTIONS

Labex IGO teams are partners initiated or participated in the following 2016-2017 national or local strategic and structural projects:

- **SIRIC (Site de Recherche Intégrée Sur le Cancer)**. Several teams of the IGO LabEx (Team 4 (M. Gregoire), Team 7 (Y. Delneste), Team 13 (M. Cherel) from the CRCINA, U1232) are involved in a SIRIC program obtained in 2017. This program aims at understanding the mechanism involved in the tumor immunosuppression in advanced cancers led to innovative and successful immunotherapies, such as immune checkpoint inhibitors (ICI). The success of ICI also demonstrates that manipulating the immune system is a powerful strategy that can be used alone or in combination with conventional therapies. Thus, objectives of the SIRIC program clearly overlap those of the IGO program, concerning tumor immunology and anti-tumor immunotherapies.
- **DHU (Département Hospitalo Universitaire)**. Most of the teams from the LabEx IGO are also part of the « DHU Oncogreffe », that is designed around three axes: 1/the immune targeting of tumors through radiotherapy, 2/ tumor escape and 3/ immune tolerance in transplantation. Besides research programs conducted in the context of the DHU Oncogreffe, clinical teams are also clearly involved in this network, facilitating the development of clinical and translational projects.
- **RHU (Recherche Hospitalo-Universitaire) KTD Innov (Kidney transplantation diagnostic innovation) et EU TRAIN (the EUropean TRAnsplantation and INnovation consortium for risk stratification in kidney transplant patients).** These two projects aim to develop integrated kidney transplant systems (iBox for RHU and EU tracer for EU TRAIN) for accurate diagnosis and treatment response to improve graft survival and patient monitoring. Labex IGO interacts with these projects since Team 4 (S. Brouard) from CRTI U1064 participates in these two projects.
- **NExT (Nantes Excellence Trajectory).** NExT is an initiative within the I-SITE call for projects which is in turn part of the Investment for the Future Programme (PIA2). The 3 founders of NExT are: University of Nantes, the Nantes University Hospital and Inserm. The goal of the NExT initiative is to accelerate the unique dynamic of the site in Nantes which is internationally famous and recognized for its expertise in research, training and innovation in two major inter-disciplinary areas: health and the Industry of the future.
- **Other Labex in the immunology area**. In November 2016 there was a fisrt half day meeting between Labex IGO, DCBIOL and Inflamex and the decision was made to organize a bigger meeting to gather members of Labex in the immunology area. So, on 13-14th November 2017, we organised the first “Inter-Labex Meeting”. This meeting fostered exchanges between Labex and created an environment conducive to the development of interactions and collaborations.

- **Project EUR** underway. The University of Nantes is working on a project of EUR (Graduate School) with Labex IGO as an essential partner. Indded, the topic of this EUR (supported by INSERM) will be "Immunintervention", and thus will strongly increase the visibility and attractiveness of LabEx IGO teams.

In 2017 we established a program to strength collaborations with international research centers. The aim is to increase for Labex IGO its scientific output, recruitment of high level post-docs and PhD students, international visibility and insertion in international research projects. The research centers to whom we will carry on this partnership are San Raffaele Institue (Milan), Charité University (Berlin) and Millenium Institute (Chile). The program will be funded by specific calls in which collaborative projects will be presented between Labex IGO groups and one of this 3 centers. The funding will pay travel expenses of Labex IGO and partner centers and common reagents and expenses (animals, antibodies, etc) and the experiments and salary of personel involved in experiments of the Labex IGO team but not of the partner. This program will be launched with the renewal of the Labex IGO proposal in 2020.

CONGRESS AND SEMINARS

Labex IGO participates to various conferences and seminars as full organiser, co-organiser or as co-sponsor.

Since 2016, Labex IGO co-organised an international congress and the first Inter-Labex meeting:

- "*22nd international NAT conference (Immunotherapies in transplantation and cancer)*" (Nantes, France, 1st-2nd June 2017), co-organised by Labex IGO and ITUN (Institut Transplantation UrologieNéphrologie / UMR1064). This conference gathered 220 scientists and PhD students from different fields.
- *"Inter-Labex Meeting"* (Val de Grâce, Paris, France, 13-14th November 2017), co-organised by Labex IGO, DCBIOL and Inflamex. This conference gathered 140 scientists and PhD students from 6 Labex in Immunology: IGO, DCBIOL, Inflamex, Immuno-Onco, MabImprove and Milieu Intérieur.

Since 2016, Labex IGO is the co-sponsor of several international congresses and workshops:

- *"21st international NAT conference (When stem cells meet immunology)*" (Nantes, France, 9-10th June 2016), organised by ITUN, LIOAD and the Loire Valley regenerative cluster Bioregate.
- *"Meeting CFCD 2016"* (Paris, France, 19-20th September 2016), organised by CFCD.
- "*FOCIS European Advanced course*" (Institut Pasteur, Paris, France, 17-19th October 2016), organised by Ignacio Anegon on behalf of FOCIS.
- *"SFI 2016: Annual Meeting of the French Society for Immunology"* (Cité des Sciences et de l'Industrie, Paris, France, 28-30th November 2016), organised by SFI (French Society for Immunology).
- *"EpiBrest 2016"* (Oceanopolis, Brest, France, 8th December 2016, organised by UBO.
- "*Advances in transgenic animal models and techniques*" (Nantes, France, 11-12th May 2017), organised by the Transgenic Rats ImmunoPhenomic (TRIP) Nantes facility, which is part of SFR François Bonamy, INSERM, ITUN and Université de Nantes.
- *"Germinal center mini-workshop"* (CHU Pontchaillou, Rennes, France, 30th June 2017), organised by UMR1236.

- *"SFI 2017: Annual Meeting of the French Society for Immunology"* (Centre des congrès, Reims, France, 7-9th November 2017), organised SFI and AFC (French Cytometry association).
- *"6th workshop immunotherapies Grand Ouest (Infection and cancer)"* (Nantilus, Nantes, France, 15th December 2017, organised by Cancéropôle Grand Ouest.

PUBLICATION EXPENSES

- Since 2016 Labex IGO has begun paying expenses linked to publications (color pictures, etc, and not the printing of manuscripts). Since March 2017, 14 publications have been payed.

FELLOWSHIPS

➢ SHORT-TERM AND LONG-TERM FELLOWSHIPS

These fellowships are awarded for the purpose of bench marking, partnership initiation, scientific collaboration, advanced training or employing techniques not available in Labex IGO labs. The steering committee decides on the amount to be given; at the end of their stay, laureates have to provide a report.

In 2016 and 2017, PhD students have sollicited this fellowship for training purposes:
- Cynthia Chauvin, PhD student in UMR892 team 1, participated to a training program on experimental surgery.
- Léa Flippe, PhD student in UMR1064 team 2, joined the team of Dr Maria Themeli (VUmc Cancer Center Amsterdam, Netherlands) for two weeks to learn a new technique of cell culture for the differentiation of induced human pluripotent stem cells (hiPSC) into T cells.

Two requests have been accepted for 2018:
- Mélanie Lancien, PhD student in UMR1064 team 1, will join the team of Pr Gianluca Matteoli (Laboratory of Mucosal Immunology, Translational Research Center for GastroIntestinal Disorders, KU Leuven, Belgium) for one week to develop a scientific collaboration.
- Cédric Ménard MCU-PH in UMR917, will joined David Weinstock group (Dana Farber Cancer Institute, Harvard Medical School, Boston, USA) for one year (September 2018 – August 2019 to work on tumoral microenvironment. He will acquire expertise in xenograft model and develop a real collaboration with de Dana Farber. This dissemination activity will benefit to the entire Labex IGO by providing new skills (PDX model), opportunities for the development of new projects on tumoral microenvironment and new collaborations, and opportunities for students exchanges.

➢ CONFERENCE TRAVEL FELLOWSHIPS

These fellowships are awarded to PhD students and post-doctoral assistants to attend to conferences for which their abstract has been accepted for an oral communication or a poster presentation. The maximum award depends on the location of the conference (500€ in France, 800€ in Europe, 1500€ out of Europe). Since 2016, 22 PhD students and 3 post-doctoral assistants (8 in 2016, 11 in 2017, 6 in 2018) have obtained this grant and several of them got a price for their presentation.

- **VISITING FELLOWSHIP**

This program aims at attracting international fellows engaged in research in the fields of immunology, oncology and transplantation. It is designed to benefit to the entire Labex IGO network by, for example, initiating or developing international collaborative research projects, enriching the research and training environment for early career (postgraduate and postdoctoral) staff, enhancing Labex IGO educational programs through courses and seminars.
Labex IGO visiting fellowships are opened to top level foreign researchers, who hold faculty appointments at other institutions abroad. Application from top level researchers, who hold positions in private companies, may be considered.
This program funds visit of foreign research staff for short period (few days to 6 weeks, in first instance; In case of success, further actions could be to propose longer hosting period, *e.g.* for research chair or academic chair).

Michael Schmueck-Henneresse (Charité Universitätsmedizin Berlin) was invited by Carole Guillonneau, from May 23rd-25th, 2016. The objective of the venue of Dr. Michael Schmueck Henneresse were to launch new collaborative projects between our center of transplantation (Team 2 of Carole Guillonneau and Ignacio Anegon) and of the Hospital Charité from Berlin on the identification of CD8 Tregs cells with stem cell properties and on the impact of anti-CD45RC on anti-viral responses in human. The medium term objectives are to apply to funding with preliminary results from these projects. In addition, Dr Michael Schmueck Henneresse gave a seminar and discussed with several members of the CRTI on projects from the CRTI and potential collaborations.

Marcello Hill (Medical School, Pasteur Institut, Montevideo, Uruguay) was invited by Maria Cristina Cuturi, from May 24th to June 5th, 2017, to have discussions about collaborative projects. He gave a seminar entitled « A regulatory role for NLRP3 inflammasome in coronavirus infection ». Finally, he was also chairman at the 20th international NAT conference, co-organised by Labex IGO.

Rodrigo Papa Gobbi (Instituto de Estudios Inmunologicos y Fisiopatologicos IIFP - CONICET
Facultad de Ciencias Exactas, Universidad de la Plata, Argentina) was invited by I. Anegon from February 17th-25th, 2018. The aim of the visit was to apply anti-CD45RC therapy in a model of intestinal transplantation in the rat. Rodrigo Papa Gobbi learnt to purify the anti-CD45RC MAb and the control isotype MAb that he will use in his experiments in Argentina and he also learnt to perform the mix of MAbs to perform multicolor cytometry to define Tregs and other immune cell populations as well as to confirm depletion of CD45RC+ cells in animals treated with the anti-CD45RC MAb. Finally, he gave a seminar describing the model and research his group has performed in Argentina. If the anti-CD45RC treatment inhibits intestine rejection it will lead to a joint publication. If the collaboration is successful, it may lead in the future to the application of other tolerogenic or immunosuppressive strategies in this transplantation model. Rodrigo Papa Gobbi's laboratory is a FOCIS Center of Excellence and thus gives visibility to Labex IGO.

RESULT EXPLOITATION AND SOCIO-ECONOMIC OUTCOMES

Between 2016 and 2018:

- An increasing number of publications
- At least 7 partnerships with private companies.
- 3 start-up were incubated.
- Results of Labex IGO research projects led to 5 patents.
- 2 clinical assays are in progress.

➢ SCIENTIFIC PUBLICATIONS

To date results of Labex IGO research projects led to 188 publications (cf. Appendix 5 : Publications).

➢ PARTNERSHIP WITH INDUSTRIES, START-UP

In order to identify the intellectual property developed in the project and the optimum route for its protection and subsequent dissemination and/or exploitation, LabEx IGO benefits from the expertise of different technology transfer offices such as SATT Ouest, INSERM Transfert and Nantes University Hospital. LabEx IGO also benefits from the expertise of Atlanpole Biotherapies, a "Competitiveness cluster" involving local biotech companies and academic laboratories from our Region. A representative of Atlanpole Biotherapies is part of the Directory Board.

Partnership with companies:

- OSE-Therapeutics (Nantes). Contracts collaboration with several teams of INSERM UMR 1064.
- TxCell (Nice). Contract collaboration with team 2 (I. Anegon and C. Guillonneau) of INSERM UMR 1064.
- Teneobio (Palo Alto, USA). Collaboration with team 2 (I. Anegon) of INSERM UMR 1064.
- genOway (Lyon). LabCom SOURIRAT with team 2 (I. Anegon) of INSERM UMR 1064.
- Biopredict (Rennes). LabCom HuLiver with team 2 (T. H. Nguyen) of INSERM UMR 1064.
- BMS, collaboration with Team 3 U1232
- Qiagen (Washington DC): collaboration with Team 3 U1232

Start-ups created within Labex IGO:

1) Epidrugs Discovery (P.F. Cartron, INSERM UMR 892) develops a new generation of epigenetic drugs. It was awarded by the French Ministery of Research and Education.
2) GoLiver (T.H. Nguyen, INSERM UMR 1064), created in 2017, develops human hepatocytes from ES/iPS cells to be used in the treatment of liver diseases. It was awarded by the French Ministery of Research and Education.
3) One new start-up, AbolerIS Pharma (C. Guillonneau and I. Anegon, INSERM UMR 1064) is also being incubated by Atlanpole and SATT. Plans are that the company will be created by the end 2018.

➢ PATENTS

During 2016 and 2017 results of Labex IGO research projects led to 5 patents. (cf. Appendix 6 : Patent list).

- **CLINICAL TRIALS**

. Two Phase I/II clinical trials of adoptive cell transfer have been authorized in December 2014 and March 2015 and first patients have been included in 2015. These trials consist either in adoptive transfer of tolerogenic DC to transplanted patients (Project T.4.2) or in adoptive transfer of melanoma specific T cells sorted and amplified according an original procedure (Project T4.1.1). During 2016-2017 the One Study on the use of tolerogenic DCs in kidney transplanted patients finished the recruitment of the intended patients and the analysis of results is underway.

. The MELSORT clinical trial (NCT02424916) is a Phase I/II study of adoptive transfer of specific melanoma antigens CD8+ T cells to 17 HLA-A2 metastatic melanoma patients. This trial is currently ongoing (6 patients treated since June 2015). This study evaluates the safety as well as the potential clinical efficacy of an adoptive transfer of CD8+ T cells, sorted with HLA-peptide multimers and specific for Melan-A and MELOE-1 melanoma antigens, to patients suffering from advanced metastatic melanoma (stages IIIc and IV). The optimization of the selection and amplifcation method, and the characterization of the functional properties of amplified T cells have been supported by the IGO program (T4.1.1).
Publication : Labarriere et al., Clin dev Immunol, 2013.

RENEWAL OF LABEX IGO IN 2020

The call for the renewal of the Labex consortiums in 2018 was announced in the spring 2017. The new Labex IGO would be created in January 1st 2020 for 5 years and funded by 2 M€. We immediately begun the organization of the new proposal with meetings that begun before the summer break and continued with a regular basis during autumn and winter 2017 and 2018.
The first important decision was the renewal of the direction of the program, with new and younger researchers as scientific coordinators. We thus created a **committee for the renewal of Labex IGO**. This committee was formed by 8 persons (UMR1064: Ignacio Anegon, Sophie, Brouard, Carole Guillonneau and Régis Josien; UMR1232: Joëlle Gaschet, Nathalie Labarrière, François Lang and Emmanuel Scotet).
Through a series of meetings and discussions it was decided to keep a rotating direction with a Director and a Deputy Director that would switch each half of the period of the new Labex. The new direction was selected unanimously to have in a first period (2020-mid 2022) as Director Emmanuel Scotet (INSERM-CNRS UMR1232-CRCINA, immuno-oncology) and as Deputy Director Régis Josien (INSERM UMR 064-CRTI, transplantation).
It was also decided to keep the scientific orientation of the Labex IGO: immunotherapy in transplantation and cancer, and autoimmunity. From a technical point of vue, we decided to keep funding the humanized rodent facility and to participate in the acquisition of an innovative high content cytofluorimeter or mass spectral cytofluorimeter, and fund salaries for bioinformatic analysis of high density data obtained from RNAseq data and non-supervised analysis of cytofluorimetry.

In 2017 we also decided to include new teams for the renewal proposal of Labex IGO in 2018. We launched a call for teams of the research units that form the Labex IGO interested in participating to the renewal of Labex IGO. We received 7 written proposals and the group leaders presented orally these proposals and answered to questions of the steering committee (11 members) and the committee for the renewal of Labex IGO (8 members including 4 not belonging to the steering committee). The selection was based on the scientific quality of the teams (publications, grants, patents, international visibility), on their research in immunology as a strong axis of their work and on their interest to integrate Labex IGO as well as in the added value for Labex IGO. This evaluation ended with an anonymous vote for the integration or not of each candidate team by the evaluation committee (15 members). Two new teams were selected for the future Labex IGO renewal: a) the team Prof. P. A. Gourraud, which works in immunogenetics and population genetics, this is an ATIP Avenir team (French government funding for new excellent teams), part of INSERM UMR 1064-CRTI and; b) the team of F. Altare which works on the role of microbiota on Tregs, immunotherapy and colon cancer development and which is part of the INSERM UMR 1232 of Nantes. The platform of cytofluorimetry and cell sorting Cytocell from the SFR/UMR1232 was also accepted to include the Labex IGO.

TABLE 1 : RESEARCH PROJECTS AND PLATFORMS INITIATED IN 2012/2013, IN 2014/2015 AND IN 2016/2017

Labex IGO funds 19 research projects for 3 years (8 projects started in 2012/2013, 6 projects selected on internal call 2014 and started in 2014/2015 and 5 projects selected on internal call 2016 and started in 2016/2017), and 2 platforms (research facilities) for 7 years (2013/2019).

Project	WORKPACKAGE RESEARCH	WP Coordinator *Project manager*
	WP1. NEW IMMUNE MARKERS AND THERAPEUTIC TARGETS FOR IMMUNOMODULATING OR DEPLETING THERAPIES	**Ignacio Anegon**
	T1. Immunobiology of common mechanisms in transplantation and cancer	
1	T1.1. Assessment of the role played by various immunoregulatory subsets in tumor immune escape and allotolerance	
1a	*T1.1.1. CD8+ Treg cells in human organ transplantation and cancer*	
	(i) Project 1: CD8+ Treg in organ transplantation	*C.Guillonneau/I.Anegon*
	(ii) Project 2: CD8+ Treg in cancer	*D.Valmori*
1b	*T1.1.2. Regulatory B cell*	*JO. Pers / S. Brouard / K. Tarte*
1c	*T1.1.3. Regulatory myeloid cells*	*Y. Delneste / MC. Cuturi*
2	T1.2. HLA-E-restricted T cell populations and immunoregulatory activity of soluble HLA-E in transplantation and in melanoma	*N. Gervois / B. Charreau*
3	T1.3. New immunosuppressive molecules	*I. Anegon*
CALL 2014	T1.4. A new anti-CMV strategy to prevent primary infection during HSCT	*F.Haspot / I. Anegon*
CALL 2014	T1.5. Functional Contribution of MICA Polymorphic variants to Immune Responses in Organ Transplantation and in Cancer	*N. Gervois / B. Charreau*
CALL 2014	T1.6. HO-1 and tolerance (HOT)	*I. Anegon / JF. Fonteneau*
CALL 2016	T1.7. Immune tolerance by IL-34: actions on macrophages and Tregs (TOL34)	*I. Anegon / MC. Cuturi*
CALL 2016	T1.8. CD8+CD28neg T cells, a threat to transplantation?	*N. Degauque / F. Haspot / C. Pecqueur*
	T2. Humanized rodent platform	
4	T2.1. Humanized mouse platform	*B. Vanhove*
	T3. Immunomonitoring platform	
5	T3.2. Immunomonitoring platform	*R.Josien/N.Labarrière*
	WP2. INNOVATIVE CELLULAR IMMUNOTHERAPIES	**François Lang**

	T4. Cell Immunotherapies	
6	T4.1. Generation of Ag specific T cells or T cells with polarized function	*N. Labarrière*
6a	*T4.1.1 Optimization of specific cytotoxic T lymphocytes (CTL) in vitro selection/amplification for therapeutic protocols*	*N.Labarrière/H. Vié*
6b	*T4.1.2 Glioblastoma immunotherapy : a model for the use of banked allogeneic T cells*	*H. Vié*
7	T4.2. Generation and characterization of human Tolerogenic Dendritic Cells for human administration	*MC. Cuturi*
CALL 2014	T4.3. Adoptive cell therapy for glioblastoma – characterization of tumor cell targets and analysis of their recognition by human t lymphocytes in vitro and in vivo	*E. Scotet / C. Pecqueur*
CALL 2016	T4.4. Engineered CAR-Tregs – licensed to specific control of immune responses in transplantation	*C Guillonneau / X. Saulquin*
CALL 2016	T4.5. Boosting anti-tumor response by conferring metabolic autonomy to T cells	*C. Louvet / B. Vanhove*
	T5. Cell tracking	
CALL 2014	T5.1. Tracking of tumor-specific T cells	*Y. Guilloux / E. Mortier*
	WP3. COMBINED THERAPIES *(New immunodepleting and immunomodulating strategies with broader indications and enhanced efficacy)*	**Jean-François Fonteneau**
	T.6. Relationship between immunoresistance of tumor and immune cells and apoptosis signaling pathways	
8	T6.1. Immunogenicity and apoptosis	*F. Vallette / PF. Cartron*
	T.7. Innovative strategies to enhance the efficacy of immunomodulating and immunodepleting therapies	
9	T7.1. CD138 Radiotargeting and Immunostimulation	*J. Gaschet*
10	T7.2. Enhancing the efficacy of anti-cancer vaccines using immunostimulants	*D. Valmori*
CALL 2014	T7.3. Melanoma vaccination : coupling optimized long peptides to a viral protein that targets dendritic cells and favors cross Presentation	*F. Lang / P. Jeannin*
CALL 2016	T7.4. Combining adoptive T cell transfer of engineered PD-1 deficient specific T cells with a-radioimmunotherapy for melanoma treatment	*N. Labarrière / E. Scotet / J. Gaschet / T. Nguyen*

REPORTS ON FUNDED RESEARCH PROJECTS AND PLATFORMS

WP1: NEW IMMUNE MARKERS AND THERAPEUTIC TARGETS FOR IMMUNOMODULATING OR DEPLETING STRATEGIES

(coordinator: I. Anegon)

This WP covers **eight projects with several tasks** aiming to accelerate and improve proof of preclinical and clinical efficacy for new immunotherapies, and two shared platforms.

1) Immunobiology of common mechanisms in transplantation and cancer.
The aim of this project is to characterize and compare new immune mechanisms regulating antitumor immunity and allograft rejection as well as tumor immune escape and tolerogenic mechanisms in allotransplantation. Particularly, γδ T cells, CD4 and CD8 T effectors and Tregs, Bregs, type II macrophages and myeloid suppressive cells, pDCs, MDSCs, and mesenchymal stromal cells and new immunosuppressive molecules (IL34, CLEC-1, IL22BP, CD45RC, HO-1, IDO, TORID,...).

2) Humanized rodent platform.
The aim is to set up new animal models as well as to generate and consolidate animal facilities. Particularly, immune system humanized mice and rats as well as new KO and KI rat models.
This platform in now fully operational and is an important tool for Labex IGO teams and for collaborations with external partners giving to Labex IGO visibility and momentum.

3) Immunomonitoring platform.
The aim is to use new technologies for cellular immune monitoring methods with very high content single cell analysis and to develop new monitoring approaches for phenotypic and functional analyses of human innate and adaptive immune cell subsets, and finally to implement a centralized data base for sharing results and standardized protocols.

T1.1 ASSESSMENT OF THE ROLE PLAYED BY VARIOUS IMMUNOREGULATORY SUBSETS IN TUMOR IMMUNE ESCAPE AND ALLOTOLERANCE

T1.1.1 CD8+ T reg cells in human organ transplantation and cancer

Subproject 1: CD8+ Treg in organ transplantation

Coordinators: Carole Guillonneau and Ignacio Anegon

Team involved: Team 2 ITUN-INSERM UMR-S 1064, Nantes

Staff funded by Labex IGO: Séverine Bézie (Engineer: IE) 2013-2015
Justine Dubé (master 2 student) 2015-2016
Antoine Freuchet (master 2 student) 2015-2016

Initiation of the project: 2013
End of the project: 2016

State of the art

Kidney transplantation is an essential therapy in patients with severe chronic renal failure. However, graft rejection requires patients to undergo extensive immunosuppressive treatment causing many side effects. There is a need for more specific therapies. There are several types of Tregs that can inhibit anti-donor immune response and the current project focuses on the analysis of one of these sub-populations among the least studied: the $CD8^+$ regulatory T cells.

Objectives

We reported previously that, in a rat MHC-mismatched heart allograft model, treatment with CD40Ig leads to indefinite allograft survival through induction of $CD8^+CD45RC^{low}$ Tregs, that themselves can induce indefinite survival. This project proposes to translate our knowledge to the clinic with in depth analysis of human $CD8^+CD45RC^{low}$ Tregs, evaluation of the potential of the $CD8^+CD45RC^{low}$ Tregs as a cellular therapy and of their specific markers and/or properties for prediction of transplant outcome.

Project progression / Results

1) We have confirmed the regulatory properties of $CD8^+CD45RC^{low}$ T cells with efficient suppression (>70%) of effector T cell proliferation after 5 days culture in presence of allogeneic T-depleted PBMCs and syngeneic CD4 effector cells. We have also demonstrated that in our culture conditions, $CD8^+CD45RC^{low}$ Tregs suppress more efficiently than $CD4^+CD25^+CD127^-$ Tregs.
2) We have also characterized human $CD8^+CD45RC^{low}$ Tregs and determined that there exists a superior suppressive subset co-secreting IFNg, IL10 and IL34 among them. We have demonstrated that blocking of these cytokines reversed the $CD8^+CD45RC^{low}$ Tregs suppressive effect. In addition, we have demonstrated that they exert little cytotoxicity.
3) Some markers including CD45RC have been already included in a clinical study of implementation of markers to the Kidney Transplant Failure Score (180 kidney transplanted patients from the DIVAT cohort have been selected). In addition, we are screening groups of kidney transplant patients from the DIVAT-cohort of Nantes CHU (acute versus stable patients) and group of bone marrow transplant patients from the BIOCORD cohort of Créteil hospital (acute versus no GVHD) for presence and functionality of $CD8^+CD45RC^{low}$ Tregs at day 0 and 12 months following transplantation.
4) We have set up a protocol of expansion of IFNg/IL10/IL34-secreting $CD8^+CD45RC^{low}$ Tregs and showed efficient expansion of human $CD8^+CD45RC^{low}$ Tregs (>1000 fold) in an antigen-dependent manner for at least 15 days. We have demonstrated that the suppressive activity of these expanded cells was increased and that they can efficiently inhibit graft rejection in model of GVHD and skin transplantation in humanized mice.

Publications and patents
See appendices 5 and 6

Collaborations
In Nantes : INSERM U1102 (D. Valmori); Team 4 of UMR1064 (M. Giral, D. Laplaud); Humanized rodents platform of the Labex IGO (B. Vanhove); Transgenic Rat Immunophenomic platform (I. Anegon); Plateforme IPSC (L. David); Plateforme recombinant protein (K. Bernardeau, F. Lang); GenoCellEdit platform (T. Nguyen).
National : Henri Mondor Hospital, Créteil (S. Maury)
International: The University of British Columbia, Vancouver, Canada (M. Levings); Department of Biochemistry and Molecular Biology, Melbourne, Australia (J. Rossjohn, S. Gras)

Perspectives of clinical or economical valorization
We have registered a patent on the phenotype of this sub-population and its role in transplantation. IL34 was patented as a new molecule with tolerogenic properties. We have more patents registered with different strategy of induction of tolerance in transplantation (one cytokine, one therapeutic depleting antibody and two allo-peptides). Another important new scientific goal will be to develop new therapeutic strategy of ex vivo expansion of CD8 Tregs. We are in discussion with industrials for licencing of these patents. In addition, I. Anegon and C. Guillonneau are incubating a start-up to develop one of these patents.
We expect to develop a better prognosis for patients before and following organ transplantation.

Added value of / for Labex IGO
The funding of the Labex IGO was essential to start the project and for the development of this project and has allowed the establishment of collaborations with team and platform of the labex and in particular the humanized rodent platform. In addition, our collaboration with D. Valmori (sub-project 2 of this task) is important to describe the role of this population and of its markers in cancer. We have also started to study this population in autoimmune disease such as multiple sclerosis (D. Laplaud) and have obtained interesting preliminary results.
Following acquisition of preliminary results, we have been able to obtain a number of important co-funding on different aspects of this project:

CoPoc INSERM 38 K€.
ARSEP 50 K€
ESOT Clinical Research Grant 50 K€
Agence de la Biomedecine 36 K€
Fondation pour la Recherche médicale 50 K€

T1.1 ASSESSMENT OF THE ROLE PLAYED BY VARIOUS IMMUNOREGULATORY SUBSETS IN TUMOR IMMUNE ESCAPE AND ALLOTOLERANCE

T1.1.1 CD8+ T reg cells in human organ transplantation and cancer

Subproject 2: IL4I1 specific CD8 Treg in cancer

(THIS PROJECT WAS NOT FINISHED; FUNDING WAS HANDED BACK TO LABEX AND WILL BE USED IN THE LAST CALL IN 2018)

Coordinator: D. Valmori

Team involved: Inserm UMR1102

Staff funded by Labex IGO: None

Initiation of the project: November 2013
End of the project: November 2016

State of the art

Even if studies on $CD4^+$ Treg have largely dominated the scene of immunoregulation during the last decade, the existence, prevalence and importance of human $CD8^+$ Treg in cancer is yet poorly appreciated.

Objectives

An L-phenylalanine oxidase called IL-4 induced gene 1 (IL4I1) was recently reported to be expressed in T_H17 cells and limit their capacity to proliferate and secrete IL-2. We have, in a project conducted by Clara Maria Scarlata PhD student, addressed the expression and role of IL4I1 in human Treg. As an extension of this project, we will address the hypothesis that IL4I1 is recognized by human CD8 Treg from cancer patients.

Project progression / Results

We have found that IL4I1 expression is induced by stimulation in *ex vivo* isolated circulating Treg and is restricted to a subpopulation that do not express Helios, a transcription factor that characterizes nTreg, but express Aiolos, that is involved in the differentiation of T_H17 and iTreg. We have also found that stimulation of Treg under inflammatory conditions increases IL4I1 expression, likely as part of a regulatory loop that attempts to limit the pathogenicity resulting from their conversion into T_H17. Together, these findings suggest that targeting IL4I1 could allow to modulate iTreg without affecting nTreg.

Publications

Clara Maria Scarlata, Clotilde Celse, Pascale Pignon, Maha Ayyoub, and Danila Valmori. Differential expression of the immunosuppressive enzyme IL4I1 in human induced $Aiolos^+$ but not natural $Helios^+$ $FOXP3^+$ Treg. Eur J Immunol. 2015 Feb;45(2):474-9.

T1.1 ASSESSMENT OF THE ROLE PLAYED BY VARIOUS IMMUNOREGULATORY SUBSETS IN TUMOR IMMUNE ESCAPE AND ALLOTOLERANCE

T1.1.2. Regulatory B cells

Coordinators: JO Pers / S. Brouard / K. Tarte

Teams involved: UMR1227 (Brest)
UMR1064 (Nantes)
UMR1236 (Rennes)

Staff funded by Labex IGO: Quentin Simon, PhD student (+50% UBO)
Audrey Mohr, PhD student (+50% Région Bretagne)
Lucas Le Lann, PhD student (+50% Région Bretagne)
Nicolas Hipp, PhD student (+50% Région Bretagne)
Léa Verdière, PhD student (+50% Région Bretagne)
Marina Boudigou, PhD student (+50% Région Bretagne)

Initiation of the project: 2012
End of the project: 2020

State of the art

Although numerous studies now contribute to strikingly demonstrate the importance of the regulatory B (Breg) cells in normal context but also in the control of cancer, transplantation, autoimmune and inflammatory responses in mice, few data are currently available in humans. However, it becomes evident that deciphering their induction and their phenotypic and functional heterogeneity could help to elaborate innovative approach to the treatments of these disorders.

Objectives

Because the field of Bregs is a complex one, we have ordered and prioritized the project with the following objectives: to identify and to characterize phenotypically and functionally subpopulations of regulatory human B cells using different types of co-cultures developed in the three laboratories. Secondarily, identification and characterization of these subpopulations will extend their monitoring in various autoimmune diseases, in cancer or in transplanted patients. We expect that these analyses will offer new tools for monitoring, diagnosis and / or prognosis for many diseases (transplantation, autoimmune diseases, chronic lymphocytic leukemia).

Project progression / Results

We have studied the nature of $CD24^{high}CD38^{high}$ transitional B cells and their potential overlaps with Breg cells. We reveal that $CD24^{high}$ $CD38^{high}$ B cells incorporate functionally distinct B cell subsets, each with distinct in vitro regulatory functions. Furthermore, our results reveal abnormal distribution of transitional B cell subsets in autoimmune diseases, bringing out new insights about Breg development and phenotype (Simon et al, J Allergy Clin Immunol, 2015).

Operationally tolerant patient (TOL) recipients show a higher frequency of $CD24^{high}CD38^{high}$ transitional B cells compared to patients with stable graft function, associated with a decreased frequency of $CD20^{-}$ $CD38^{+}$ $CD138^{+}$ differentiated plasma cells, suggestive of abnormal B cell differentiation. B cells from TOL proliferate normally but produce more IL-10 suggesting that a balance between B cells producing IL-10 and a deficiency in plasma cells may encourage a favorable environment to the tolerance maintenance (Chesneau et al. Am J Transplant, 2014).

In parallel, we have also analyzed the role of B cells from operationally tolerant patients, on T cell suppression. We showed that B cells inhibit CD4+CD25- effector T cell response in a dose dependent manner. This effect required B cells to interact with T-cell targets and was achieved through a

granzyme B (GzmB)–dependent pathway. Tolerant recipients harbored a higher number of B cells expressing GzmB. Finally, GzmB+ B-cell number was dependent on IL-21 production, and B cells from tolerant recipients but not from other patients positively regulated both the number of IL-21+ T cells and IL-21 production, suggesting a feedback loop in tolerant recipients that increases excessive B cell activation and allows regulation to take place. These data provide insights into the characterization of B cell–mediated immunoregulation in clinical tolerance and show a potential regulatory effect of B cells on effector T cells in blood from patients with operationally tolerant kidney graft (Chesneau et al. JASN 2015).
We showed that patients with chronic antibody-mediated rejection (cAbMR) display a unique B-cell phenotype with a reduced ratio of activated to memory B cells associated with an impaired immunosuppressive activity. Thus, phenotypic and functional analyses of the B-cell compartment may be indicated in the follow-up after transplantation and drive therapy in the establishment of transplant tolerance processes (Nouel et al. Kidney Int, 2013 and Ann Rheum Dis, 2014 and J Autoimmunity, 2015).
Follicular helper T (Tfh) cells are instrumental in the development of humoral responses. We have demonstrated that human Breg cells can modulate the development of humoral responses through the control of the Tfh cell polarization and of the Tfh-dependent terminal differentiation of B cells (Achour et al, J Allergy Clin Immunol, 2017).
To understand the heterogeneity of B cells and the heterogeneity of B cell responses to T-cell signals, we initiated spatiotemporal single-cell transcriptomic analysis from transitional and mature human naive B cell up to their terminal differentiation. We aimed to dissect the molecular mechanisms involved in B cell fate decision and function, and used single cell analysis to counteract the averaging caused by B cell heterogeneity. Results demonstrated that *in vitro* differentiation of transitional or naive B cells is a suitable model to study early signal integration such as IL-2 produced by T cells (PhD project of Nicolas Hipp). We showed that the timed repression of BACH2 through IL-2-mediated ERK/ELK1 signalling pathway directs plasma cell lineage commitment (Hipp et al. Nat Commun. 2017). This study provides insights into the temporal regulation of BACH2 and its targets for controlling the differentiation of human naive B cells.

Besides this research program, focused on B reg cells, the Team of JO Pers also study B cells in a peculiar autoimmune disease, the Sjögren's syndrome (SS), characterised by mouth and eye dryness due to irreversible destruction of glandular tissue by infiltrated lymphocytes. B cell hyperactivity is a hallmark of SS and results in secretion of auto-antibodies and production of various pro-inflammatory cytokines. In this context, the thesis project of Marina Boudigou (funded by the Labex IGO) aims to understand how the microenvironment influences B cell differentiation and their subsequent functional response and how it could contribute to the pathogenesis of autoimmune diseases.

Publications

-Chesneau M et al. Front Immunol. 2013;4:497.
-Nouël A, et al. Kidney Int. 2014 Mar;85:590-9..
-Chesneau M, et al. Am J Transplant, 2014;14:144-55.
-Nouël A, et al. Ann Rheum Dis. 2014;73 Suppl 1:A89.
-Nouël A, et al. Front Immunol. 2014;5:11.
-Nouël A et al. J Autoimmun, 2015, 59:53-60.
-Simon Q et al. J Allergy Clin Immunol. 2015 Oct 30. pii: S0091-6749(15)01352-4.
-Chesneau M, et al. J Am Soc Nephrol. 2015 Oct;26(10):2588-98.
-Mohr A et al. OncoImmunology. 2016 May;5(5):e1132977.
-Achour A et al. J Allergy Clin Immunol. 2017 Jul;140(1):215-222.
-Hipp et al. Nature Communications 2017 Nov 13;8(1):1443

Collaborations

UMR1227 is involved since 2014 in the European IMI Project PRECISESADS (Grant Agreement N°115565). The objective of the project is to determine a new taxonomy of autoimmune diseases based on OMICS approaches. The Breg phenotype will be a part of this new classification. A PhD student (Lucas le Lann) involved as computational immunologist has been hired by UMR1227.

Perspectives of clinical or economical valorization

The development of pharmacological methods for inhibiting expression of immune suppressive molecules specifically in B lymphocytes might reverse tumor-mediated regulation and significantly improve treatment of B cell lymphoma and leukemia. In addition, immune suppressive B cells could constitute a useful tool for therapeutic induction of tolerance in solid organ transplantation but also in autoimmune diseases. Developing new and improved methods to identify, purify and understand the functions of regulatory B cell subsets in humans should help advance the possibilities of translating this research into clinical practice.

Added value of / for Labex IGO

One grant/year has been allocated to the Labex IGO by Région Bretagne (50% of co-funding) since 2013. These grants have allowed UMR1227 (from Brest) and UMR1236 (from Rennes) to recruit and form PhD students involved in the project. Several co-fundings have been obtained on this project: Agence Nationale de la Biomédecine (Phenotypical and functional study of B cells in cAbMR patients) : 15,000 euros in 2014 and Ligue nationale contre le cancer (Identification and role of Bregs in chronic lymphocytic leukemia): 30,000 euros in 2015. Through the Labex visibility, UMR1227 is now heavily involved in the European Consortium "Innovative Medicine Initiative" (IMI) "Molecular Reclassification to Find Clinically Useful Biomarkers for Systemic Autoimmune Diseases (PRECISESADS)". No. 115565.

Derivative projects

Léa Verdière's PhD thesis

B cell behavior is not only influenced by the crosstalk with T cells but also with lymphoid stromal cells that express ECM components, cell surface markers, and soluble factors able to modify normal and malignant B cell growth and polarization. One of the main limitations to the study of B-cell/stromal cell crosstalk is the lack of characterization of human lymphoid stromal cell subsets. Léa Verdière started her PhD in October 2016 (Labex IGO/Région Bretagne co-funding) in order to study the heterogeneity of human lymphoid stromal cells. She performed already RNAseq on 4 sorted lymphoid stromal cell subsets and single-cell RNAseq (10X Genomics) is starting. In parallel, ATAQseq experiments are ongoing to better understand the relationships and the cell trajectories between and within these subsets. Finally cocultures of stromal cells and B cells are performed and 2D and 3D models have been optimized. Such work will provide the first characterization of B-cell/lymphoid stromal cell crosstalk in human and paves the way to the understanding of how stromal cells modulate B-cell fate and could eventually be used for therapeutic purposes.

Lucas Le Lann's PhD thesis

Lucas Le Lann's PhD thesis is part of the European project IMI PRECISESADS. This project aims to re-classify systemic autoimmune diseases according to molecular characteristics and not only clinical aspects. Indeed, the clinical symptoms being similar from one autoimmune disease to another, the diagnosis remains difficult to establish. To achieve a reclassification of these diseases according to biological criteria, several European laboratories in different scientific disciplines will generate various OMIC data (epigenomics, transcriptomics, metabolomics, proteomics ...). The INSERM Unit U1227 in Brest is responsible for coordinating the implementation and the analysis of the flow cytometry data acquisition obtained from the blood of 2900 individuals who are included in the study by 11 European laboratories. Flow cytometry allows the identification and counting of different populations (granulocytes, monocytes and lymphocytes) of the peripheral blood and allows determining the density of expression of the characteristic proteins of each of these cell populations.

The objective of Lucas LE LANN's PhD thesis is to harmonize the results obtained by the 11 centers and to analyze these data using bioinformatic tools. Individuals are divided into 3 cohorts. The cohort I comprises 300 individuals including 50 controls and 250 patients of known diagnostic who benefit from an extensive flow cytometry analysis. The cohort II comprises 2000 individuals including 1400 diagnosed patients and 600 controls who benefit from a partial flow cytometry analysis. Finally, the prospective cohort consists of 200 new patients who are followed at 6 months and 18 months with an extensive flow cytometry analysis.

Lucas LE LANN elaborated a bioinformatics approach allowing the analysis of more than 350 parameters, for each of the 2900 individuals, based on packages and scripts developed under R. Specifically, the use of OPENCYTO and flowCore packages makes it possible to automate the analysis strategy. Beforehand, a first series of non-automated analyzes on the 300 individuals of the cohort I has been carried out, in order to generate a databank allowing to compare and validate the strategy of the future automated analysis.

Publication :

Jamin, C., Le Lann, L., Alvarez-Errico, D., Barbarroja, N., Cantaert, T., Ducreux, J., ... & Trombetta, E. Multi-center harmonization of flow cytometers in the context of the European "PRECISESADS" project. Autoimmunity reviews, 15(11), 1038-1045.2016

T1.1 ASSESSMENT OF THE ROLE PLAYED BY VARIOUS IMMUNOREGULATORY SUBSETS IN TUMOR IMMUNE ESCAPE AND ALLOTOLERANCE

T 1.1.3. Regulatory myeloid cells

Title: Mechanisms of action and therapeutic potential of Myeloid-Derived Suppressive Cells (MDSCs)

Coordinators: Cédric Louvet and Maria Cristina Cuturi

Teams involved: Team 1 UMR1064, Nantes
Team 7 UMR892, Angers

Staff funded by Labex IGO : Garo Erwan (Engineer: IE) March 2013-August 2013, Angers
Aurélie Lemoine (Engineer Assistant: AI) April 2014-March 2016, Nantes

Initiation of the project: September 2012
End of the project: March 2016

State of the Art
Myeloid-derived suppressor cells (MDSCs) are a clinically applicable source of suppressive cells for their potential use in cell therapy. A translational view implicates the development of a clinically acceptable method for the production of these cells as well as a better understanding of their development, stability and function.

Objectives
The objective of this project is to combine the expertise and models of the two teams to develop in vitro generation of MDSC and evaluate their therapeutic potential in mouse models of autoimmunity and allograft transplantation. Moreover, we aim to investigate the function of a novel ion channel encoded by the gene *Torid/Tmem176b*.

Project progression / Results
Our results indicated that GM-CSF+IL-6-induced MDSCs bear therapeutic potential for allograft survival (**Drujont *et al.* PLoS One 2014**), in fact, similarly or better than other types of distinct BM-derived regulatory myeloid cells including tolerogenic DCs (tolDCs), regulatory macrophages (Mreg) (**Carretero-Iglesia *et al.* Transplantation 2016**).
The finding that the TMEM176B cationic channel function could be compensated by its co-regulated homolog TMEM176A prompted us to successfully develop a double KO mouse using CRISPR/Cas9 directly in C57BL/6 mouse zygotes (in collaboration with Francina Langa, Institut Pasteur, Paris) (**Lemoine *et al.* The Journal of Genetics and Genomics 2016**).
Unexpectedly, we discovered that *Tmem176a* and *b* genes were strongly co-expressed in Th17 cells as well as other cells of the RORγt+ family that are pivotal in type 17 immunity. We found that *Tmem176b* single-deficiency partially but significantly reduced imiquimod-induced psoriasis-like skin inflammation. We reported these observations and further data in a manuscript (**Drujont *et al.* Scientific Reports 2016**). Importantly, preliminary experiments using our double KO mice indicate an alteration of the Th17 compartment in the absence of both homologs. These results suggest that *Tmem176a/b* exert important functions in the immune system in intriguingly different but selected cells (immature myeloid cells versus RORγt+ cells).
Thus, during the course of this work, our initial hypothesis of a major role of *Torid/Tmem176b* in regulatory myeloid cells has been extended to the RORγt+ family including Th17 cells. It adds another

layer of complexity since both types of cells are involved in the development of anti-tumor immunity or autoimmune diseases.

Publications
See appendix 5

Collaborations
The Labex IGO has boosted an active collaboration between our team (Team 1, UMR1064, Nantes) and the team of Yves Delneste (Team 7, UMR892, Angers). This project has allowed the development of an academic collaboration with Francina Langa, Institut Pasteur (Paris), to successfully generate *Tmem176a/b* double KO mice.

Perspectives
This project led us to unexpectedly extend the implication of the gene *Torid/Tmem176b* in the immune system to RORγt+ cells. The high expression of TMEM176A and B cation channels in these cells is intriguing and could represent in the future a novel therapeutic entry point for treating immune-mediated inflammatory diseases.

Added value of / for Labex IGO
The Labex IGO has been central to the development of Lucile Drujont's PhD and the current position of Marcelo Hill (Institut Pasteur, Montevideo, Uruguay), with whom we maintain collaborative projects regarding the study of *Tmem176a/b*. Moreover, the Labex IGO is key to Cédric Louvet's aim to obtain a CNRS/INSERM researcher position. Finally, it has allowed funding by IHU Cesti to generate conditional double KO mice.

T1.2. HLA-E-RESTRICTED T CELL POPULATIONS AND IMMUNOREGULATORY ACTIVITY OF SOLUBLE HLA-E IN TRANSPLANTATION AND IN MELANOMA

Coordinators: Nadine Gervois and Béatrice Charreau

Teams involved: UMR892, Team 3
UMR1064, Team 5

Staff funded by Labex IGO: None

Initiation of the project: 2013
End of the project: 2018

State of the art:
A growing amount of data highlights an important immunoregulator role of non-classical MHC class I molecules in different pathologies. If the immunosurveillance and immune regulation functions of HLA-E as ligand for CD94/NKG2 are well established, its role as ligand for $\alpha\beta$ TCRs has only more recently become appreciated. We reported existence and allogeneic cross reactivity of HLA-E-restricted CD8 T cell response to CMV peptides that could be detrimental to graft endothelial cells and thus a risk factor to consider in kidney transplantation.

Objectives:
The general aim of this project is to re-evaluate the role of cell-bound and soluble HLA-E in both innate and acquired immune responses. Based on our recent work, one objective is to assess the frequency and the possible adverse impact of HLA-E-restricted immune response to CMV on renal graft outcome. This project also aims to investigate the existence of anti-tumor HLA-E-restricted T cells in melanoma. As we also preliminary demonstrated a frequent expression of soluble HLA-E molecules by vascular endothelial and tumor cells, another objective is to study the properties of soluble HLA-E in the context of transplantation, CMV infection and melanoma, including the potential predictive and prognostic value of sHLA-E levels in the blood of patients.

Project progression / Results:

On the one hand, we developed a dedicated flow cytometry multistaining (using HLA-E-CMV peptide tetramers) for the screening of CMV-committed HLA-E-restricted $CD8^+$ T cells in blood samples. This allowed us to characterized *ex vivo* by a qualitative and quantitative approach the unconventional UL40-specific HLA-E-restricted CD8 T lymphocytes (LT HLA-E_{UL40}) in kidney transplant patients (n = 119) and healthy volonteers (n = 25). We showed that their development was related to HCMV infection of the recipient and is specific for the infectious viral strain. LT HLA-E_{UL40} cells are quasiclonal populations present in approximately 30% of the $HCMV^+$ individuals and can account for up to 40% of circulating CD8 T cells. In addition, it appears that the *HLA-A*02* allele and the *HLA-E*01:01/01:03* genotype are factors associated with the generation of these populations. LT HLA-E_{UL40} are effector-memory CD8 T cells capable of cytotoxicity and cytokine production (TNF-α, IFN-γ, IL-2). In addition to the nominal peptide, these cells recognize a set of relatively close nonamers, including signal peptides derived from HLA-I proteins presented physiologically by HLA-E, thus raising the question of potential autologous and/or allogeneic reactivity. Overall, these results highlight the interest of studying the degree of involvement of LT HLA-E_{UL40} in the protection against HCMV and their role in a transplant context (Jouand N, Plos Pathogens, in revision).

On the other hand, we documented, in collaboration with Pr. C. Bossard, that intra-epithelial $CD94^+$ tumor-infiltrating lymphocytes in a context of HLA-E overexpression predicts poor prognosis in colorectal carcinomas (CRC) and define CD94/NKG2A as a potential new druggable inhibitory immune checkpoint in these cancers *(Eugène J. in preparation)*.

Retrospective dosages of HLA-E (using an in-house ELISA) in serum of melanoma patients enrolled in immunotherapy with TIL (in collaboration with Pr. B. Dréno) and of MSS/MSI colorectal cancer are also ongoing to assess its value as a biomarker and/or prognosis marker.
Finally, we have shown that non-classical HLA-E/peptide monomers (as well as classical) can efficiently activate antigen-specific CD8 T cells *in vitro*, via a mechanism that involves the passive transfer of monomer-derived peptide to cell-bound HLA-I molecules *(Allard M., J Immunol 2014)*. It remains to examine whether HLA-E molecules naturally produced by vascular endothelial cells and tumor cells have the same function.

Publications and patents (published / in press / in preparation)
- Allard M, Oger R, Benlalam H, Florenceau L, Echasserieau K, Bernardeau K, Labarrière N, Lang F and **Gervois N**. Soluble HLA-I/peptide monomers mediate antigen-specific CD8 T cell activation through passive peptide exchange with cell-bound HLA-I molecules. ***J Immunol**, 2014, 192 (11): 5090-5097.*
-Djaoud Z., Riou R., Gavlovsky P.J., Mehlal S., Bressollette C., Gérard N., Gagne K., **Charreau B**., Retière C. Cytomegalovirus-infected primary endothelial cells trigger NKG2C^{+} Natural Killer cells, **J Innate Immun**, 2016, 8(4):374-85.
- International extension of Patent "Methods and Kits for determining whether a cytomegalovirus infection in a transplanted patient is susceptible to induce allograft rejection" **Nadine Gervois**, **Béatrice Charreau** and Mathilde Allard, EP2872888A1, US20150192570 in 2015.
- Jouand N, Bressollette-Bodin C, Gérard N, Giral M, Guerif P, Rodallec A, Oger R, Parrot T, Cesbron-Gautier A, **Gervois N** and **Charreau B**. HCMV triggers frequent and persistent UL40-specific unconventional HLA-E-restricted CD8 T-cell responses with potential autologous and allogeneic peptide recognition, 2018, ***Plos Pathogens***, in revision.
- Eugène J, Jouand N, Dansette D, Ducoin K, Leveque E, Meurette G, Podevin J, Matysiak T, Bezieau S, Volteau C, Chettritt J, Kerdraon O, Fourquier P, Thibaudeau E, Mosnier JF, Toquet C, Jarry A, **Gervois N**, Bossard C. Intra-epithelial CD94^{+} tumor-infiltrating lymphocytes in a context of HLA-E overexpression predicts poor prognosis in colorectal carcinomas: a potential new druggable inhibitory immune checkpoint, in preparation.

Additional publications in the context of LabEx program:
- Pabois A., Pagie S., Gérard N., Laboisse C., Pattier S., Hulin, P., Nedellec S., Toquet C. **Charreau B**., Notch signaling mediates crosstalk between endothelial cells and macrophages via Dll4 and IL-6 in cardiac microvascular inflammation, ***Biochemical Pharmacology***, 2016, 104:95-107.
- Rouger C, Pagie S, Derbré S, Le Ray AM, Richomme P, **Charreau B.** Prenylated Polyphenols from Clusiaceae and Calophyllaceae with Immunomodulatory Activity on Endothelial Cells. ***PLoS One***. 2016 Dec 1;11(12):e0167361.
- Delville M, **Charreau B**, Rabant M, Legendre C, Anglicheau D. Pathogenesis of non-HLA antibodies in solid organ transplantation: Where do we stand? ***Hum Immunol.*** 2016 Nov;77(11):1055-1062.
- Parrot T, Oger R, Benlalam H, Raingeard de la Bletiere D, Jouand, N, Coutolleau A, Preissier L, Khammari A, Dreno B, Guardiola P, Delneste Y, Labarriere N, **Gervois N**. CD40L confers helper functions to human intra-melanoma class-I-restricted CD4CD8 double positive T cells. ***OncoImmunol.*** 2016 ; e1250991.
- Pagie S, Gérard N, **Charreau B.** Notch signaling triggered via the ligand DLL4 impedes M2 macrophage differentiation and promotes their apoptosis. ***Cell Commun Signal***. 2018, 10;16(1):4.

Collaborations (inside and outside Labex IGO)
- Pr. M. Giral, MD, PhD, head of the Nantes's Clinical Investigation Center in Transplantation
- Dr. C. Bressollette, MD, PhD, Nantes University and Virology Laboratory Hospital
- Pr. C. Bossard, MD, PhD, Nantes University and Pathological Anatomy Department Hospital
- Cytometry facility "CytoCell", Recombinant protein production facility and Cellular and Tissular Imaging Core Facility "MicroPICell" of the SFR Santé, Nantes

Perspectives of clinical or economical valorization

Defining the possible adverse impact of HLA-E-restricted immune response to CMV and regulatory mechanisms should provide a proof of concept identifying a new risk factor associated with CMV infection and shape approaches for immunotherapy. Our ultimate goal is to develop a predictive, innovative, non-invasive test for patient monitoring and treatment. Our finding proposing a unifying model of T cell activation by HLA-I/peptide monomers reappraise the potential involvement of soluble HLA-I molecules in the global immune response. Moreover, our results strongly suggest that HLA-E/CD94/NKG2A interaction represents a new inhibitory immune checkpoint preferentially up-regulated in MSI CRC, but also in a subgroup of MSS CRC. These findings would be of important clinical relevance since HLA-E^+ and/or $CD94^+$ CRC could be eligible to the new humanized anti-NKG2A monoclonal antibody.

Added value of / for Labex IGO

This project strengthened interactions between the two involved partners within the fields of immunology, cancer and transplantation. Thanks to LabEx IGO, the project “Impact of host immune control of CMV on allospecific endothelial injury and transplant outcome” has been supported by the Biomedicine Agency (30 000€) in 2013. Both partners on this collaborative project share a student, who has obtained a ministerial graduate research allocation on this project and defended his thesis on 2 February 2018.

T 1.3. NEW IMMUNOSUPPRESSIVE MOLECULES

Coordinator: Ignacio Anegon

Teams involved: all teams of WP1

Staff funded by Labex IGO: None, the objectives were only reagents

Initiation of the project: 2012
End of the project : 2015

Topic of the project and state of the art
The characterization of cells or molecules with immunosuppressive actions and that need new reagents to be better defined.

Objectives
The objective of this task is to make available reagents to analyze cells or molecules with a immunosuppressive actions. The accent is on the generation of new reagents not available through commercial or academic sources and gain intellectual property on new molecules. Some of these reagents can be generated by local academic facilities (production of tetramers by the Nantes Protein Production facility, http://www.sfrsante.univ-nantes.fr/68392562/0/fiche___pagelibre/) or by companies (tetramers and mouse MAbs).

Project progression / Results
1) Immunization of mice with human CLEC-1-derived peptides for the production of mouse MAbs initiated with a company (project of E. Chiffoleau, INSERM 1064, company Biotem). This has been finished and several anti-human CLEC-1 mouse MAbs were generated and their specificity and capacity to recognize the natural form of the protein confirmed by FACS.
2) Production (by Nantes Protein Production facility) and purchasing of tetramers with rat and human MHC-Ia for projects involving rat and human CD8+CD45RC- Tregs (C. Guillonneau/D. Valmori, INSERM 1064 and 892). This has been finished and resulted in one publication in the rat model (1). The work for the identification of relevant tolerogenic peptides in the human system is ongoing and thus tetramers were not funded for this part of the project. Human tetramers (HLA-A2) loaded with other peptides (from heme oxygenase-1 and IDO) have been purchased and are being used in human cells to identify CD8+ Tregs recognizing these peptides, using cells from both normal and kidney transplanted patients.
3) Tetramers using MHC-Ib (HLA-E) have been generated (by Nantes Protein Production facility) for projects involving CD8+ T cells in model of melanoma and transplantation (N. Gervois/B. Charreau, INSERM 892 and 1064). Using HLA-E-CMV peptide tetramers, a dedicated flow cytometry multistaining for the screening of CMV-committed HLA-E-restricted $CD8^+$ T cells in blood samples was developed. This allowed the identification of such cells in two pilot cohorts of renal transplant recipients according to CMV infection. The presence and frequency of CMV-reactive HLA-E restricted CD8 T cells will be next analyzed in correlation with clinical data. It was also shown that non-classical HLA-E/peptide monomers (as well as classical) can efficiently activate antigen-specific CD8 T cells in vitro, via a mechanism that involves the passive transfer of monomer-derived peptide to cell-bound HLA-I molecules. It remains to examine whether HLA-E molecules naturally produced by vascular endothelial cells and tumor cells have the same function
4) Generation of mouse Mabs anti-human CD45RC (project of C. Guillonneau and I. Anegon, INSERM 1064, company Biotem). We generated human CD45RC as a protein and we purchased peptides derived from human CD45RC. The peptides were used to immunize mice and rats. We obtained MAbs directed against the peptide but they did not recognize the natural form of the protein as

assessed by cytofluorimetry. Immunization with the protein of CD45RC did not result in the generation of MAbs. This project is pursued using as immunization the cDNA enconding for human CD45RC and genetic immunization but it is not covered by the Labex IGO funding.

Publications and patents
See appendices 5 and 6

Collaborations
Besides the collaborations between the teams directly funded by the project, other collaborations include: **1)** the production of tetramers by the Nantes Protein Production facility of INSERM 892; **2)** Determine the role of CLEC-1 in DC and DC-10 and endothelial cells (between E Chiffoleau, M. C. Cuturi and B Charreau, INSERM 1064).

Perspectives of clinical or economical valorization
1) Generation of tolerogeneic DC with agonistics anti-human CLEC-1 Abs as a treatment for kidney transplanted patients. **2)** using tetramers/peptides in vitro and in vivo expand human $CD8^{+}CD45RC^{-}$ Tregs as a treatment for kidney transplanted patients. **3)** Defining the possible adverse impact of HLA-E-restricted immune response to CMV and regulatory mechanisms should provide a proof of concept identifying a new risk factor associated with CMV infection and shape approaches for immunotherapy. Our ultimate goal is to develop a predictive, innovative, non-invasive test for patient monitoring and treatment. Our finding proposing a unifying model of T cell activation by HLA-I/peptide monomers reappraise the potential involvement of soluble HLA-I molecules in the global immune response. **4)** The use of an anti-human CD45RC Mab could eliminate effector cells preserving CD8+ and CD4+ Tregs and could serve as a prognostic marker of transplant outcome.

Added value of / for Labex IGO
Labex IGO allowed funding for reagents that otherwise would have been very difficult to generate or to purchase.

T1.4. A NEW ANTI-CMV STRATEGY TO PREVENT PRIMARY INFECTION DURING HSCT

Coordinators: Fabienne Haspot and Ignacio Anegon

Involved teams: Teams 1, 3 and 2 UMR1064, Nantes

Staff funded by Labex IGO: Janina Gergen (PhD student), October 2014-December 2017
Nathan Elain-Duret (M1 trainee)

Initiation of the project: October 2014
End of the project: December 2017

State of the art
Human cytomegalovirus (HCMV) infection is characterized by lifelong persistence in the host organism as an asymptomatic chronic infection. However, in immunocompromised transplant recipients, HCMV primary infection and/or reactivation are significant cause of morbidity and mortality. Anti-viral strategies with the use of pre-emptive or prophylactic drugs only target the replicating viral pool, by blocking viral DNA polymerase, and have no effect on the virus in a latent state. The aim of our project is to develop a new antiviral strategy that will be effective on latent as well as on replicating HCMV.

Objectives: The aim of the project is to develop a new antiviral strategy that will be effective on latent as well as on replicating cytomegalovirus. We are using gRNA/CAS9 to directly alter the HCMV genome and to induce its degradation or modification leading to abortive HCMV cycle.

Project progression / Results:
We designed two anti-viral CRISPR/Cas9 strategies to target the *UL122/123* gene, a key regulator of lytic replication and reactivation from latency. The singleplex strategy contains one gRNA to target the start codon. The multiplex strategy contains three gRNAs to excise the complete *UL122/123* gene. Primary fibroblasts and U-251 MG cells were transduced with lentiviral vectors encoding Cas9 and one or three gRNAs. Both strategies induced mutations in the target gene and a concomitant reduction of immediate early (IE) protein expression in primary fibroblasts. Further detailed analysis in U-251 MG cells showed that the singleplex strategy induced 50% of indels in the viral genome, leading to a reduction in IE protein expression. The multiplex strategy excised the IE gene in 90% of all viral genomes and thus led to the inhibition of IE protein expression. Consequently, viral genome replication and late protein expression were reduced by 90%. Finally, the production of new viral particles was nearly abrogated. In conclusion, the multiplex anti-*UL122/123* CRISPR/Cas9 system can target the viral genome efficiently enough to significantly prevent viral replication.
We started to work on CD34+ cells and were able to transduce them with a LV encoding the GFP and harboring the pseudotype HF. After having issues with our transduction and FACS sort on MRC5, we decided to change our strategy for the sort of our transduced cells. We are changing the GFP in our plasmid with a truncated NGFR that will be stably express at the surface of all transduced cells. Thanks to anti-NGFR miltenyi beads we will be able to select our transduced cell as soon as Day 2 post transduction in our P3 laboratory and directly continue our experiments. This strategy will be applied to CD34+ cells.

Publications and patents (published / in press / in preparation)
F.Haspot, J. Gergen, F. Coulon and F.Halary. METHODS AND COMPOSITIONS FOR RNA-GUIDED TREATMENT OF HUMAN CYTOMEGALOVIRUS (HCMV) INFECTION. June 10th 2015 EP 15305886.2; December 15th International Application

Multiplex CRISPR/Cas9 system impairs HCMV replication by excising an essential viral gene. Janina Gergen[1,2,3], Flora Coulon[1,2], Alison Creneguy[1,2], Nathan Elain-Duret[1,2,3], Alejandra Gutierrez[4], Olaf Pinkenburg[5], Els Verhoeyen [4,6], Ignacio Anegon[1,2,3], Tuan Huy Nguyen[1,2], Franck Albert Halary[1,2,‡] and Fabienne Haspot [1,2,3,] ***PlosOne (2018) in press***

Collaborations

Inside Labex we are collaborating with Tuan Huy Nguyen
We are collaborating with the CellGenoEdit platform here in Nantes
We are collaborating with Els Veroyen (Lyon, FRance) for the HF pseudotype LV
We are collaborating with the EFS (Etablissement Français du Sang) to obtain frozen G-CSF mobilized blood as a source of $CD34^+$ cells, we will soon have access to fresh G-CSF mobilized blood. We also work with the Hospital Maternity Service to obtain cord blood $CD34^+$ cells.

Perspectives of clinical or economical valorization

We have a patent EP 15 305 886.2 «Methods and compositions for RNA-guided treatment of human cytomegalovirus (HCMV) infection»
Clinical application would need the development of strategies allowing the delivery of the CrispR-Cas9 to all targeted CD34+ cells or to a recipient.

Added value of / for Labex IGO

- Labex IGO's funding was the only funding of our project so without it we could not have conducted this research.
- Sustained funding to recruit an international PhD Student for 3 years.
- Strengthen internal collaboration among different teams of the Labex IGO
- Visibility of the Labex IGO through publications in peer-review international journal

Janina Gergen successfully obtained her PhD on December 15th 2017.

T 1.5. FUNCTIONAL CONTRIBUTION OF *MICA* POLYMORPHIC VARIANTS TO IMMUNE RESPONSES IN ORGAN TRANSPLANTATION AND IN CANCER

Coordinators: Béatrice CHARREAU and Nadine GERVOIS

Involved teams: ITUN, UMR 1064 (Team5) INSERM /Université Nantes
CRCNA, UMR 892 INSERM Team 3/6299 CNRS / Université Nantes

Staff funded by Labex IGO: Nicolas PETIT, post-doc, 15 months from 01.01.2016 to 07.04.17
Yveline HAMON, post-doc, 12 months starting on 01.11.2017

Initiation of the project: 2014
End of the project: 2018

State of the art

This project explores the emergent concept that some MICA gene polymorphisms encode for non-conventional MICA proteins which may affect MICA expression, regulation and functions. Our study focus on the genetic variant MICA A5.1 that associates with MICA*008, the most common allele, and on two MICA alleles encoding novel MICA isoforms that we recently identified (**Charreau B.** et al., EP13305955.0 5/7/2013, *patented*). **Overall these 3 MICA alleles account for around 70% of the general population and of our transplant donors**. Our hypothesis is that **these *MICA* polymorphisms and MICA allele mismatch between transplant donor and recipient may play a role in MICA alloimmunization and in both innate and adaptive responses for the control of cancer progression.**

Objectives

The **principal aim** of this project is to determine how some frequent *MICA* gene polymorphisms encoding for non-conventional/truncated MICA proteins will affect MICA expression (membrane-bound and circulating proteins) and regulation on endothelial cells and melanoma cells, NKG2D-dependent NK cell activation and CD8 T cell costimulation, and their contribution to the control of tumor progression and MICA alloimmunization in kidney transplantation.

Project progression / Results:

1. **MICA genetic variants in melanoma.** The frequency of MICA A5.1 genetic variant in a panel of melanoma cell lines (n=49) has been determined by the sequencing of *MICA* exon5 region (**Fig.1A**). We found an allele frequency for MICA A5.1 in melanoma cell lines of 56.1% that slightly exceeds the frequency found in a control cohort of healthy volunteers (48.4%, n=92). Overall 40 out of the 49 melanoma cell lines (81.6%) carried at least one MICA A5.1 alleles (15/49, 30,6% being homozygous and 25/49 (51%) being heterozygous for MICA A5.1). In the control cohort 73 out 92 HV (79.3%) carried at least one MICA A5.1 alleles (20/92 (20.8%) were homozygous and 53/92 (55.2%) were heterozygous for MICA A5.1). In an attempt to correlate MICA5.1 with quantitative changes in MICA expression on melanoma cell lines, transcript steady state levels and surface expression were analyzed by QRT-PCR and flow cytometry, respectively. Our results show no statistical difference between MICA A5.1$^{+/+}$ and MICA A5.1$^{-/-}$ melanoma cells at mRNA (**Fig 1B**) or surface level. Consequently, we found no statistical difference between MICA A5.1$^{+/+}$ and MICA A5.1$^{-/-}$ melanoma cells in terms of NK and T cell activation. Our current experiments investigate the possible impact of MICA A5.1 variant on soluble and exosomal MICA forms released by melanoma and their immunoregulatory function toward cancer immunity. Interestingly, among the 49 lines tested, one melanoma cell line (M131) carries a MICA*017 allele. In this melanoma cell line, the presence of a set of MICA isoforms was observed (**Fig. 1C**) confirming the association between MICA*017 and alternative splice transcripts that we recently reported in vascular endothelial cells (Gavlovsky et al. *JI*, 2016). Overall, these findings indicate a high prevalence of the variant MICA

A5.1 in melanoma but not significantly different from the frequency found in healthy controls. Our data also report the detection of MICA alternative transcripts associated with MICA*017 in melanoma. The biological significance of these MICA variants on tumor immunity still requires investigations.

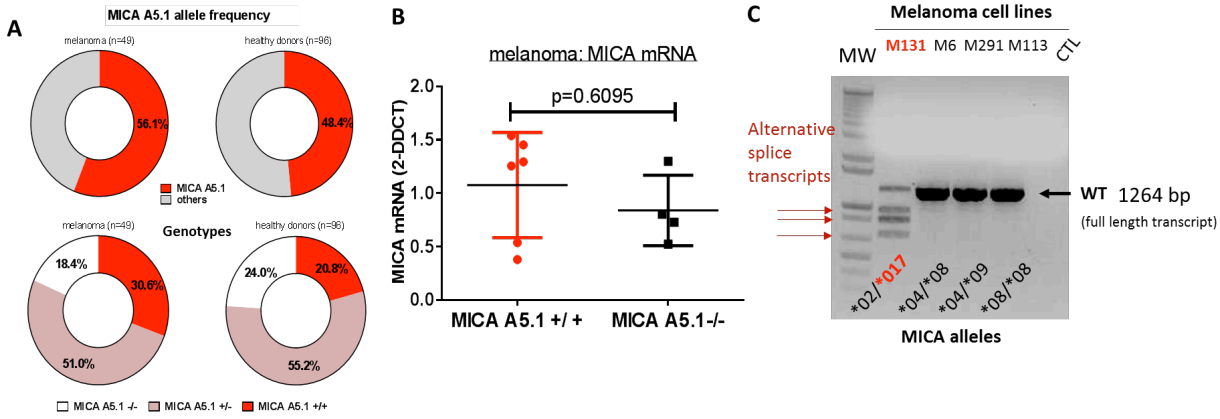

Fig.1. Analysis of MICA genetic variants in melanoma. (**A**) Comparative analysis of MICA A5.1 allele frequency in a panel of melanoma cell lines (n=49) and a cohort of healthy donors (n=92). (**B**) QRT-PCR quantifying MICA mRNA in MICAA5.1+/+ (n=6) and MICA A5.1-/- (n=4) melanoma cell lines. Results are normalized toward RPLP0 housekeeping gene and are expressed as $2^{-\Delta\Delta Ct}$. (**C**) Identification of MICA alternative splice transcripts in a melanoma cell line (M131) carrying the MICA*017 allele.

2. **First functional characterization of new MICA isoforms toward NKG2D-dependent NK cell activation.** We identified five novel MICA isoforms: MICA-A, -B1, -B2, -C, and -D. Structurally, the α3 domain is deleted in all isoforms, and the α2 domain in the majority of isoforms (A, B1, C, and D). We demonstrated that MICA-B1, -B2 and -D bound NKG2D by surface plasmon resonance (coll. Pr. B. Mac Farland, Seattle University, US) and were expressed at the cell surface. Functionally, MICA-B2 contains two extracellular domains (α1 and α2) and is a novel potent agonist ligand for NKG2D. We found that MICA-D is a new truncated form of MICA with weak affinity for NKG2D despite lacking α2 and α3 domains. MICA-D may functionally impair NKG2D activation by competing with full-length MICA or MICA-B2 for NKG2D engagement. These findings establish that new truncated MICA isoforms exhibit a range of functions that may drive unexpected immune mechanisms and provide new tools for immunotherapy (Gavlovsky PJ *et al*., *J. Immunol., 2016*). In 2016/2017, we produced and purified a panel of recombinant MICA proteins (WT, B2 and D) both in *E. coli* and in CHO to investigate *in vitro* the regulatory functions of these truncated proteins as well as the impact of N-glycosylation. Our preliminary functional studies highlight (1) the ability of MICA recombinant proteins to efficiently costimulate CD8 T cells and activate NK (2) the ability to efficiently modulate NKG2D signaling by disabling N-glycosylation and/or α2/α3 domains of MICA proteins. Crystallography analysis of NKG2D/MICA -B2/-D interactions will be performed in 2018. Specific impact on innate and adaptive immunity will be further defined *in vitro* and, if suitable, using *in vivo* models.

3. **Impact of MICA5.1 prevalence on viral infection post-Transplantation.** We designed a retrospective observational study questioning the role of MICA in the occurrence and severity of BK Polyomavirus (BKPyV) reactivation after kidney transplantation. Our findings associate the frequency of MICA A5.1 in transplant donors with a lower incidence of BKPyV reactivation. Our study indicated that MICA A5.1 allele can be either protective or detrimental toward BKPyV reactivation when carried by the transplant or by the recipient, respectively. These findings probably reflect a specific control of MICA A5.1 on immune responses including MICA immunization and soluble MICA in the host (Tonnerre P. *et al., J. Infectious Diseases*, 2016). Interestingly, we found an inverse correlation between soluble MICA and the level of anti-MICA antibodies in the sera of transplant recipients suggesting a functional interplay between BKPyV infection and MICA that still remain to be defined. These findings are summarized in **Fig.2**. Consequently, in the future,

this project will explore how anti-MICA immunization and release of soluble MICA could be involved in pathogen control.

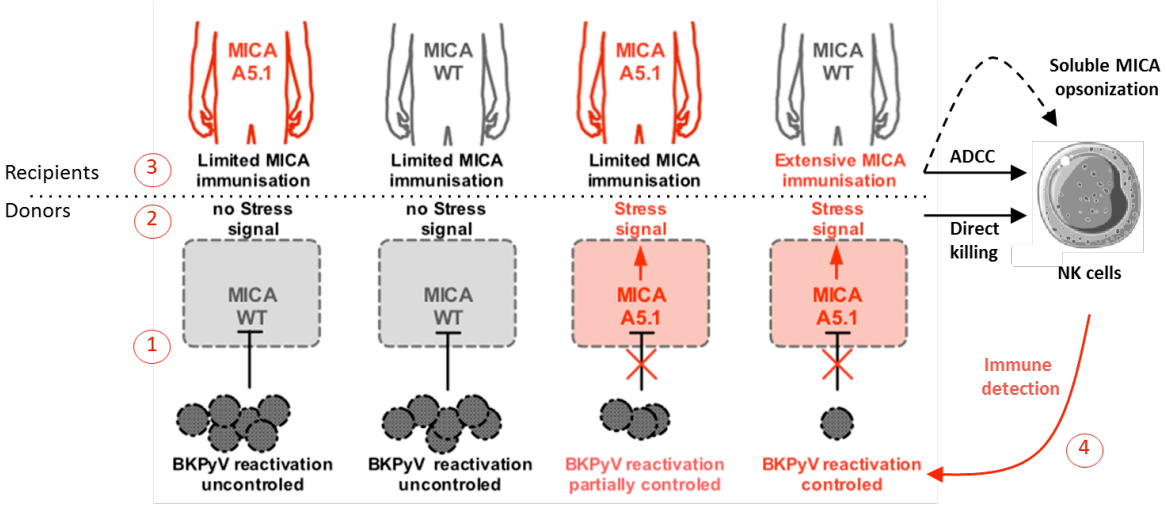

Fig 2.Schematic representation of findings and hypothesis. Donor and recipient pairs can be divided into 4 combinations according to *MICA A5.1* genotypes: DWT/RA5.1 ; DWT/RWT ; DA5.1/RA5.1 and DA5.1/RWT. We suggest that BKPyV might be equipped to prevent MICA WT expression, but not the A5.1 variant, as it was previously reported in other virus infection (Zou et al. J immunol. 2005 ; Thomas et al. Proc Natl Acad Sci USA. 2008 ; Ashiru et al. J Virol. 2009) **(1)**. If true, that would lead to an impaired stress signal **(2)**, as would be the detection of donor's infected cells in DWT/RA5.1 and DWT/RWT combinations. As we previously reported (Tonnerre et al. J Am Soc Nephrol), we found higher MICA Immunization in DA5.1/RWT as the result of both an increase of MICA antigens in the graft (ie due to MICA expression after BKPyV reactivation) and a mismatch for MICA genotypes between donors and recipients (here DA5.1/RWT) **(3)**. We propose a positive effect of anti-MICA antibodies on NK cell-mediated immune surveillance through a direct action on targeted cells (ie ADCC) and/or, indirectly, by opsonizating shedded soluble MICA. In this model, an optimal control of BKPyV reactivation occurs when both surface MICA expression and anti-MICA immunization are combined (DA5.1/RWT), a partial control of BKPyV reactivation when only membrane MICA expression is induced (DA5.1/RA5.1), and uncontroled BKPyV reactivation when both menbrane-MICA expression and anti-MICA antibodies are missing (DWT/RA5.1 ; DWT/RWT) **(4)**.

Publications

- Tonnerre P, Gérard N, Chatelais M, Poli C, Allard S, Cury S, Bressollette C, Cesbron-Gautier A, **Charreau B**. MICA variant promotes allosensitization after kidney transplantation***. J Am Soc Nephrol.*** 2013 May;24(6):954-66.
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Collaborations

An **international collaboration** with Pr. B. Mac Farland (Seattle University, US) allowed us to define the affinity of MICA isoforms for NKG2D receptor using surface plasmon resonance. A **national collaboration** with phytochemists Dr. S. Derbré and Dr. P. Richomme (Sonas, University of Angers, Fr) has been developed to identify new bioactive molecules from natural products specifically able to regulate HLA and MICA expression on endothelial and immune cells (an article published in 2015).
Finally, an **internal collaboration** with the recombinant protein core facility (P2R, IRS-UN, Nantes, Fr) allows the production of soluble recombinant MICA isoforms for analysis and recombinant NKG2D for crystallography.

Perspectives of clinical or economical valorization

Prospective cohort studies to validate the clinical role of MICA A5.1 mismatch in kidney transplantation as a risk factor for recipient sensitization and a protective factor toward BKPyV reactivation will be organized for the next years with the kidney transplant unit (Pr. M. Hourmant, Nantes) and the virology unit (Dr. C. Bressollette, Nantes).
The importance of truncated MICA isoforms for the development of therapeutic drugs and strategies targeting NKG2D-mediated immune responses in transplantation and cancer will be explored with biotech companies.

Added value of / for Labex IGO

This collaborative project strengthened interactions between the two involved partners within the fields of immunology, cancer and transplantation. Both partners on this collaborative project share a

postdoc granted with research allocation on this project. The Labex IGO funding allowed the subsequent obtention of a grant (2017/2018: 37500€) from the IHU CESTI *Valorisation* for the "Evaluation of new MICA isoforms as novel immune checkpoint regulators".

T1.6. HEME OXYGENASE-1 AND TOLERANCE (HOT)

Coordinators: Ignacio Anegon (INSERM UMR 1064)
Jean-François Fonteneau (INSERM UMR 892)

Involved teams: Team 2 INSERM UMR 1064 (Part1: HO-1 and transplantation)
Team 4 INSERM UMR 892 (Part2: HO-1 and cancer).

Staff funded by Labex IGO: Maud MAQUINEAU (technician for part1)
Nadège VIMOND (technician for part2)

Initiation of the project: Part 1: May 2015
Part 2: March 2015

End of the project: Part 1: May 2017 for the transplantation experiments
Part 2: January 2016 for part2

State of the art

Heme oxygenase (HO) catalyzes the degradation of free heme in carbon monoxide (CO), biliverdin and iron. Among HO isoforms, HO-1 is the only inducible enzyme by inflammatory stimuli. HO-1 is an anti-oxidant, anti-inflammatory and pro-tolerogenic molecule (1-3). As previous work of team 2 on the role of HO-1 in transplantation, we showed that HO-1 gene transfer or overexpression inhibited acute (4) and chronic rejection (5, 6) of vascularized organs in rodent models.

The precise underlying mechanisms for HO-1-based immunoregulatory properties are being uncovered. Both team partners (Team 2 and 4) described in 2005 that DCs express HO-1 and that its enzymatic activity rendered DCs less immunogenic (7). Team 2 of INSERM 1064 described that DCs expressing HO-1 were resistant to TLR and CD40-mediated maturation of DCs as well as defined CO as the principal mediator of these effects (8). We further showed that antigen presentation is impaired in DCs expressing HO-1 and the mechanism underlying these effect was the inhibition of antigen processing by blockade of endosome-lysosome fusion (9). We also described that CO treated DCs tolerize naïve CD8+ T-cells by inhibiting their migration into tissues (10).

As a direct preliminary work to this project, in a paper just submitted, we described in mouse models of autoimmunity (autoimmune diabetes and EAE) and in baboons that administration of HO-1 inducer and cognate antigen in the skin inhibited specific immune responses (11).

After intradermal immunization with CoPP and OVA, HO-1 was induced only in the skin draining lymph node (DLN, not in spleen or pancreatic lymph node) and almost only in MHC-II^{low} F4/80^{+}CD11c^{int}CD64^{+}FceRI^{+}CD11b^{+}Ly6C^{hi} cells (data not shown), a phenotype characteristic of inflammatory DCs (12) also called monocyte-derive DCs (MoDCs) (11).

It is of particular interest that until now MoDCs/inflammatory DCs have been described as being immunogenic (12). Thus, this is the first description of MoDCs, expressing HO-1 as potent tolerogenic DCs.

In cancer, HO-1 has been largely described by us and other groups as expressed by tumor cells with inhibitory or activator effects on tumor cells proliferation (1-3, 13). Very few manuscripts have analyzed HO-1 expression by antigen presenting cells in a tumoral environment, such as in tumor M2 macrophages (14) and myeloid-derived suppressor cells (15), in both cases with immunosuppressive effects. In vivo treatment with an inhibitor of HO-1 accelerated tumor rejection (16). It has been shown that individuals with genetically-defined higher expression of HO-1 have higher risk of developing mesothelioma (17) but it was not defined if this was due to a direct pro-proliferative effect on tumor cells or to decreased anti-tumor immune responses. Thus, it is not known whether tumor-associated APCs express HO-1 and its effects on anti-cancer immune responses.

Furthermore, it has very recently been shown that classical chemotherapeutic anthracycline treatment in cancer mouse models induces the migration of monocytes and their differentiation into inflammatory DCs/MoDCs capable of rejecting tumors (18). Several reports have shown that HO-1 expressed by tumor cells is protective of anthracycline-induced apoptosis (19). However, there are no conclusive reports on the effect of anthracyclines on cellular HO-1 levels and none in the case of monocytes or DCs.

Objectives

This project focuses on increasing heme oxygenase-1 (HO-1) in transplantation to induce tolerance and inhibiting HO-1 to augment anti-tumoral immune responses.

More precisely, the objectives in the transplantation projects are to define whether after intradermal injection of donor antigens and CoPP: a) mouse alloantigen presentation by HO-1+MoDCs induces tolerance to mouse skin.

The objectives for the part 2 "HO1 and Cancer" are:: a) the expression of HO-1 by different types of APCs present in pleural effusion of malignant pleural mesothelioma (MPM) or lung adenocarcinoma (ADCA) patients; b) to determine if pleural liquids from MPM or ADCA patients differentiate monocytes into HO-1+ myeloid cells; c) to determine how human HO-1+ DCs tolerize effector CD8+ T cells using tumor antigens specific T cells; d) to explore whether human DCs treated with anthracycline show lower levels of HO-1;

Using 8 panels of 12 monoclonal fluorescence conjugated antibodies, we made an inventory of cells present in pleural effusion of 25 MPM and 16 ADCA patients by flow cytometry with a focus on antigen presenting myeloid cells, such as macrophages, CD1c+ dendritic cells or tolerigenic HO-1+ dendritic cells.

Project progression / Results

Part 1. HO-1 and transplantation:

1) we set up in our team the male-to-female (C57BL/6) skin transplantation model (in the back of the mouse). This model is now reproducible and we routinely obtain skin rejection between days 20 and 40 (it should be realized that this rejection time frame does not allow a fast evaluation of the different conditions) whereas female to female grafts are accepted indefinitively. **2)** As we did in the RIP-OVA and EAE models, we performed intradermal administration in the upper and lower back of the mice of a potent HO-1 inducer (CoPP) and different doses of 3 peptides derived from the HY antigen (Uty, Smcy and Dby, the first 2 for T CD8 and the last one for T CD4+, respectively). In the skin transplantation model we performed one injection with different amounts of HY peptides (of each of the 3: 10, 5, 2.5 or 1.25 mg/mouse) or control peptide (OVA) along with PBS or CoPP (70 mg/mouse)(n=4-7 per condition, total grafts=80). The day after, we transplanted full male skin on the back of the recipients (between the 2 injection points). We performed visual inspection of the graft and determined the time of rejection when over 75% of the graft was necrotic. The results for all groups (10 to 1.25 mg/mouse) showed a prolongation of graft survival for mice injected with HY peptide vs. controls for both PBS and CoPP groups. Thus, we observed a partial effect of the HY peptides alone and no clear effect of CoPP. In the EAE model we performed 3 injections in days 0, 3 and 6 of MOG peptide (each 20 mg/mouse) with or without CoPP (each 70 mg/mouse). Ongoing experiments will increase the number of injections up to 3/mouse (at days -1, 2 and 5), we will initiate them later (day 13, 16 and 19) since the time between injection at the moment of transplantation and rejection maybe too far apart (in the EAE and diabetes model there is 1 week interval between injection of CoPP+autoantigen and initiation of pathology). We will also initiate a new model in which C57-H2-Kbm1 will be skin donors and C57-H2-Kb will be recipients. Both strains differ in 3 aminoacid mutations of the MHC-I antigen only and this results in ~12 days rejection of skin transplants mainly mediated by T CD8+ cells. Peptides including these 3 mutated aminoacids will

be injected intradermically with CoPP at days 0, 3 and 6. We will combine treatment with suboptimal or optimal doses of immunosuppressors (rapamycin or tacrolimus) for a short course (10 days).
None of these treatments resulted in a statistical significant effecto of HO-1 and peptides over that obtained with the peptides alone.

Part2: HO-1 and cancer:

We found a high heterogeneity in the cellular composition of pleural effusions ranging from patients with a large majority of myeloid cells to patients with a large majority of lymphoid cells. Using the MPM tumor cells specific markers podoplanin and cytokeratin 5/6, we found variable low amounts of tumor cells in MPM associated pleural liquids. The main population of myeloid cells from 33/41 pleural liquids harbor an immunosuppressive M2 macrophage phenotype and produce IL-10 and no IL-12 in response to LPS stimulation. In 8/41 pleural effusions with a majority of lymphoid cells, the main population of myeloid cells were the inflammatory CD1c+ dendritic cells. We detected low frequency of HO-1+ myeloid cells inferior to 10% of myeloid cells in 5 patients. HO-1 is an intracytoplasmic marker and we tried without success to find a surrogate surface marker to sort these HO-1+ cells by flow cytometry. In addition, we found that myeloid cells and tumor cells from pleural effusion express the molecule PD-L1 in a large majority of patients. Finally, we also found a low expression of PD-1 on T cells freshly isolated from pleural liquids, but this expression can be increased by culture with IL-2.
This large expression of PD-L1 by myeloid cells and tumor cells may participate to the immunosuppressive tumor environment and may justify the use of anti-PD-1 or anti-PD-L1 for the treatment of MPM.

Publications

Part1: HO-1 and Transplantation and in other models:

1) S. A. Riquelme, J. Pogu, I. Anegon, S. M. Bueno and A. M. Kalergis. Carbon monoxide impairs mitochondria-dependent endosomal maturation and antigen presentation in dendritic cells. **2015. Eur. J. Immunol. 45:3269-88.**

2) Thomas Simon, Julien Pogu, Séverine Rémy, Frédéric Brau, Sylvie Pogu, Maud Maquigneau, Jean-François Fonteneau, Bernard Vanhove, Gilles Blancho, Eliane Piaggio, Ignacio Anegon* and Philippe Blancou*. Inhibition of effector antigen-1 specific T cells by intradermal administration of heme oxygenase-1 inducers. **2017. J. Autoimmunity. 81:44-55. * both senior and corresponding authors.**

3) Janyra A. Espinoza, Miguel A. Leon, Pablo F. Cespedes, Roberto S. Gomez, Gisela Canedo-Marroquın, Sebastian A. Riquelme, Francisco J. Salazar-Echegarai, Phillipe Blancou, Thomas Simon, Ignacio Anegon, Margarita K. Lay, Pablo A. Gonzalez, Claudia A. Riedel, Susan M. Bueno and Alexis M. Kalergis. Heme Oxygenase-1 Modulates Human Respiratory Syncytial Virus Replication and Lung Pathogenesis during Infection. **2017. J. Immunology. 199(1):212-223.**

4) Paula Carasi′, Ernesto Rodríguez, Valeria da Costa, Sofía Frigerio, Natalie Brossard, Verónica Noya, Carlos Robello, Ignacio Anegón and Teresa Freire**.** Heme-oxygenase-1 expression contributes to the immunoregulation induced by fasciola hepatica and promotes infection. **2017. Frontiers in Immunol. 8:883.**

Part2: HO-1 and cancer:

Vimond N, Roulois D, Gueugnon F, Deshayes S, Anegon I, Deleneste Y, Jeannin P, Cellerin L, Gregoire M and Fonteneau JF. High expression of PD-L1 by tumor and myeloid cells of pleural effusions from malignant pleural mesothelioma (MPM) and lung adenocarcinoma (ADCA). In preparation.

Collaborations

Part1: HO-1 and transplantation. If we obtain prolongation of skin allograft survival, we will collaborate with the rodent humanization platform of the Labex to transplant human skin in NSG mice that will also receive allogeneic human PBMCs, following protocols already stablished in INSERM 1064.

Part2: HO-1 and cancer: We have started a collaboration with the team of Dr Cristina Cuturi. We provided her with sample of cells from pleural liquid to compare with their panel of markers the phenotype of their tolerogenic DC (tolDC) that are evaluated in phase I clinical trial to suppress the rejection of human kidney graft. We will probably start a collaboration with Dr Nadine Gervois from Team3 of INSERM UMR1232, to provide her with double positive CD4+CD8+ T cells that we found in pleural liquid of several patients. Finally, we started a collaboration with team 7 INSERM U1232 (Yves Delneste)

Perspectives of clinical or economical valorization

Part1: HO-1 and Transplantation: The application of HO-1 to induce tolerance in transplantation by the simultaneous intradermal administration of antigen and an HO-1 inducer resulted in a patent (EP14306723.9): "COMPOSITIONS AND METHODS FOR ANTIGEN-SPECIFIC TOLERANCE". I. Anegon, P. Blancou, T. Simon, J. Pogu., with an international extension in September 2015. Discussions are ongoing among the persons implicated in this work and patent to create a start-up to develop the application of this approach. At the same time, we will initiate contacts with companies in this area that could be interested to have an exploitation license.

Part2: HO-1 and cancer: This work will allow us to better understand the heterogeneity of the immune cells associated with MPM. Furthermore, we have now a good source of primary tumor cells and tumor-associated immune cells to better understand the mechanism and the efficacy of the therapeutic approach that we want to develop in team 4.

In collaboration with Dr Frederic Tangy from Pasteur Institute, Paris, we have notably patent an oncolytic immunotherapeutic treatment for MPM by the attenuated Schwarz strain of measles virus (MV), which is spontaneously oncolytic against MPM (22). Recently, we showed that 15 out of 22 MPM cell lines are sensitive to MV oncolytic activity, due to defects in their capacity to develop an antiviral type I interferon response when exposed to the virus (23). To transfer this approach to the clinic, we are often asked if primary tumor cells freshly isolated from patient are as sensitive as MPM tumor cell lines. We will be able to respond to this question by purifying fresh tumor cells from pleural liquid where we found a high fraction of these cells and assess their sensitivity to MV oncolytic activity.

We also showed that MV infection causes the immunogenic cell death of tumor cells that induce a strong activation of myeloïd and plasmacytoïd blood DCs (24, 25). We found in three pleural liquids more than 10% of pDC. We will sort these pDC, but also the myeloid cells present in other liquids and determine if these cells are still able to be activated by MV or MV infected cells.

This study allowed us to set up some new methods to sort antigens presenting cells from tumor environment and we will now study capacity of cells to mount an antitumor response and strategy to stimulate them, notably by oncolytic viruses (Vaccinia virus from Transgene^{SA} and Schwarz Measles virus from Dr F Tangy, Pasteur institute).

Added value of / for Labex IGO

The funding from Labex IGO allowed collaboration between 2 teams, one with a perspective of transplant immunology and the other of cancer, which is the very essence of the Labex IGO project, the yin-yang of a given molecule or cell type.

The project also consolidated momentum in the analysis of myeloid/DC subpopulations that will likely be of interest in transplantation, cancer and other situations.
Some of the data may have industrial or clinical applications and the publications submitted or in preparation will enrich the scientific production of the Labex.

Part1: HO-1 and Transplantation: The fact that immune responses can be tolerized by simultaneous administration in the skin of antigen and an HO-1 inductor has initiated a collaboration with the team of Alexis Kalergis to increase immune responses in vaccination by the simultaneous administration of a viral vaccine and an inhibitor of HO-1. The initial data is encouraging, showing increased protection in mice treated with the viral vaccine and an inhibitor of HO-1 vs. the viral vaccine alone.

Part2: HO-1 and cancer: our bio-collection of cells from pleural liquid of MPM and ADCA patients represent a great source of primary tumor cells and tumor-associated immune cells to study the anti-tumor immune response. We will start a collaboration with Dr Nadine Gervois to provide her with tumor-associated double positive T cells that we found in some pleural liquids. We have been also solicited by the laboratory of Dr Yves Delneste and Dr Pascale Jeannin that study tumor associated macrophage (TAM) to provide them with some myeloid cells from pleural liquids. We already started a collaboration with the laboratory of Dr Maria Cristina Cuturi by providing them with samples of cells from pleural liquid that they want to compare with their DCtol. We also make available our eight panels of 12 mAbs to member of the IGO Labex to study immune cells associated to other types of cancer.

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T1.7. IMMUNE TOLERANCE BY IL-34: ACTIONS ON MACROPHAGES AND TREGS (TOL34)

Coordinator: **I. Anegon**

Teams involved: Team 1 (M. C. Cuturi) and team 2 (I. Anegon) INSERM UMR 1064
Jordi Ochando, Department of Immunology of Transplants, Instituto de Salud Carlos III, Madrid, Spain.

Staff funded by Labex IGO: Antoine Freuchet (PhD student) 36 months starting on 01.10.2016

Initiation of the project: September 2016

End of the project: September 2019

State of the art:
Interleukin-34 (IL34) is a newly discovered cytokine that binds to CSF1R (the M-CSF receptor), CD138 and PTPz and involved in differentiation and survival of myeloid cells. Until recently, no link with T cell biology or transplantation had ever been reported for IL34. We previously showed that the cytokine IL34 is expressed by rodent $CD8^+CD45RC^{low}$Tregs, human $FOXP3^+CD8^+$ and $CD4^+$ Tregs. In addition, its overexpression using an AAV encoding IL-34 was able to induce long-term allograft tolerance in rat through the modulation of monocytes into pro-regulatory macrophages and the induction of Tregs (Bézie et al. J. Clin. Invest. 2015).

Objectives: To define the role of IL34 in immune responses.

Project progression / Results:
IL34 KO rats. To further understand the function of this cytokine in the T cell biology and alloreactive immune responses, we generated IL34-deficient rats using the CRISPR/Cas9 technology. Phenotyping of these deficient rats showed an increase of NK cells and a significantly decrease of $CD8^+$ and $CD8^+CD45RC^{hi}$ T cells in the spleen and $CD8^+$ and $CD8^+FoxP3^+$ T cells in the blood compared to WT animals. In addition, we observed that deficient rats presented an impaired microglia in the brain vs. WT rats. We did not observe differences in the suppressive function of $CD8^+CD45RC^{low}$ T regulatory cells both *in vitro* and *in vivo* (in a model of wasting disease in Rag-/- rats injected with T cells) between deficient and WT rats. However, we observed that $CD4^+CD25^+CD127^{low}$ T regulatory cells from deficient rats were not able to control the development of the wasting disease *in vivo* compared to $CD4^+$ Tregs cells from WT, suggesting that IL-34 is essential for the suppressive function of $CD4^+$ Tregs. M-CSF was significantly increased in IL34 KO animals and this could be a compensatory mechanism for the lack of IL34. These preliminary results if confirmed will be important and new as compared to the results described for IL34 KO mice and will be the core of a future manuscript describing these IL34 KO rats.

Role of IL34 in GVHD. To analyze the potential of IL34 in human transplantation, we used a model of GVHD in NSG mice. We demonstrated that human IL34 protein administration into NSG mice infused with human PBMCs efficiently delayed in a dose dependent-manner GVHD occurrence when associated with a suboptimal dose of rapamycin during 10-days. These results will be the first description for a tolerogenic role of IL34 in GVHD and part a future manuscript together with the analysis of immune mechanisms underlying this effect.

In vitro effect on macrophages. In vitro, we showed that after 6 days of culture with IL-34, human $CD14^+CD16^-$ and $CD14^+CD16^+$ monocytes are more prone to be differentiated into macrophages with an M2-like phenotype (CD36+ CD163+) than $CD14^-CD16^+$ monocytes. Their phenotype was

comparable to the one observed with the culture in M-CSF+IL10 or IL-4 and different from the M1-like observed with GM-CSF and IFNg. The phenotype of $CD14^{+}CD16^{-}$ cultured in IL34 vs. M-CSF showed a lower percentage of CD80+ and CD163+ cells. In addition, IL34 treated $CD14^{+}CD16^{-}$ macrophages expanded more efficiently CD8+FOXP3+ Tregs compared to allogeneic APCs (>100-fold) and a phenotype that showed an augmentation of the percentage of TGFb+, GITR+, PD-1+ IL-34+, IFNg+ Tbet+, HLA-DR+, L'IL-10+ and FoxP3+ cells. These CD8+ Tregs showed a potentiation of their suppressive activity since lower ratios of IL-34-expanded Tregs were sufficient to delay GVHD development in humanized mice compared to anti-CD3/CD28 polyclonally expanded Tregs.

IL34 as a biomarker in organ transplantation and GVHD. We dosed IL34 by ELISA in serum samples kidney transplant recipients' cohorts with different outcomes (University Hospital Nantes, n=289). Analyses of these data is underway. M-CSF was also analyzed in these patients and statistically different levels were observed.
We are testing the phenotype of CD8+ and CD4+ Tregs in a cohort of bone marrow transplanted patients with or without GVHD (University Hospital H. Mondor, Paris; n=67) and among the markers analyzed by cytofluorimetry is IL34. The number of patients is still low to draw conclusions and we plan to analyze IL34 in the sera of patients with or without GVHD before and after bone marrow transplantation.

Oral presentations, publications and patents.

Oral presentations. A. Freuchet « IL34 as a tolerogenic cytokine ». NAT meeting in June 2017 and in December 2017 in the French society for transplantation (SFT).

Publication: C. Guillonneau, S. Bézie, I. Anegon. Immunoregulatory properties of the cytokine IL-34. 2017. Cellular and Molecular Life Science. 74: 2569-2586.

Patent: WO2016009041, 17/07/2014: C. Guillonneau, I. Anegon, S. Bézie « An isolated interleukin-34 polypeptide for use in preventing transplant rejection and treating autoimmune diseases ».

Collaborations

M. C. Cuturi, team 1 CRTI-INSERM, Nantes.
J. Ochando, Madrid Spain.
D. Heymann (Nantes) and Diaclone (Besançon).
S. Maury (Univ. Hospital Henri Mondor, Paris)
M. Giral (Kidney Transplantation Department, Univ. Hospital Nantes).

Perspectives of clinical or economical valorization.

Start-up. The patent on IL-34 is part of the intellectual property assets of a start up that is being incubated by regional agencies and that will be created in 2018.

Development of a commercial kit for the isolation of IL34 producing Tregs. Since IL34 is produced among lymphocytes only by CD4+ and CD8+ Tregs (Bézie et al. J. Clin. Invest. 2015), we reasoned that it could be a marker for the isolation of Tregs using anti-IL34 MAbs. To this end, we started a collaboration with Prof. Dominique Heymann (Nantes University) and with the company Diaclone (Besançon, France) who have already generated mouse anti-human IL34 MAbs. These MAbs will be used to generate a kit for Treg enrichment in which conjugated anti-CD3 and anti-IL34 antibodies will capture Tregs and an anti-IL34 against a second epitope different from the one recognized by the capture one will be used to bind beads.

Added value of / for Labex IGO

For the Labex: future publications and generation of intellectual property.

T1.8. CD8+CD28NEG T CELLS, A THREAT TO TRANSPLANTATION?

Coordinator: **Nicolas Degauque**

Teams involved : CRTI, Team 4 (Nicolas Degauque)
CRTI Team 3 (Fabienne Haspot)
CRCiNA Team 9 (Claire Pecqueur)

Staff funded by Labex IGO: Tra-My Doan Ngoc (PhD Student) 36 months starting on 01.10.2016
Virginie Huchet (Technician) 18 months starting on 01.06.2017

Initiation of the project: 10/2016

End of the project: 2019

State of the art:
Preventing the occurrence of acute rejection in kidney transplanted patient is not anymore an issue. However, minimal success had been obtained to prevent late chronic rejection and current immunosuppressive drugs are associated with increased infections, viral reactivations and malignancies. It is thus critical to design innovative strategies to control the pathogeneic cells involved in chronic rejection. We and others have accumulated evidences that the direct pathway of allorecognition is functional months after transplantation and that the composition of CD8 T cell subsets is modified in patients with chronic antibody-mediated rejection (CABMR) : with strong alterations of their TCR Vβ repertoire and an expansion of Effector Memory cells re-expressing CD45RA (EMRA) despite the stability of their kidney graft function. Patients with an expansion of TEMRA CD8 T cells exhibit a 2-fold higher risk of kidney dysfunction. These results have been confirmed in a new cohort of kidney transplant recipients analyzed 12-month post-transplantation (unpublished data). Immune check-point inhibitors such as Belatacept (CTLA4-Ig) had been recently approved for kidney transplantation. However, costimulation blockade resistant patients in which a sizable frequency of $CD8^+CD28^-$ T cells have been evidenced exhibit polyfunctional cells including multiple cytokines secretion and high level of cytotoxic molecules (PERF and GZM-b) in the context of alloreactive assays. Collectively, these results highlight that pre-existing or neo-formed pathogeneic $CD8^+CD28^-$ T cells are a barrier for long-term graft acceptance that cannot be control by actual IS drugs nor by the newly developed costimulation blockade drugs.

Objectives:
The aim of the project is to characterize the development of pathogeneic $CD8^+CD28^-$ T cells after kidney transplantation and to evaluate in humanized mouse model the ability to control their immune function by metabolic interferences. The project is divided into 3 tasks

- **Task #1.** To investigate the adaptation of the metabolism of CD8 T cell subsets after kidney transplantation
- **Task #2.** To develop humanized mouse models to study $CD8^+CD28^-$ T cells pathogenicity
- **Task #3.** To test the ability of metabolic interferences to control the immune response of $CD8^+CD28^-$ T cells in preclinical models.

Project progression / Results:

- **Metabolic fitness of TEMRA CD8 from Kidney transplant recipients and their involvement in.** We have identified that IL-15 is potent stimulatory cytokine for TEMRA CD8 T cells purified from Kidney transplant recipients (KTx). We have shown that IL-15-stimulated TEMRA from KTx promote an inflammation by inducing the expression of CX3CL1 by endothelial cells in an IFN-γ- and TNF-α-dependent manner. The responsiveness of TEMRA to IL-15 is not restricted to chronic

stimulation, as TEMRA from healthy volunteers respond earlier and faster when compared to effector memory (EM). IL-15 induces anti-apoptotic signals and promotes proliferation dependent of PI3K/Akt, MAPK, and ERK pathways. Without *ex vivo* stimulation, TEMRA cells are metabolically more active than naive and EM, as shown by their high ATP reservoir and a high expression of genes involved in glycolysis, glutaminolysis, and the Pentose Phosphate Pathway. Upon stimulation, TEMRA adapt their metabolism by sustaining an increased mitochondrial respiration and glycolysis. Finally, we show that the inhibition of glycolysis is highly effective in preventing endothelial in inflammation induced by TEMRA from KT recipients. Together, our findings highlight the metabolic fitness that tightly regulates the immune function of TEMRA in physiological and pathogenic situations.

- **Development of an in vivo humanized mouse model to investigate the pathogenicity of TEMRA CD8.** We aimed to setup an in vivo model to investigate the pathogenicity of TEMRA CD8 from KTx. This experimental setup will be later use as a screening platform to screen metabolic interference drugs for their ability to control TEMRA CD8. A model of GVHD using NOD/SCID/IL2gRKO (NSG) mice expressing the HLA class I molecule HLA-A*0201 was used. Different experimental groups have been tested
    - Injection of TEMRA CD8 T cells purified from KTx with autologous non-T cells to HLA-A*0201 NSG mice irradiated on day-1
    - Injection of TEMRA CD8 T cells purified from KTx with autologous non-T cells to HLA-A*0201 NSG mice
    - Injection of TEMRA CD8 T cells purified from KTx to HLA-A*0201 NSG mice
    - Injection of TEMRA CD8 T cells purified from KTx to HLA-A*0201 NSG mice irradiated on day-1

  Mice developed GVHD with weight loss, organs are under analysis for human T cell infiltrate.

Publications and patents (published / in press / in preparation)

1. Tilly G, Doan-Ngoc T-M, Yap M, Caristan A, Jacquemont L, Danger R, Cadoux M, Bruneau S, Giral M, Guerif P, Nicol B, Garcia A, Laplaud DA, Brouard S, Pecqueur Hellman C, Degauque N. IL-15 Harnesses Pro-inflammatory Function of TEMRA CD8 in Kidney-Transplant Recipients. Front Immunol. Frontiers; 2017 Jun 30;8:778. PMCID: PMC5492498

Collaborations

Internal collaboration.

Sarah Bruneau (Team 3 – CRTI). A model of transmigration using primary endothelial cell (HDMEC) had been setup to characterize the ability of TEMRA CD8 from KTx and HV to transmigrate in order to screen the ability of metabolic interferences drugs to prevent this transmigration of TEMRA cells.

Core facility MicroPiCell. A model of time-lapse assay has been setup to characterize the mobility of TEMRA CD8 from KTx. The initial setup has been completed using TEMRA CD8 from HV (n=5) and the analysis of TEMRA CD8 from KTx is ongoing.

External collaboration.

A collaboration with **Sian Henson (WRHI – London, UK)** has been initiated to functionally characterize TEMRA CD8 from KTx in a shear-stress model.

Added value of / for Labex IGO

- Strengthen internal collaboration among different teams of the Labex IGO
- Visibility of the Labex IGO through publications in peer-review international journal
- Sustained funding to recruit an international PhD Student and a technician and to support research program for 3 years.

T2.1 HUMANIZED RODENT PLATFORM

Coordinator: Fabienne Haspot

Involved team: CRTI, Teams 1, 2, 3 and 4 (Nantes)
CRCiNA Team 1 (Nantes)
LBAI (Brest)

Staff funded by the Labex IGO:
animal care assistants :
- Chrystelle DUMET 2013-2015
- Malek JAOUACHI 2016-2017
- Marjolaine DUGUE since June 2017.

Initiation of the project: September 2012
End of the project: December 2019

State of the art:
Research "from bench to bedside" requires a first Proof of Concept in a disease-relevant model. However, biologics targeting the immune system such as monoclonal antibodies, recombinant proteins, or cellular therapies are often species-specific and cannot be tested in classical animal models. To circumvent this hurdle, IGO/WP1-Project P2 is setting up humanized mouse (NSG) and rat models, i.e. immunodeficient animals grafted with human immune cells or hematopoietic precursor cells for the generation of a stable human immune system. IGO/WP1-Project P2 also developed novel models in immune-inflammation.

Objectives:
The humanized rodent platform will be used for the preclinical evaluation of molecules and therapeutic strategies identified/developed by IGO participants, which modify immune responses by promoting or dampening immune tolerance, finding direct applications in organ, cell and gene transplantation or by promoting anti-cancer immune responses. Humanization of rats is a novel tool to be developed, based on the inactivation of RAG and IL2Rg genes, as recently described

Project progression / Results:
Equipment/logistics:
The platform is currently breed NSG mice (20 breeders), NSG-SGM3 mice (10 breeders) and NSG-HLA-A*0201 mice (5 breeders) thanks to MTA with Jackson laboratory and Charles River.

Models used
1) Infusion of human PBMC (or specific blood subpopulation) and development or control of a xenogeneic Graft-Versus-Host disease.
2) Infusion of human cord blood CD34+ cells (agreement with the Nantes CHU/Obstetrics for a weekly delivery of cord blood) for a sustained reconstitution of the >T cell compartment.
3) The human skin graft on NSG mice model has been successfully implemented on the platform (an agreement with the Nantes CHU/plastic surgery has been setup).
4) Induction of TNBS/ethanol induced colitis in CD34+ cells reconstituted NSG mice. This is the first ever model showing a clinical colitis in humanized mice.
5) Immunodeficient Rag KOrats have been obtained by the BioGenOuest TRIP platform and are being reconstituted by infusion of human cord blood CD34+ cells.

Models in development / Future collaborations

1) Role of microenvironment for mantle cell lymphoma (CRTI; not Labex Team David Chiron)
2) Arthrose development in CD34-humanized NSG mice (RMES; not Labex Team, Claire Vignatier)
3) iPSC derided CD34+ cells for humanization of NSG-SGM3 mice (CRTI, Labex teams, Laurent David)
4) In vivo evaluation of regulatory B cells (CRTI and INSERM-Brest, Labex teams, Sophie Brouard and Sophie Hillion)
5) A Muliple sclerosis model in humanized NSG mice (CRTI, Labex teams, Arnaut Nico)
6) ANCA-associated vasculitis (CRTI, Labex team, Sarah Bruneau)

Scientific advice:

The platform together with the different PIs co-writes the ethical application for the animal experimentation authorization.

Publications and patents

Bézie S., Meistermann D., Boucault L., Daguin V., Autrusseau A., Bellier-Waast F., Duteille F., David L., Anegon I. and **Guillonneau C.** *Cell therapy with human CD8$^+$ Tregs secreting IFNg, IL-10, IL-34 and TGFb efficiently inhibit human transplant rejection*. **In press Frontier in Immunolog**y.

Picarda E., Bézie S., Boucault L., Autrusseau E., Kilens S., Meistermann D., Martinet B., Daguin V., Donnart A., Charpentier E., David L., Anegon I. and **Guillonneau C**. *Transient antibody targeting of CD45RC to induce transplant tolerance and sustained antigen-specific regulatory T cells.* **JCI Insights.** 2017 Feb 9;2(3):e90088.

Collaborations inside Labex IGO

-CRCINA Team 1, E. Scotet obtain his NSG mice from the platform to validate a cell therapy using intrabrain infusion human γδ T cells to NSG mice implanted with human glioma.
-CRTI Team 1, Aurélie Moreau collaborate with the platform for a xeno-GVDH model and a human-skin graft model to evaluate the potential of her tolerogenic DCs.
-CRTI Team 2, Carole Guilloneau collaborate with the platform for a xeno-GVDH model and a human-skin graft model to evaluate the potential the anti-CD45Rc Ab, of CD8 tolerogenic T cells.
-CRTI Team 3, Nicolas Poirier and Bernard Vanhove developed a colitis model together with the platform in which they tested their anti-IL7R blocking mAb. Fabienne Haspot, is evaluating the pathogenicity of TEMRA CD8 T cells from kidney transplanted patients in a xeno-GVHD model. She is also addressing the role of CD28-H and SIRPg (2 molecules which are not expressed on mouse nor in rats) on human T and NK cells.
-CRTI Team 4, Sophie Brouard started to address the role of the GanzymB+ regulatory B cells to control human skin graft rejection.

Collaborations outside Labex IGO

David Chiron develops a project to evaluate the role of the microenvironment for mantle cell lymphoma.
Claire Vignatier develops a project of Arthrose induced in CD34-humanized NSG mice.

Perspectives of clinical or economical valorization

The humanized mouse model is a preclinical model allowing products and/or cell therapies to be able to reach clinical application and therefore economical valorization. One therapeutic mAb targeting CD28 (FR104) and 1 cell therapy based on the use of immature dendritic cells (The One project) have been tested on the Labex IGO Humanized Rodents Platform before entering clinical phase I development in 2015. Another mAb/drug candidate targeting CD127 and 3 other cell therapies are in preclinical development for transplantation/autoimmunity. Their assessment on the platform will

contribute to the demonstration of safety/efficacy of these therapies and will facilitate the clinical translation of the corresponding drug candidates.

Added value of / for Labex IGO
Publication, oral communication, collaborations inside and outside the LABEX.

T3.2 IMMUNOMONITORING PLATFORM

Coordinators : Régis Josien, MD, PhD
Nathalie Labarrière, PhD

Teams involved : UMR1232
UMR1064

Staff funded by Labex IGO :

- Karine Amouriaux, PhD – Engineer (IE) from 15/07/2013 to 31/03/2015 (*permanent position obtained at CRB, CHU Nantes*)
- Nina Salabert, PhD – Engineer (IE) started on 01/04/2015

Initiation of the project: July 15th 2013
End of the project: December 2019

State of the art

Measuring and following the immune response and its cellular/molecular effectors (immunomonitoring) are crucial for the development of new immunotherapeutics in the context of cancer, transplantation or autoimmune diseases. The complexity and the diversity of immune responses together with the large numbers of genetic polymorphisms in the population are a hurdle to the definition of immune status and patient selection. This project gathers teams with long term expertise in the biology of various compartment of the immune response (T cells, B cells, DC, macrophages, stromal cells, MSC, etc..), their use and monitoring in cancer, transplantation or inflammatory diseases. It offers new avenues for implementing innovative and transversal monitoring approaches in patients.

Objectives

The two main objectives of this platform are: 1. To develop and implement monitoring approaches for in depth phenotypic and functional analyses of human innate and adaptive immune cell subsets (Task 3.2a); 2. To create a centralized database for sharing standardized immune monitoring protocols and data (task 3.2b). The project is centralized at the Center for Immunomonitoring Nantes Atlantic (CIMNA), a CHU and UMR1064 platform. Extensive and innovative analyses of immune effector cells will be performed using the combination of various technologies (multiparametric flow cytometry, cell sorting, multiplex assays, Elispot, functional analyses) already available in the context of an efficient quality system environment (ISO15189) and possibly standardized at CIMNA for using in different pathological situations (Transplantation, Cancer, Autoimmune diseases) to identify common immune status or signatures.

Project progression / Results

We first focused on the validation of flow cytometry panels for analyzing immune cells in whole blood samples. 8-10 colors panels have been set up in the context of a collaborative FP7 project, called The One study, a phase I/II international clinical trial of various cell therapy products in kidney allograft recipients. The Labex IGO funding was critical for implementing the immunomonitoring part of this trial which has been completed in 2017 (8 patients included having received TolDCs). It allowed us to established strong collaborations with La Charité in Berlin Germany and provided visibility in Europe. The CIMNA is now involved in another FP7 program called BioDrim that aims at minimizing immunosuppression in transplanted patients according to immune monitoring (ongoing). We participated to the validation of new dry flow panels and to the international validation of an anti-donor Elispot assay. In addition, we have set up internally a 10-color panel for the identification and numeration of non classical lymphocytes (MAIT cells, iNKT cells, NKT cells) and innate lymphoid cell subsets (ILC1, 2 and 3). Both of these panels have been used to demonstrate an alteration of

MAIT cells in ANCA-associated vasculitis (AAV) (**J Autoimmun 2016**). We also set up new panels to assess the phenotype of blood myeloid cells (monocytes, neutrophils, eosinophils and basophils). Using these assays, we reported that basophils, are an important and new source of RANKL (**Immunobiology, 2014**) and more recently we demonstrated that human basophils do not respond to TSLP (**J Allergy Clin Immunol 2017**). We also set up whole blood assays to assess innate functions of circulating DCs and demonstrated a complex dysregulation of these function in AAV (**Frontiers Immunol 2017**). All these flow cytometry panels are available for other studies. The platform also participated to international projects aiming at standardizing immunomonitoring procedures (**Clin Immunol 2018, Cytom B Clin Cytom 2018**) and provided technical expertise in local collaborative project (**Blood Adv 2017, J Immunol 2016**).
The CIMNA also realized, develop and validate quality controls for the Unit for Cell and Gene Therapy at Nantes CHU (UTCG), for instance for TolDC cell therapy products (phenotype, contaminants) and for TILs immunotherapy in melanoma patients (phenotype, contaminating tumor cell, potency assays) These activities also benefit from the LabEx funding especially for the development and validation steps.

Publications

1. HLA-DR expression in neonates after cardiac surgery under cardiopulmonary bypass: a pilot study. Chenouard A, Braudeau C, Cottron N, Bourgoin P, Salabert N, Roquilly A, Josien R, Joram N, Asehnoune K. **Intensive Care Med Exp. 2018** Jan 11;6(1):1. doi: 10.1186/s40635-017-0166-x. PMID: 29327145
2. A standardized flow cytometry procedure for the monitoring of regulatory T cells in clinical trials. Pitoiset F, Barbié M, Monneret G, Braudeau C, Pochard P, Pellegrin I, Trauet J, Labalette M, Klatzmann D, Rosenzwajg M. **Cytometry B Clin Cytom. 2018** Jan 6. doi: 10.1002/cyto.b.21622. [Epub ahead of print] PMID: 29316248
3. Thymic stromal lymphopoietin does not activate human basophils. Salabert-Le Guen N, Hémont C, Delbove A, Poli C, Braudeau C, Fantou A, Amouriaux K, Bériou G, Martin JC, Colas L, Soumelis V, Josien R. **J Allergy Clin Immunol. 2017** Dec 2. pii: S0091-6749(17)31876-6. doi: 10.1016/j.jaci.2017.11.012. PMID: 29208546
4. Standardized whole blood stimulation improves immunomonitoring of induced immune responses in multi-center study. Duffy D, Rouilly V, Braudeau C, Corbière V, Djebali R, Ungeheuer MN, Josien R, LaBrie S, Lantz O, Louis D, Martinez-Caceres E, Mascart F, Ruiz de Morales JG, Ottone C, Redjah L, Guen NS, Savenay A, Schmolz M, Toubert A, Albert ML; Multinational FOCIS Centers of Excellence. **Clin Immunol. 2017** Sep 21. pii: S1521-6616(17)30198-5. doi: 10.1016/j.clim.2017.09.019. PMID: 28943400
5. Cell-surface C-type lectin-like receptor CLEC-1 dampens dendritic cell activation and downstream Th17 responses. Lopez Robles MD, Pallier A, Huchet V, Le Texier L, Remy S, Braudeau C, Delbos L, Moreau A, Louvet C, Brosseau C, Royer PJ, Magnan A, Halary F, Josien R, Cuturi MC, Anegon I, Chiffoleau E. **Blood Adv. 2017** Mar 22;1(9):557-568. doi: 10.1182/bloodadvances.2016002360. eCollection 2017 Mar 28. PMID: 29296975
6. Braudeau C, Néel A, Amouriaux K, Martin JC, Rimbert M, Besançon A, Giraudet S, Terrien C, Aliaga M, Salabert-Le Guen N, Hémont C, Hamidou M, Josien R. Dysregulated Responsiveness of Circulating Dendritic Cells to Toll-Like Receptors in ANCA-Associated Vasculitis. **Front Immunol. 2017** Feb 9;8:102. doi: 10.3389/fimmu.2017.00102. PMID: 28232832
7. N. Poirier, G. Blancho, M. Hiance, C. Mary, T. Van Assche, J. Lempoels, S. Ramael, W. Wang, V. Thépenier, C. Braudeau, N. Salabert, R. Josien, I. Anderson, I. Gourley, J.-P. Soulillou, D. Coquoz & B. Vanhove. First-in-Man Study in Healthy Subjects with FR104, a pegylated Monoclonal Antibody Fragment Antagonist of CD28. **J. Immunol**. **2016.** *Nov 14. pii: 1601538. [Epub ahead of print]*. PMID: 27849166
8. Braudeau C, Amouriaux K, Néel A, Herbreteau G, Salabert N, Rimbert M, Martin JC, Hémont C, Hamidou M, Josien R. Persistent deficiency of circulating mucosal-associated invariant T

(MAIT) cells in ANCA-associated vasculitis. **J Autoimmun. 2016** Jun;70:73-9. doi: 10.1016/j.jaut.2016.03.015. Epub 2016 Apr 18. PubMed PMID: 27102145.

9. Poli C, Martin JC, Braudeau C, Bériou G, Hémont C, Charrier C, Guérin S, Heslan M, Josien R. Receptor activating NF-κB ligand (RANKL) is a constitutive intracellular protein in resting human basophils and is strongly induced on their surface by interleukin 3. **Immunobiology. 2015** May;220(5):692-700. doi: 10.1016/j.imbio.2014.11.009. Epub 2014 Nov 21. PubMed PMID: 25433635.

Collaborations

- **Local**
  - M. Hamidou, A. Neel. Department of Internal Medicine, CHU Nantes
  - E. Chiffoleau, CRTI UMR1064, Nantes
  - K Asehnoune, Department of Anesthesiologu and Intensive Care, CHU Nantes
  - A Chenouard, Department of Pediatrics, CHU Nantes
  - N Poirier and B Vanhove, OSE Immunotherapeutics, Nantes

- **National**
  - V. Soumelis, Institut Curie, Paris, France
  - D. Duffy, Institut Pasteur, Paris, France
  - M. Rosenzwajg, La Pitié, Paris, France

- **International**
  - B. Sawitzki, Charité, Berlin, Germany
  - P. Reinke, Charité, Berlin, Germany

Perspectives

The next step will be to: 1. Implement systematic immunomonitoring of patients included in new clinical trials in the context of immunotherapy in cancer, as done in transplantation; 2. Validate new panels for exploration or rare cells such as circulation tumor cells or antigen specific T cells.

WP2 : INNOVATIVE CELLULAR IMMUNOTHERAPIES

(coordinator: F. Lang)

There is a general consensus that T cells responses are critical for both tumor and graft rejection. In graft rejection, it is well documented by the efficacy of immunosuppressive drugs on prevention of acute graft rejection whereas in cancer, this is underscored by the clinical efficacy of check point inhibitors and some adoptive cell transfer clinical trials. Nonetheless, there is still room for improvement in terms of long-term tolerance after organ transplantation and in terms of tumor rejection. The common feature of the 4 projects (funded in 2014 and 2016) that follow is to use transfer of T lymphocytes to achieve this goal.

The first project led by Dr Scotet and Dr Pecqueur (T4.3, funded in 2014) explored the recognition of glioblastoma by Vγ9Vδ2 T cells to determine which subtype of glioblastoma was the most sensitive to human γδ T cells and how this various subtypes of glioblastoma disseminated in the brain in mice. In addition, by transcriptomic and phenotypic analysis, they identified surface molecules that may be involved in this Vγ9Vδ2 T cell recognition. This opens the way to clinical use of these T cells in situ as *per operative* adjuvant of surgery. The project has ended mid-november 2017 and resulted in 2 accepted publications, one in revision and one submitted.

The second project by Pr Guilloux and Dr E. Mortier (T5.1) aimed at developing non invasive tracking methods for antigen specific T cells used for ACT purposes. Three methods were used to label OT-1 specific T cells: fluorescent nanoassemblies (fluo@mag) produced by Pr Ishow's team (CEISAM - UMR CNRS 6230 / University of Nantes), fluorescent liposomes produced by the team, and metabolic engineering of modified substrates and click-chemistry. Only liposomes and click-chemistry result in stable labeling *in vitro* (up to 5-days) while preserving the functions of specific T cells. *In vivo* persistence of liposome-labeled OT-I-specific T cells has also been assessed, in tumors from mice engrafted with 5T33-OVA MM cells. The same experiment will be performed with click-chemistry. The final goal of this project is to radiolabel specific T lymphocytes with the click method, either before ACT or using a two-step strategy, with the infusion of engineered-T cells with click function, followed by administration of a radiolabeled-clickable compound. The fate of T lymphocytes will be followed by microTEP imaging (CIMA Imaging platform, available in 2018).

The third project led by Dr Guillonneau and Dr Saulquin (T4.4, funded in 2016) aimed at redirecting regulatory T cells towards graft rejection sites by making them express a CAR specific for HLA molecules expressed by the graft. They successfully generated CAR Tregs against HLA-A2, expanded them in vitro and demonstrated that they retained their regulatory phenotype, and that they could inhibit skin graft rejection in a mouse model, thus providing proof of concept of this therapeutic approach. They also progressed in the development of new human anti-HLA-A2 antibodies that will be used to design new CARs with selected affinities. This work led to a publication in press, and constitutes the core of the collaboration with the company TxCell and a patent is being filed on the new anti-HLA-A2 antibody. This project is on-going.

The fourth project developed by Dr Louvet and Dr Vanhove (T4.5, funded in 2016) was to render T lymphocytes independent on the cysteine provided by dendritic cells by transfecting them with the enzyme cystathionine-γ-lyase (CSE). The idea was that they could thus be resistant to cysteine deprivation which occurs in the tumor microenvironment. To achieve this goal, as a first step, they developed a recombinant retrovirus containing the enzyme cDNA that could successfully infect a majority of mouse T lymphocytes. They then determined in a model of mouse bearing B16-OVA melanoma what were the best conditions (total body irradiation and IL2 injections) to allow survival of transferred T lymphocytes against OVA. Treating the tumor-bearing mice with CSE-transfected T cells led to enhanced control of tumor growth but only transiently. Other enzymes may be necessary to achieve complete cysteine independence. This project is on-going.

T4.1 GENERATION OF ANTIGEN SPECIFIC T CELLS WITH OPTIMIZED FUNCTIONS

T4.1.1. Optimization of specific cytotoxic T lymphocytes (CTL) in vitro selection/amplification for therapeutic protocols

T4.1.2. Glioblastoma immunotherapy : a model for the use of banked allogeneic T cells

Coordinators: N. Labarrière/H. Vié

Teams involved: Team 3 UMR1232
Team 1 UMR1232
Team 6 UMR1232
Team 14 UMR1232

Staff funded by Labex IGO: Sylvain Simon (PhD Student), 01/10/13 – 31/09/15
Ulrich Jarry (Post-Doc), 01/03/2013 – 28/02/2015

Initiation of the project: October 2013
End of the project: September 2016

State of the art
Among the many approaches for the production of specific T lymphocytes for adoptive transfer in oncology and transplantation, peptide stimulation followed by immunomagnetic sorting represents an interesting choice in terms of technological feasibility, safety and efficacy. Nonetheless, these methods need to be challenged and improved according to several parameters such as the initial precursor frequency, the effector functions of produced T cells and the cost of a complete production process.

Objectives
The objectives of this program were
(1) to implement procedures for selection and amplification of specific T cells
(2) to optimize the effector functions of the selected T lymphocytes
(3) to achieve the selection and amplification of T lymphocytes whose repertoire is more restricted (T cell specific for glioblastoma epitopes)

Project progression / Results
Objective 1: In the field of melanoma immunotherapy we developed a clinical grade procedure leading to the production of more 5.10^8 polyclonal CD8 T lymphocytes specific for two melanoma antigens (Melan-A and MELOE-1), within 31 days of culture ***[publication ≠1],*** using a selection method based on peptide stimulation followed by HLA-Peptide specific multimer sorting. These T lymphocytes are fully specific, polyclonal and tumor reactive. A clinical trial has been funded and validated by regulatory agencies and the first two patients have been included in November 2015 (MELSORT NCT 02424916).
We also demonstrated that for the healthy donors whose CMV precursors CTL frequency were above 1%, the direct amplification of these CTL after peptivator stimulation (Miltenyi Biotec) compared favorably with a protocol including a sorting procedure. This straightforward process has now been validated in GMP conditions after three blank runs at Atlantic Bio GMP (ABG). Production of the first clinical batch of a bank of CMV-specific CTL will be scheduled before spring.

Objective 2: A fraction of selected and amplified melanoma T cells expresses the PD-1 molecule, while another fraction remained negative, even after activation. We documented for the first time the existence of melanoma specific T cell clones unable to express PD-1. This stable feature was due

to the persistent methylation of the *PDCD1* promoter. These PD-1^{neg} clones were of lower avidity than their PD-1^{pos} counterparts, showing that PD-1 expression identifies antigen-specific T cell clonotypes of high functional avidity. We demonstrated that PD-1 blockade during the *in vitro* selection process of Melan-A specific T cells favored the amplification of higher avidity T cell clonotypes. This preferential amplification of high avidity memory T cells upon PD-1 blockade resonates with the expansion of reactive T cells observed in anti-PD-1 treated patients while providing new insights for adoptive transfer treatments ***[publication ≠2]***.

Objective 3: In line with the selection of the rare CTL specific for antigens associated with glioblastoma, because of the very low frequency of these precursors as well as the discrepancy between their structural and their functional characteristics (some of the selected populations that were recognized by the peptides-loaded tetramer did not reacted against peptides-loaded target-cells), we could not consider the possibility to develop this strategy for clinical purposes.

Publications and patent
See appendices 5 and 6

Clinical trial
MELSORT NCT 02424916 : "Phase I/II clinical trial of adoptive transfer of CD8^{+} T lymphocytes specific for Melan-A and MELOE-1 melanoma antigens, selected with HLA-peptide multimers, in the treatment of metastatic melanoma."
Principal investigator: B. Dreno, Associated scientific investigators: N. Labarrière, F. Lang. Sponsor : CHU de Nantes. Start : November 2015.

Collaborations
- This program allowed to strenghten the collaborations between Team 1 and 3 from the CRCNA and also with the Unit of Cell Therapy of Nantes Hospital, and the clinical team of B. Dreno (Onco-dermatology).
- A collaboration with BMS emerged from this program, based on the use of PD-1 antibody in combination with adoptive T cell transfer.
- This program allowed initiating the production of a bank of CMV specific CTL in a local pharmaceutical environment (Atlantic Bio GMP).

Perspectives of clinical or economical valorization
This project will improve T cell production procedures to amplify specific T cells to be used for adoptive transfer in the field of transplantation and cancer. Based on the results obtained on PD-1 and T cell avidity, a future clinical trial combining the adoptive cell transfer of sorted melanoma specific T cells with high functional avidity and anti-PD-1 treatment is considered in association with the BMS company.
The bank of CTL will be used first in the context of a clinical protocol to treat CMV diseases after allo HSCT (PI Pr. Patrice Chevallier, service Hématologie CHU Nantes).
Furthermore, the ability to select very simply virus-specific CTLs when their frequency is high enough (without the need of immunomagnetic separation), will also be considered for the selection of EBV-CTLs.

Added value of / for Labex IGO
The collaboration between Team 1 and 3 from the CRCNA is fruitful in terms of complementary skills and interests and allowed the development of new research programs.
On a clinical perspective and based on the results obtained on PD-1 and T cell functional avidity, Team≠3, in collaboration with the clinical group of B. Dreno, has investigated the quantitative and qualitative variations within Melan-A specific T cell repertoire from Nivolumab treated patients (Simon et al 2017) and started a collaboration with Qiagen.

T4.2 : OPTIMIZATION OF CELL THERAPY PROTOCOL USING TOLEROGENIC DENDRITIC CELLS IN TRANSPLANTATION

Coordinators: Aurélie Moreau and Maria-Cristina Cuturi

Teams involved: Team 1 ITUN-INSERM UMR-S 1064, Nantes

Staff funded by LABEX IGO: Marcelo Hill (Post-Doc) 09/11/12 – 08/05/13
Cédric Louvet (Post-Doc) 01/01/14 – 31/07/15
Amandine Even (master 2 student) 15/06/15 – 12/07/15

Initiation of the project: September 2012
End of the project: September 2016

State of the art

The use of immunosuppressive drugs to treat transplant recipients has markedly reduced the incidence of acute rejection but fails to prevent chronic allograft dysfunction. In this context, therapies-based on the adoptive transfer of regulatory cells are promising strategies to induce indefinite transplant survival. Our pre-clinical studies in animal models have demonstrated that administration of autologous tolerogenic dendritic cells (TolDC) prolongs graft survival (Peche et al., Am. J. Transpl, 2005; Segovia et al., Am. J. Transpl, 2014). As part of the ONE Study clinical trial project, human autologous TolDC will be administrated in kidney transplant patients in association with a minimized immunosuppressive regimen. The feasibility and the safety of TolDC injection will be evaluated in this first in human clinical trial.

Objectives

1) to develop and characterize TolDC from blood samples from healthy volunteers (HV),
2) to compare TolDC obtained from HV with those of patients with impaired renal function,
3) to validate the protocol in GMP conditions,
4) to assess the safety, efficacy and homing of TolDC in humanized mice,
5) to define the mechanisms of action of these tolerogenic DCs.

Project progression / Results

We have first developed a protocol to generate human TolDC from monocytes of healthy volunteers (Aim 1). These cells display an immature phenotype and are highly resistant to maturation stimuli. These properties are essential to ensure that they will not mature and become immunogenic once injected into patients. About the function of TolDC, they are hypostimulative cells as they induce a very low level of T cell proliferation when cultured with allogeneic CD3+ T cells. Lastly, the proliferation of mature DCs-stimulated T cells is suppressed by autologous TolDC, suggesting a high efficacy of our cells to control T cell proliferation. This characterization of human TolDC was set up from blood samples of healthy donors and then confirmed in TolDC generated from renal insufficient patients receiving or not dialysis (Aim 2). We also transferred the TolDC manufacturing process to a GMP facility able to produce human TolDC in clinical conditions and obtained the authorization to use TolDC in clinic in September 2014 (Aim3). Three patients enrolled in our clinical trial received their own TolDC so far. Our first experiments in humanized mice models showed that ATDC delay the development of GVHD (Graft versus Host Disease) in these mice. Furthermore, our results also demonstrated that ATDC survive at least 2 weeks after injection, migrate preferentially to the spleen and preserve an immature phenotype in vivo (Aim 4). In order to define the mechanisms of action of TolDC (Aim 5), we are currently trying to understand ***i)*** how TolDC suppress T cell proliferation and also ***ii)*** how ATDC delay GVHD development in vivo.

Publications

- Segovia M, Louvet C et al., Autologous dendritic cells prolong allograft survival through*Tmem176b*-dependent antigen cross-presentation *Am. J. Transplantation*. 2014
- A second manuscript on human tolerogenic DCs is in preparation.

Collaborations

Nathalie Labarrière – CRCNA – Labex IGO – France
Bernard Vanhove/humanized rodent platform – ITUN INSERM1064 – Labex IGO – France
Edward Geissler- University of Regensburg – Germany
Barry Sharp – University of Loughborough – UK

Perspectives of clinical or economical valorization

Three patients were enrolled in the clinical trial and received autologous TolDC without signs of toxicity associated with cell administration. To anticipate further trials in which the efficacy of TolDC will be the ultimate aim, it is essential to in-depth characterize our TolDC to understand very precisely how they will act in patients.

Added value of / for Labex IGO

Thanks to Labex IGO, we set up collaborations with two others partners of Labex IGO, N. Labarriere's team (T4.1) and the humanized rodent platform (B. Vanhove).
The funding also allows us to in-depth study human TolDC. This is an essential step for further clinical trials using these cells.

T4.3. ADOPTIVE CELL THERAPY FOR GLIOBLASTOMA – CHARACTERIZATION OF TUMOR CELL TARGETS AND ANALYSIS OF THEIR RECOGNITION BY HUMAN T LYMPHOCYTES IN VITRO AND IN VIVO

Coordinators: Emmanuel Scotet (Team 1 - UMR1232)
Claire Pecqueur-Hellman (Team 9 - UMR1232)

Teams involved: Team 1 UMR1232 (E. Scotet)
Team 9 UMR1232 (F. Vallette)

Staff funded by Labex IGO: Cynthia Chauvin, PhD student, 36 months from October 2014 to September 2017

Initiation of the project: 1st October, 2014
End of the project: November, 2017

State of the art

This program relied on both developed *in vitro* and *in vivo* experimental models and preliminary observations from participating teams. It aimed at providing a better characterization and understanding of the biology of different GBM subtypes. A major objective was to to establish novel physiological orthotopic human GBM models in mice, which recapitulated the extensive invasive and hypervascular features of GBM and could be exploited for next testing immunotherapies. This experimental program also focused on identifying pathways and mechanisms used by human effector T cell subsets involved in the recognition and elimination of these GBM tumor cells, both *in vitro* and *in vivo*.

Objectives

The main objectives of this research program were :
(i) to study the phenotype, metabolism and molecular diversities of human GBM cells in primary cultures from GBM tumors, in correlation with the tumor classification and the disease outcome in patients;
(ii) to analyze if these primary GBM cultures reflect *in vitro* biological and pathological characteristics of the stem cell component and to compare the ability of the different GBM tumor cell subtypes to recapitulate parental human tumors, after intracranial implantation into immunodeficient mice (NSG strain);
(iii) to assess, through various complementary functional and videomicroscopic assays, the recognition of primary GBM cultures *in vitro* to various human effector cell subsets, which can be used in adoptive immunotherapy strategies;
(iv) to study the effects of current therapy lines, such as chemotherapy (eg, temozolomide) and radiotherapy on primary human GBM cultures and the impact or the activation of different human effector T cell subsets;
(iv) to analyze the feasibility and efficiency of intracranial adoptive immunotherapies targeting human effector T cell subsets in immunodeficient mice xenografted with characterized primary GBM cultures.

Project progression / Results

The research program achieved the following results :
1) Human GBM tumor cells collected from patients (biopsies) have been characterized and next classified for their metabolic activity and their phenotype, including the expression of various known stemness markers (e.g., expression of CD133);

2) The extensive analysis of the reactivity of humanq allogeneic Vγ9Vδ2 T cells (PBMC of healthy donors) against primary GBM cells has revealed that GBM from the mesenchymal subtype represent preferential natural cell targets for some of the generated γδ T cell lines *in vitro*;
3) The ability of human primary GBM tumors to develop and disseminate within the brain of NSG mice after implantation has been analyzed (IHC). These parameters have been compared and correlated to the phenotypic and metabolic classification established above;
4) A comparative transcriptomic analysis of these primary GBM lines has led to the identification of some candidate molecular pathways (eg, NKR-related molecules) implicated in this natural tumor recognition process by human γδ T cells;
5) The expression of several candidate molecules has been analyzed by multicolor flow cytometry (using fluorochrome-labelled mAbs or recombinant ligands). This currently performed study tends to confirm that mesenchymal GBM express a particular set of NKRL which contribution to the natural recognition process by human γδ T cells should be soon assessed.

Publication

Jarry U, Joalland N, Chauvin C, Clémenceau B, Pecqueur C, Scotet E. Adoptive transfer by stereotaxy of cytotoxic immune cells in murine models of orthotopic human glioblastoma multiforme xenografts. 2018. *Submitted*

Joalland N*, Chauvin C*, Oliver L, Vallette FM, Pecqueur C, Jarry U, Scotet E. IL-21 increases the reactivity of allogeneic human Vγ9Vδ2 T cells against primary glioblastoma tumors. Journal of Immunotherapy. 2018, *in revision*. * co-authors

Oizel K*, Chauvin C*, Oliver L, Gratas C, Geraldo F, Jarry U, Scotet E, Rabe M, Alves-Guerra MC, Teusan R, Gautier F, Loussouarn D, Compan V, Martinou JC, Vallette FM, Pecqueur C. Efficient Mitochondrial Glutamine Targeting Prevails Over Glioblastoma Metabolic Plasticity. Clin Cancer Res. 2017 Oct 15;23(20):6292-6304. doi: 10.1158/1078-0432.CCR-16-3102. Epub 2017 Jul 18. PubMed PMID: 28720668. * co-authors

Jarry U*, Chauvin C*, Joalland N, Léger A, Minault S, Robard M, Bonneville M, Oliver L, Vallette FM, Vié H, Pecqueur C, Scotet E. Stereotaxic administrations of allogeneic human Vγ9Vδ2 T cells efficiently control the development of human glioblastoma brain tumors. Oncoimmunology. 2016 Mar 30;5(6):e1168554. doi: 10.1080/2162402X.2016.1168554. eCollection 2016 Jun. PubMed PMID: 27471644; PubMed Central PMCID: PMC4938356. * co-authors

Collaborations
This joint research program strengthened the collaborations between Teams #1 and #9 from CRCINA (co-supervision of the PhD). *In vivo* experiments first relied on interactions with Dr B. Vanhove from INSERM UMR 1064 ITUN. A collaboration with group of C. Retière (EFS, Nantes and Team #1 since 2017) has been established (analysis of NKR/NKRL expression). Collaborations have also been established with Teams #13 (Labex IGO and IRON) and #14 from CRCINA to analyze both *in vivo* localization of injected human effector cells, the impact of radiotherapeutic lines and the efficacy of GD2 CARs expressed by human γδ T cells.

Perspectives of clinical or economical valorization
The project has provided key insights into the biology and in-depth metabolic and phenotypic characteristics of GBM cell subtypes, such as glioma stem cells which are likely involved in tumor relapse. Moreover, the results, which were also supported by those from previous complementary programs (e.g., IGO WP2-T4.1.2) supported the relevance of adjuvant *per operative* adoptive immunotherapy targeting various human effector T cell subsets. By combining a complementary set

of *in vitro* and *in vivo* experimental approaches, this program highlighted the modalities of T cell activation against GBM and some tumor escape mechanisms, as well as the positive *vs.* deleterious impact of chemo-radiotherapy on immune responses. Altogether, the results from this study paved the way to the development of original and more refined therapeutic strategies, such as adjuvant immunotherapy, to prevent or delay relapse in GBM. A clinical trial is currently in preparation (Angers CHU hospital)

Added value of / for Labex IGO

Interactions between Teams #1 and #9 from the CRCINA has been fruitful in terms of complementary skills and interests and has allowed the submission of joint research programs (eg, EU FP7/ERA-NET TRANSCAN JTC2015). The efficient and rapid development of animal models of human tumor xenografts and cellular immunotherapies, crucially supported by the Labex IGO, has led to the establishment of recent collaborations with local (e.g., Teams #3 and #8 from CRCINA, INSERM U957 Nantes) and international teams (e.g., M. Eberl Cardiff University, GB).

T4.4. ENGINEERED CAR-TREGS – LICENSED TO SPECIFIC CONTROL OF IMMUNE RESPONSES IN TRANSPLANTATION

Coordinator: Carole Guillonneau

Teams involved : Team 2 UMR1064 (Carole Guillonneau)
Team 1 UMR1232 (Xavier Saulquin)
Humanized rodent platform

Staff funded by Labex IGO: Séverine Bézie (Engineer:IR) 2016-2017

Initiation of the project: 2016
End of the project: 2019

State of the art:

Kidney transplantation is an essential therapy in patients with severe chronic renal failure. However, graft rejection requires patients to undergo extensive immunosuppressive treatment causing many side effects. There is a need for more specific therapies. There are several types of Tregs that can inhibit anti-donor immune response and the current project focuses on one of these sub-populations: the $CD8^+$ regulatory T cells that we recently described as capable to inhibit graft rejection in immune humanized NSG mice transplanted with human skin. However, adequate numbers of antigen-specific Tregs are difficult to achieve for adoptive therapy. Chimeric antigen receptors (CAR) have been used to redirect CD4+ Tregs in autoimmune diseases, but so far there is no description of applicability of CAR-Tregs for transplantation. Moreover, there has been no attempt to redirect CD8+ Tregs, even in autoimmunity. We hypothesize that redirecting Tregs toward donor molecules in transplantation will prevent immune attacks against the organ and induce tolerance.

Objectives:

The project aims at targeting molecules expressed at the cell surface of donor allograft transplant in order to direct CAR-engineered regulatory T cells on inflammatory sites and promote graft survival and tolerance. To do so, this proposal will address the following objectives:
WP1) Proof of concept of the ability to graft an antigenic specificity to a regulatory T cells using an already available HLA-A2 specific CAR and their efficacy to induce allograft tolerance.
WP2) Genome editing of engineered CAR-Tregs to improve specificity, safety and efficacy
WP3) Production of new antibodies targeting allogeneic immune responses for further use as CARs.
WP4) Generation of new CARs and analyses of CAR-Tregs in vitro and in vivo.

Project progression / Results:

We have produced lentivirus expressing HLA-A2-specific CAR and set up a protocol of transduction of CD8+ Tregs with the lentivirus, using a Her2-specific CAR as control and an empty vector. We have been able to obtain up to 30% of transduced cells. Upon sorting and expansion during 14 days with high dose IL-2 and IL-15 we are able to demonstrate that HLA-A2-specific CAR CD8+ Tregs are superior in suppressive capacity than Her2-specific CAR CD8+ Tregs in a dose dependent manner in presence of $HLA\text{-}A2^+$ APCs. We have demonstrated that their phenotype was preserved and that they did not acquire cytotoxic activity upon transduction. Our preliminary results in vivo suggest that they can inhibit graft rejection at low Treg/Teff ratio in a model of skin transplantation in humanized NSG mice.
In parallel, HLA-A2 specific B cells were sorted from healthy individuals and single cell sequencing was performed. The sequences were cloned and the antibodies produced and analyzed for specificity and affinity. One specific anti-HLA-A2 human antibody of low affinity was obtained. To improve the

affinity, affinity-maturation using CrispR/Cas9 was performed, the antibody was successfully mutated in the CDRs and the mutated sequences obtained are currently analyzed for potential as CAR.

Publications and patents
Partner 1 and 2 are currently drafting a patent with Inserm-Transfert to protect the sequence of the antibody obtained that will be used to generate a CAR.
In preparation:
#HLA-A2 specific CAR CD8+ Tregs can efficiently protect against transplant rejection. Séverine Bézie, Ignacio Anegon and Carole Guillonneau.
#Advances on CD8+ Tregs and their potential in transplantation. Séverine Bézie, Ignacio Anegon and Carole Guillonneau.
Published:
1 publication in press: Bézie et al, Frontiers in Immunology (see appendix 5).

Collaborations (inside and outside Labex IGO)
International: Megan Levings, Canada
National: TxCell S.A., France

Perspectives of clinical or economical valorization
We have registered a patent on the phenotype of this sub-population and its role in transplantation. We will register new patent with the antibodies obtained. The patent has been licensed and we expect to start a clinical trial in transplantation by the end of the Labex IGO project.

Added value of / for Labex IGO
The funding of the Labex IGO was essential to start the project and for the development of this project and has allowed the establishment of collaborations with team and platform of the labex.
Following acquisition of preliminary results, we have been able to obtain an important co-funding from TxCell S.A. and licensed our patent.

T4.5. BOOSTING ANTI-TUMOR RESPONSE BY CONFERRING METABOLIC AUTONOMY TO T CELLS

Coordinator: Cédric Louvet, Bernard Vanhove

Teams involved : CRTI UMR1064 Teams 1 and 3

Staff funded by Labex IGO : Technician. Lucile Guéno, 1 year (Nov. 2016 – Oct. 2017)

Initiation of the project: 2016

End of the project: 2019

State of the art:
Impressive results have been recently obtained in patients with cancer by engineering T cells with anti-tumor T cell receptors (TCRs) or chimeric antigen receptors (CARs) upon adoptive cell transfer (ACT). Most of the attention is currently focused on tumor antigen specificity, precision, expression/signaling tuning and safety of the infused T cells. However, additional therapeutic weapons will be needed to reach long-term remissions, especially in solid cancers where the tumor microenvironment can exert strong suppressive effects on T cells. Beyond CTLA-4 and PD-1 blockade, multiple other targets likely remain to be discovered and could be overcome by original T cell engineering. Cysteine deprivation within the tumor environment may represent a major inhibitory mechanism favored by tumor cells and/or suppressive myeloid cells. Cysteine (along with its oxidized dimeric form, cystine) is the least abundant of the amino acids in the extracellular space and is normally mainly provided to T cells by dendritic cells.

Objectives:
In this work, we explore the therapeutic potential of engineering genes of the transsulfuration pathway in T cells with the aim to confer cysteine production autonomy. We address this hypothesis in a preclinical mouse model of antigen-specific antitumor ACT.

Project progression / Results:
Our first objective was to optimize large scale mouse T cell infection in order to proceed with in vivo injection. Through collaboration with Nahzli Dilek (Olivier Michielin lab, Lausanne, Switzerland), we successfully generated ecotropic gamma-retrovirus encoding the cystathionine-γ-lyase (CSE) enzyme (along with Thy1.1 reporter gene) and routinely achieved >50% transduction efficacy. From April 2016, we particularly focused our attention on setting up the best experimental conditions for a preclinical model of antitumor ACT therapy. We found that pre-conditionning of tumor-bearing mice (B16-OVA melanoma, s.c. injection 10 days before treatment) with whole body irradiation (6 Gy) and IL-2 injection (i.p., $6x10^4$ IU, 3 consecutive days) was critical to allow persistance of infused T cells, as observed in patients in the clinic. Finally, we tested whether CSE expression in antitumor T cells ($CD8^+$ T cells from OT-1 transgenic mice) could be beneficial to boost the effect of ACT in this model. In line with this hypothesis, our results showed that this strategy could significantly enhance, although transiently, the control of tumor growth. While these results are encouraging, we are now aiming at achieving a long-lasting and stronger effect with combination therapies (notably anti PD-L1 treatment). We will also explore the potential of combining different enzymes besides CSE in T cells. Indeed, we postulate that CSE alone may not be able to fully recapitulate the transsulfuration pathway on its own without significantly increasing the expression of one or other additional key enzymes. Finally, we will take advantage of the cell tracking system (we bred OT-1 mice with Ly5.1 mice to allow expression of the CD45.1 allelic variant on infused T cells, thus distinguishable from CD45.2+ host cells) to investigate the fate and function (localization, numbers, proliferation status, IFNγ production etc…) of transduced T cells in vivo, notably in the tumor. In conclusion, after one

year of development of this project, these preliminary results reinforce the potential importance of this cysteine metabolic checkpoint during the antitumor response.

Collaborations:
Nahzli Dilek (Olivier Michielin lab) : Molecular Modeling Group, Swiss Institute of Bioinformatics (SIB), Lausanne, Switzerland. The Ludwig Institute for Cancer Research, University of Lausanne, Epalinges, Switzerland.

Perspectives of clinical or economical valorization :
This gene-engineering approach to enhance the function of T cells could be implemented in the context of antitumor TCR or CAR-transduced T cells currently used in the clinic and could be particularly relevant in the context of solid cancers where the tumor microenvironment can exert strong suppressive effects on T cells, often considered as counter-regulatory mechanisms triggered by the T cells themselves.

Added value of / for Labex IGO:
This project stems from an original collaboration allowed by the Labex IGO between the team of Bernard Vanhove who initiated works on the role of cysteine metabolism in alloimmune response in transplantation (Vuillefroy de Silly *et al.* Blood 2012) and Cédric Louvet, interested in developing novel gene-engineering approaches to enhance antitumor effect of T cells.

T5.1. TRACKING OF TUMOR SPECIFIC T CELLS

Coordinators: Yannick Guilloux and Erwan Mortier

Involved teams: CRCINA team 13 (M. Chérel) Nantes/ CRCINA team 1 (E. Scotet) Nantes

Staff funded by Labex IGO: Dr Nolwenn Fichou Post-Doctoral fellow, Team ≠13 (9/1/2015 until 05/15/2016)
Pascal Aumond, Engineer, 18 months, Team ≠1 (8/1/2016 until 12/31/2017)

Initiation of the project: September 1, 2015
End of the project: December 31, 2017.

State of the art

Ionizing radiation induces direct and indirect killing of cancer cells and for long has been considered as immunosuppressive. However, this concept has evolved over the past few years with the demonstration that irradiation can increase tumor immunogenicity and can actually favor the implementation of an immune response against tumor cells. Adoptive T-cell transfer (ACT) is also used to treat cancer and several studies have shown that the efficacy of this immunotherapy was enhanced when combined with radiation therapy. We thus hypothesized that, in the setting of α-Radioimmunotherapy (α-RIT), an immunotherapy like ACT, could benefit from the immune context induced by irradiation. Then we evaluated the therapeutic efficacy in the mice treated with α-RIT, using an anti-CD138 antibody coupled to bismuth-213, followed by an adoptive transfer of OVA-specific CD8+ T cells (OT-I CD8+ T cells) in multiple myeloma (MM) murine model. We observed a significant tumor growth control and an improved survival in the animals treated with the combined treatment. These results demonstrate the efficacy of combining α-RIT and ACT in the MM model we established.

Objectives

The main objectives of the program are:
1/ to document the impact of ionizing radiation on different aspects of the injected cells: motility, migration, activation status and proliferation.
2/ to investigate T cell trafficking using three kinds of stainings (liposomes, click chemistry or non-doped nanosystems fluo@mag).

Project progression / Results

For the past ten years, team 13 of the CRCINA is developing news approaches to treat cancer: RIT. In order to potentialize the efficacy of RIT, we decided to study the combination of RIT with adoptive transfer of tumor specific T-lymphocytes to boost anti-tumor immunity. This combination treatment was assessed in the C57BL/KaLwRij /5T33 MM immunocompetent murine model. 5T33 is a murine MM cell line, which expresses CD138. This cell line has been transduced to express ovalbumin (OVA) and is therefore called 5T33-OVA. RIT was performed using an anti-CD138 antibody radiolabeled with bismuth-213, an α-particle emitter. ACT consisted in the injection of *in vitro* activated CD8+ T-lymphocytes specific for OVA (OT-I T-cells). We have shown *in vitro* that OT-I are highly specific for 5T33-OVA cells. In mice engrafted with 5T33-OVA MM cells and treated with the combination treatment α-RIT + ACT, we observed a significant inhibition of tumor growth and increased survival compare to the mice treated with α-RIT alone or ACT alone (Ménager et al. 2015).
Using flow cytometry, we have demonstrated that the transferred OT-I T-cells migrate to the tumor site. Animals receiving the combined treatment were the only one to exhibit a stimulation of their immune system, as shown by a delayed tumor growth and a larger immune cell infiltrate within the tumor. Overall, our data demonstrated efficacy of the combination treatment (Ménager et al. 2015).

This preclinical study provided encouraging results with this type of therapeutic combination to treat cancer. It also shows that ACT alone is inefficient to control tumor development *in vivo* compare to α-RIT + ACT. However, all the data related to the biodistribution and behavior of injected T- cells implied the sacrifice of animals and sampling of organs for analysis.
Therefore, the aim of this project is to develop a strategy to follow the fate of T-cells after ACT using non-invasive modalities. The second objective will be to perform a real time follow-up of the injected cells, their biodistribution and thus getting access to new informations. Finally the development of such *in vivo* imaging strategy could also be applied to follow any type of cell after injection.
In order to follow T-lymphocytes by intravital imaging on live animals, we already initiated several cell labeling approaches. To this end, we analyzed diverse parameters of the labeling: impact on cell viability (toxicity), efficacy and intensity, stability and consequences on the cell function. In a research project funded by the LabEx IGO (immunology graft and Oncology), we started to evaluate 3 different methods to label T-cells (fig1):

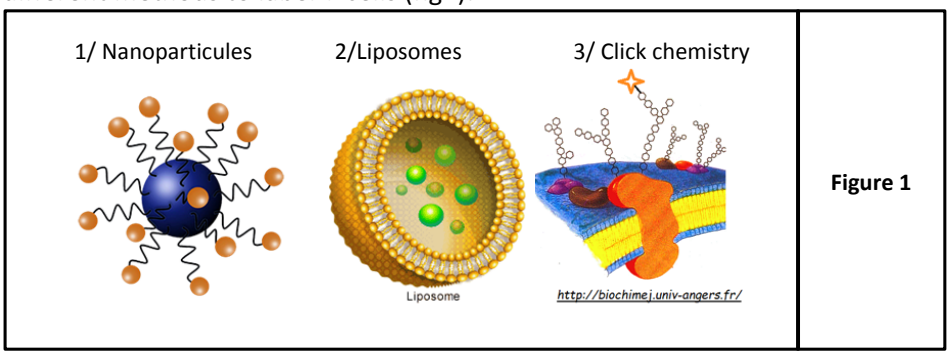


Figure 1

1) Using magnetic and fluorescent nanoassemblies (fluo@mag) produced by Pr Ishow's team (CEISAM - UMR CNRS 6230 / University of Nantes),
2) Using fluorescent liposomes produced at the CRCINA in our team by Dr M. Mougin-Degraef,
3) Using metabolic engineering of modified substrates (azido-sugars) and click-chemistry
The three labeling approaches have been assessed. So far, only liposomes and click chemistry have provided labeling results, which fulfill the parameters, we initially defined. Indeed T-lymphocytes labeling is stable enough to allow easy detection *in vitro* for 5 days and the cells remain highly specific and cytotoxic for the tumor cells (Fig 2 for liposome and Fig 3 for click chemistry)

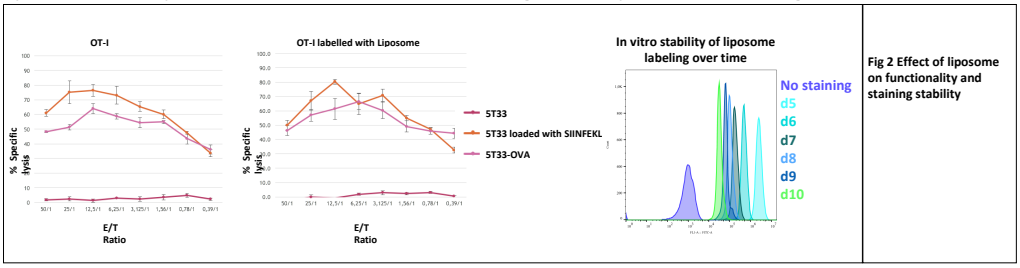


Fig 2 Effect of liposome on functionality and staining stability

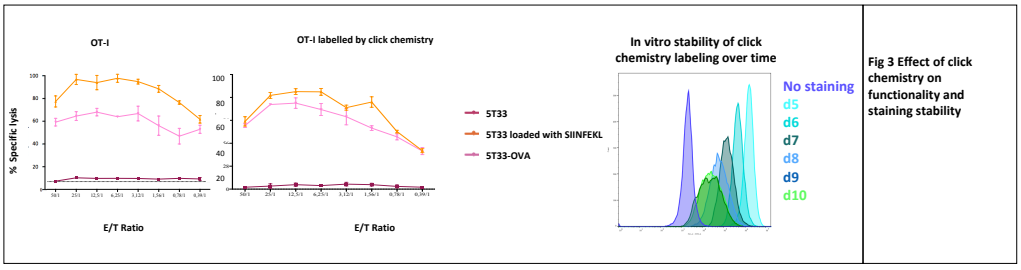


Fig 3 Effect of click chemistry on functionality and staining stability

We then wanted to define the time that OT-I T cells had to reach the tumor in-vivo. Mice were injected with OT-I T cells labeled by liposomes. Two types of tumors were grafted orthotopically: 5T33 or 5T33-OVA multiple myeloma cells. The mice were sacrificed at different times. Tumors were retrieved to detect the presence of OT-I T cells. The results are presented in Figure 4 and show that OT-I T cells can be detected ex-vivo 48h after injection until 72h.

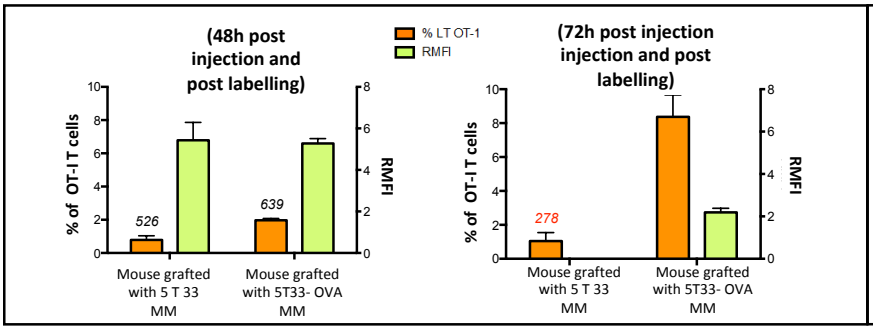


Fig 4: Ex vivo OT-I T cells détection in tumors by cytometry

The same experiment will be performed soon with OT-I T cells labeled by click chemistry and injected in vivo in mice grafted with multiple myeloma.

In the last part of the project, the biodistribution of OT-I T cells will be performed with the radioactive tracer. For the 1-step strategy, the clickable tracer or the liposome will be labeled with the long-lived isotope zirconium-89 ($T_{1/2}$ = 78h) to allow detection several days after injection of the labelled T-cells. In this case, the clickable tracer will be ligated to the engineered T-cells **before** their injection. For the two-step strategy, T-cells were firstly injected and the fast-clearing radioligand is then injected in a second step, after allowing sufficient distribution of T-cells. In this case, a shorter half-life isotope could be used (Copper-64, $T_{1/2}$ = 12.4h). The kinetics of distribution of the injected T lymphocytes will be evaluated by nuclear imaging performed on a microPET / CT (CIMA imaging Platform). This final imaging study will allow us to access to a semi-quantitative approach to tumor infiltration by T lymphocytes and their precise distribution in whole-body imaging. The possibility to visualize homogeneity degree of engineered T-cells distribution within the tumor will also be investigated.

Oral presentation

Workshop Canceropole GO, 15-16 March 2018

Lucine Marotte (PhD student in Team ≠3): “Inactivation of PDCD1 gene in melanoma specific CTL clones

Collaborations

Locally: Pr Elena Ishow' CEISAM- UMR CNRS 6230 Nantes,

National level: Dr Emmanuel Donnadieu INSERM 1016 - Institut Cochin Paris,

International level: reinforced our collaboration with Dr Alfred Morgenstern and Dr Frank Bruchertseifer.

Perspectives of clinical or economical valorization

Developing *in vivo* cell tracking tools will be useful to our team as well as other LabEx partners in different settings to study the interaction between immune cells (dendritic cells, T lymphocytes and macrophages) and tumors by nuclear molecular imaging techniques. Since the field of nanomedicine represents nowadays a momentous turn in terms of treatment and diagnostics, studying the fate of functional nanoparticles in vivo will bring essential information that can be shared for the future clinical transfer of innovating theranostic tools.

Knowledge about the effects of ionizing radiation on immune cells and/or tumor microenvironment may find potential applications in the context of clinical protocols.

Added value of / for Labex IGO

The Labex IGO helped us to rise other funding:

- Ligue contre le cancer, 2015 grant: 30.000 €
- Grant for thesis: Bourse Ministere enseignement supérieur for 3 years The Labex IGO helped us to rise other funding (Ligue contre le cancer) and to prepare new grant dedicated to Bioorthogonal chemistry – Next project)

WP3: COMBINED THERAPIES (NEW IMMUNODEPLETING AND IMMUNOMODULATING STRATEGIES WITH BROADER INDICATIONS AND ENHANCED EFFICACY

(coordinator: J.F. Fonteneau)

WP3 aims at evaluating (i) the influence of novel drugs on tumor cell death and tumor-infiltrating immune cells (Task 6) and (i) the impact of manipulating/targeting immune cells to increase the efficacy of conventional or immune-based therapies (Task 7).

The ongoing project in P6 is based on the impact of original drugs on (i) the nature of cell death and acquisition of resistance and (ii) on the phenotype and functions of effector cells; one important aspect of the Task is on the induction of immunogenic cell death and the consequences on establishing memory antitumoral T cell responses.
Even though promising, most immunotherapies are faced to limited results, probably as a result of using one strategy. Accumulating evidence shows that manipulating the immune system may enhance the efficacy of conventional treatments. The impact of targeting regulatory immune cells/mediators on the therapeutic benefit is clearly demonstrated in tumors. Task 7 thus encompasses projects that aim at designing optimized depleting or immumodulating protocols with broad indications.

Task 6 – Apoptosis and Immunity
The project "Influence of cancer cell death induced by chemo- and/or radio- therapies on the tumor and immune cells", supervised by M Vallette et PF Cartron, started in October2012. This project is focused on the epigenetic modifications induced by PGE2, a potent immunomodulatory molecule released in the microenvironment after chemo- and/or radio- therapies, on glioma cells and immune cells (B and T lymphocytes, pDC). This project allowed identifying new molecular pathways of the PGE2-induced epigenetic modifications and of molecular actors governing the methylation status of genes encoding co-stimulatory and co-inhibitory molecules.
In term of scientific production, two articles have been submitted and one manuscript is in preparation. In addition to strengthening intra-LabEx collaborations, this project also allowed to establish national and international collaboration as well as privileged collavboration with a start-up in development.

Task 7 - Innovative strategies to enhance the efficacy of immunomodulating and immunodepleting therapies
Three projects are supported by the LabEx IGO in Task 7.

Task 7.1: CD138 Radiotargeting and Immunostimulation
This project, including 3 teams of the CRCNA, aims at assessing the relevance of combining radioimmunotherapy (RIT) using a radiolabelled anti-CD138 mAb, with different approaches to stimulate immunity, in a syngeneic murine model of multiple myeloma (MM). Results obtained on the combination of RIT with an IL-15 superagonist have been published in Frontiers in Medicine. An in vivo model of MM, set up within this program, allowed to demonstrate that transferring specific T cells increases the efficacy of RIT (PLoS One; 2015). The third aspect of the project (combining vaccination and RIT) has been postponed as a consequence of delays in producing vaccine.
In addition to 2 articles and 1 review already published, 2 articles are in preparation. This project allowed strengthening national and international collaboration. This project allowed getting funding from charities.

T7.2: Enhancing the efficacy of anti-cancer vaccines using immunostimulants

This project, started in November 2013, is mainly driven by D Valmori (UMR Inserm 1102), in collaboration with two teams of the CRCNA. The main objective is to assess spontaneous immunogenicity of selected cancer testis antigens (CTA) in non-small cell lung cancer (NSCLC). Results evidenced humoral and cellular XAGE-1b-specific responses in some patients. This data supports the relevance of XAGE-1b in caucasians NSCLC patients with adenocarcinoma, and the implementation of future immunotherapies. Results have been submitted for publication to PLoS One (in revision).

For internal strategic reasons, this project is redirected on the study of the expression and function of PD-1/PD-1L in ovarian cancer (as compared to lung cancer); discussions with the partners have been initiated to redirect this collaborative project.

T7.3. Melanoma vaccination: coupling optimized long peptides to a viral protein that targets dendritic cells and favors cross-presentation

This project, leaded by F Lang, is developed by two CRCNA teams. The rational of this project, initiated in october 2014, is to combine selected synthetic long peptides (SLP) containing both CD4 and CD8 epitopes, with vaccination (coupling SLP to vaccine vehicles selected on their capacity to induce cross-presentation of coupled antigens). Preliminary results are very encouraging and the in vivo evaluation of the candidate SLP, combined or not to a vaccine vehicle, will be performed shortly (use of HLA-A2/DR1 transgenic mice).

A manuscript describing the relative capacity of synthetic SLP in stimulating both CD4+ and CD8+ T cells clones is under preparation. This project also allowed strengthening national collaboration on SLP and optimization of vaccine vehicles. Patenting linker optimization of SLP is in progress.

T6.1 CANCER CELL DEATH AND MODULATION OF THE TUMORAL NICHE

Title: Influence of cancer cell death induced by chemo- and/or radio- therapies on the tumoral and immune cells

Coordinators: FM Vallette and PF Cartron

Teams involved: Team 9 UMR1232

Staff funded by Labex IGO: Romain Pacaud (PhD Student) *(1/2 salary from Labex IGO +1/2 salary from MENR)* (2012-2015)
Mélanie BEZARD (master 1 Student)

Initiation of the project: October 2012
End of the project: Février 2016 (=thesis defence of R Pacaud).

State of the art
Our research was focused on:

- the epigenetic modifications occurring in glioma cells, lymphocytes T, B and pDC and induced by PGE2 (since PGE2 is release in tumor microenvironment after chemo- and/or radio-therapies. The novelty of this project is due to the identification of molecular pathways of the PGE2-induced epigenetic modifications occurring in glioma cells, lymphocytes T, B and pDC.
- the identification of molecular actors governing the methylation status of CTA and/or genes coding for the positive and negative co-stimulation molecules. The novelty of this project is associated with the fact that DNMT inhibitors currently available induced the demethylation/reactivation of CTA (MAGEA-A1, XAGE,…) and positive co-stimulation molecules (ICOSL,…) but also the demethylation/reactivation of negative co-stimulation molecules (PD-L1,…).

Objectives
The goal of our project is:

- the identification of molecular pathways of the PGE2-induced epigenetic modifications occurring in glioma cells, lymphocytes T, B and pDC.
- The identification of molecular actors governing the methylation status of CTA and/or genes coding for the positive and negative co-stimulation molecules to develop against these actors a selective epidrugs (Our team already publishes several articles supporting this point (PMID: 25111481 and 23809695)).

Project progression / Results
Our studies have permitted to determine that:

- PGE2 influence apopto-resistance phenotype through the histone H3 phosphorylation (publication#1).
- The PGE2/mPGES autoamplification loop seen in tumor and microenvironment cells is dictated by a dynamic methylation/demethylation process of mPGES gene (publication#2).
- PGE2 modulates the methylome/demethylome machinery of tumor and micro-environment cells (publication#3).

The development of the project "identification of molecular actors governing the methylation status of CTA and/or genes coding for the positive and negative co-stimulation molecules" permits today to:

- Validate the idea that epidrugs of 1st and 2nd generations (decitabine and procainamide) can promote the demethylation/expression of CTA and positive co-stimulation molecules but

also the demethylation/expression of negative co-stimulation molecules. Thus, these drugs can promote opposite effects on the activation of NK cells against GBM cells. (publication#4).

- Identify DNMT/Protein-X complexes implicated in the epigenetic regulation of MAGE-A1, XAGE-3, LLT1 and other (in progress).

Publications

- PGE2 induces XIAP-mediated apopto-resistance phenotype in glioma cells through the MSK1-mediated histone H3 phosphorylation. Pacaud Romain, Nadaradjane Arulraj, Perruche Sylvain, Vallette François M and Cartron Pierre-François (submitted).

- AP2 ✔/TET2-directed DNA demethylation and 5-hydroxymethylcytosine-dependent recruitment of Pax5 on mPGES-1 promoter are two processes governing the mPGES-1/PGE2 amplification loop. Pacaud Romain, Nadaradjane Arulraj, Cartron Gwenola, Briand Joséphine, Oliver Lisa, Vallette François M and Cartron Pierre-François (submitted).

- PGE2 controls the methylation level of PD-L1 promoter in B cells through TET2-DNMT3L-Elk1 complex. Pacaud Romain, Nadaradjane Arulraj, Cartron Gwenola, Perruche Sylvain, Vallette François M and Cartron Pierre-François (in preparation).

- Effect of decitabine on expression of positive and negative co-stimulation molecules in NK and glioma cells. Bézard Mélanie, Cartron Gwenola, Nadaradjane Arulraj, Vallette François M and Cartron Pierre-François (in preparation).

Collaborations

In addition to "intra-Labex" collaborations, we have established 3 collaborations outside to the Labex perimeter with Dr S Perruche (Université de Besançon), Dr C De Smet (Université de Louvain, Belgique) and Dr AR Karpf (Nebraska University, USA).

Perspectives of clinical or economical valorization

The development of our two research programs laid the foundations for the identification of molecular actor of governing the methylation/expression of CTA and of positive and negative co-stimulation molecules of immune system (PDL1/PD1, OX40L/OX40, ICOSL/ICOS,….). Thus, the detection of these actors as biomarkers could permit:

- to identify patients likely to respond to therapies using anti-PD1 blocking agent (clinical valorization).
- to identify epigenetic actors as targets for the development of epidrugs of third generation targeting selectively these actors. The development of this epidrugs could be realized via a putative collaboration with the star-up "EpiDrugs Discovery" (a start-up in development) and could generate the deposit of patent.

Added value of / for Labex IGO

The introduction of the epigenetic field in Labex-IGO permitted to:
- address questions of basic research on the epigenetic reprogrammation of immune cell.
- establish new international collaborations: Dr C De Smet (Université de Louvain, Belgique) and Dr AR Karpf (Nebraska University, USA).
- organize an "immunology session" in the EpiNantes-2015 meeting.

T7.1 CD138 RADIOTARGETING AND IMMUNOSTIMULATION

Coordinator: Joëlle Gaschet

Teams involved: CRCNA Team 6 (Yannick Jacques)
CRCNA Team 7 (Yves Delneste)
CRCNA Team 13 (Michel Chérel)

Staff funded by Labex IGO: Fichou Nolwenn, post-doctoral fellow

Initiation of the project: 01/01/2013
End of the project: 31/12/2015

State of the art

Even though RIT has demonstrated its efficacy in some hematological malignancies (e.g. B lymphoma), combination strategies are necessary to improve its efficacy and to limit toxicity of this approach. This project is original since it is the first project assessing different type of immunotherapies in combination with RIT. T-cell transfer is a well know type of immunotherapy but the RLI molecule and vaccination with tumor antigen coupled to carrier proteins are also quite novel approaches.

Objectives

The project aimed at studying the relevance of combining radioimmunotherapy (RIT) using a radiolabelled anti-CD138 mAb, with different approaches to stimulate immunity, in a syngeneic murine model of multiple myeloma (MM). The working program included 3 different combination treatments :

1) RIT followed by injection of RLI, an interleukin-15 agonist
2) RIT followed by adoptive transfer of tumor specific T-cells
3) RIT followed by vaccination with tumor antigen coupled to carrier proteins

Project progression / Results

The first part of the project has been entirely developed and completed by the post-doc fellow hired by the Labex. The results obtained show that combining RIT and RLI is very efficient (70 % long term survival of the animals) compare to RIT alone (42% survival) and to RLI alone (no efficacy). Impact of the RIT and/or the RLI on the immune cells and immune system was performed. A manuscript is in preparation. In parallel, a study comparing two types of RIT to optimize the combined treatment was performed and published in Frontiers in Medicine in 2015.

A tumor model had to be set up to perform the second and third part of the project, meaning that we had to modify our existing MM model in order to have a tumour that express one specific antigen which could be recognized by tumor specific T-cells and which could also be used for the vaccination approach. The modified tumor model has been set up by a PhD student who also developed the second part of the project. The results show that combining RIT and transfer of tumor specific T-cells improve animal survival compared to RIT alone or specific T-cells alone (median survival = 101.5 vs. 70 and 60.5 days respectively). Only the animals receiving the combined treatment exhibited stimulation of their immune system with production of IL-1α pro-inflammatory cytokine. Finally, in the animals treated with combined treatment, the transfered T-cells showed a better migration in the lymph nodes and a better tumor cell recognition of compared to mice treated with T-cells alone. Setting up the tumor model was published in Plos One in 2015, the rest of the study is the subject of a manuscript in preparation. In parallel, a review describing interconnection between ionizing radiation and the immune system was written and is in press in Médecine & science.

The third part of the project has been postponed. Modifying our existing MM model was more difficult and time consuming than expected. Time was also needed to optimize the vaccine production. Both aspects forced us to delay this study.

Publications

- Ménager J et al. Plos One. 2015 Jun 22;10(6):e0130249. doi:10.1371/journal.pone.0130249
- Fichou N et al. Front. Med (Lausanne). 2015 Nov 4;2:76. doi: 10.3389/fmed.2015.00076
- Ménager J et al. Médecine & Science. 2016 *in press*
- Ménager J et al. *in preparation*
- Fichou N et al. *in preparation*

Collaborations

Through the Labex IGO, collaborations have been established:
- locally with different IGO partners (CRCNA teams 6a, 7, 3, 1)
- at the national level with Pr E. Tartour, PARCC-INSERM UMR_S970
- at the international level, this project reinforced our collaboration with the Institute for transuranium elements in Karlsruhe, Germany

Perspectives of clinical or economical valorization

These preclinical studies shall open interesting therapeutic perspectives in the context of MM that could extend to other malignant pathologies. Indeed, the possibility to raise an anti-tumor immune response with RIT combined to different immune-based strategies would provide the advantage to set up an immunologic memory that could benefit the patient facing a cancer recurrence and to adapt to the genomic instability of tumor cells.

Added value of / for Labex IGO

The Labex IGO helped us to raise other fundings: Ligue contre le cancer, 2013 grant: 35.000 €

T7.2: ENHANCING THE EFFICACY OF ANTI-CANCER VACCINES USING IMMUNOSTIMULANTS OR/AND INHIBITION OF IMMUNOSUPPRESSION: A PRECLINICAL IN VITRO PRIMING MODEL

(THIS PROJECT WAS NOT FINISHED; FUNDING WAS HANDED BACK TO LABEX AND WILL BE USED IN THE LAST CALL IN 2018)

Coordinator: Danila Valmori

Team involved: UMR1102

Staff funded by Labex IGO: Sardaro Alessandro (PhD student) November 2013-January 2015
Pascal Aumond (Engineer : IE) March 2015-December 2015

Initiation of the project: November 2013
End of the project: November 2016

State of the art / Objectives

Assessment of the spontaneous immunogenicity of selected cancer testis antigens (CTA) in non-small cell lung cancer (NSCLC). Lung cancer is the leading cause of cancer-related mortality worldwide, with non-small cell lung cancer (NSCLC) accounting for approximately 85% of all lung cancer cases. Despite recent improvements in therapeutic strategies, NSCLC constitutes therefore one of the major public health problems. Emerging immunotherapeutic strategies for cancer, including NSCLC, are those using checkpoint blockade specific antibodies that have shown clinical efficacy in subgroup of patients. Recent data suggest that these responder patients are those that harbour spontaneous immune responses to the autologous tumor. Other immune based strategies in NSCLC include cancer vaccination approaches using CTA proteins encoded by genes normally expressed in germ cells in testis and fetal ovary and in some cases in placental trophoblasts, silenced in normal adult tissues, but aberrantly re-expressed in various types of cancer. Some CTA are highly immunogenic and are therefore considered among the most attractive targets for the development of cancer vaccines. Cancer vaccines as monotherapy are currently under evaluation in NSCLC and could be particularly effective in patients with minimal residual disease. Despite the first clinical trials applying this type of strategy have not met their clinical endpoint, combination of vaccination with checkpoint blockade therapies are very promising. However, the use of these strategies requires the identification of tumour specific antigens expressed by and immunogenic in a significant fraction of NSCLC.

XAGE-1b the main transcript expressed in tumours is encoded by the XAGE-1 gene that is located in the Xp11.22 region of the X chromosome. XAGE-1b encodes an 81 amino acids long protein. XAGE-1b has been reported reported to induce antibody and T cell responses in Japanese NSCLC patients. The purpose of our study was to extend these findings to the Caucasian population to evaluate the potential of XAGE-1b based immunotherapy in Caucasians.

Project progression / Results

We assessed the presence of XAGE-1 specific antibodies in a cohort of 141 Causasian patients with NSCLC using a full length synthetic XAGE-1b protein. We found spontaneous serological responses to XAGE-1b in 9% of the patients. Importantly, these responses were limited to and represented 13% of patients with adenocarcinoma tumors, the most frequent histological subtype, for which immunotherapy approaches are under development. Using a set of overlapping peptides spanning the entire XAGE-1b protein, and in support of the serological data, we detected significant XAGE-1b specific $CD4^+$ T cell responses in all XAGE-1b seropositive patients and identified several $CD4^+$ T cell epitopes. Altogether, our results support the relevance of XAGE-1b in Caucasians NSCLC patients with adenocarcinoma, and the implementation of future immunotherapies exploiting the high immunogenicity of the antigen, in this patient population.

T7.3. MELANOMA VACCINATION : COUPLING OPTIMIZED LONG PEPTIDES TO A VIRAL PROTEIN THAT TARGETS DENDRITIC CELLS AND FAVORS CROSS-PRESENTATION

Coordinators: François Lang (Partner 1) /P. Jeannin (Partner 2)

Teams involved: CRCINA team 3 (N. Labarrière), CRCINA team 7 (Y.Delneste)

Staff funded by Labex IGO: Laetitia Florenceau, (Engineer Assistant: AI)

Initiation of the project: October 2014
End of the project: December 2017

State of the art

Numerous class I and class II T cell epitopes from tumor antigens have been characterized and exploited in various vaccination protocols. Initial trials revealed that relying solely on short class I epitopes to activate CD8 T lymphocytes failed to elicit strong clinical responses while the simultaneous recruitment of CD4 T cells greatly enhanced vaccine efficacy. Thus, recent vaccination trials use synthetic long peptides (SLP) to activate both CD4 and CD8 T cells. In most instances, the long peptides used consisted in a mix of selected class I epitopes extended (1) to require DC internalisation for presentation and (2) to contain an undefined class II epitope. However, on native tumor antigens, the immunodominant class I and class II epitopes can be either separated by hundreds of amino acids or on the contrary, overlap which could impair their processing efficiency.

Objectives

The principal objective of this program was to design synthetic long peptides from melanoma tumor antigens

i) that can induce an efficient activation of both CD4+ and CD8+ specific T cells following uptake by DC *in vitro* and
ii) that can generate anti-tumor CD4+ and CD8+ T cell responses *in vivo* in a preclinical mouse model.

This will be explored following two paths :

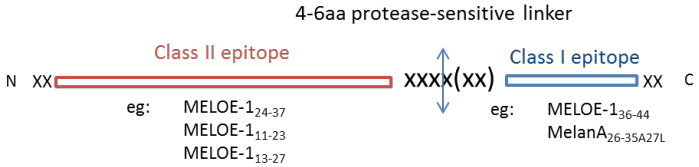
i) the design of an optimal linker between the class I and II epitope

the exploration of the potential advantage of coupling SLP to NS3 (hepatitis C virus protein non-structural protein 3) to enhance DC presentation.

Project progression / Results

Task 1: Design, testing and choice of a long peptide from MELOE-1 (Partner 1)

We have tested over 40 different SLP comprising various linkers for their capacity to stimulate both CD4+ and CD8+ T cell clones (see figure below). We tested 3 different class II epitopes and 2 class I epitopes from MELOE-1 tumor antigen and 1 class I epitope from melanA/MART1 tumor antigen. We show that the nature of the linker used to connect the class II and the class I epitope is critical for optimised antigen presentation by DC and have identified an optimal linker sequence that improves cross-presentation in all the tested epitope combinations.



SLP: 30-35aa

Figure1: Schematic representation of the SLP used in this study.

Task 2 : Production of chimeric NS3-SLP and Task 3 : Comparison of SLP and NS3-SLP on DC maturation and on T cell clone stimulation (Partner 1 and 2)
Dr. Piller has provided us with 2 batches of chimeric NS3-SLP produced in recombinant E.coli showing variable batch to batch activities on T cell activation. We think that this was most likely due to the fact that the chimeric protein had the tendency to aggregate, which influenced the route of internalization of the antigen. We did not have easy technical solutions to solve these issues in the course of this project. Moreover, this task is quite cumbersome as it requires a new recombinant preparation for every peptide tested. For all these reasons, in the frame of this study, we decided to not pursue this axis.
Task 4 : Evaluation of the ability of SLP to prime naive T cells (Partner 1)
We confirmed that SLP optimized for cross-presentation are more efficient than native antigen to expand specific CD8 T lymphocytes from whole PBMC in vitro. We compared the optimized SLP to either the natural sequence or to a SLP designed with an unfavorable linker for their capacity to induce MELOE-1 or MelanA/MART1 positive microcultures (tested using HLA A*02/peptide tetramer complexes). We were able to amplify specific T cells for 5 donors (out of 6 tested), and in all of them the optimized SLP enabled increased frequencies of positive microcultures.
Task 5 : Immunogenicity of SLP and NS3-SLP in HLA-A2/DR1 transgenic mice (Partner 2)
In a humanized mouse model (HLA A*0201/HLA DRβ1*01), our data indicate that immunization with optimized SLP tends to increase cross-presentation in vivo. In 3 independent experiments, we compared the optimized SLP to either the natural sequence or to a SLP designed with an unfavorable linker for their capacity to induce MELOE-1 specific CD8+ T splenocytes (tested using ELISPOT assays).
Task 6: Production of a stable MELOE-1 expressing SARC-A2 cell line (Partner 1 and collaboration)
We set up a collaboration with Dr. Latouche (CHU Rouen) who provided us with this SARC cell line stably transfected with full length meloe. After cloning the bulk cell line, we tested its antigen presentation capacity to CD4+ and CD8+ cell clones. As expected, this cell line is highly recognised by CD8+ T cell clone although it lacks the capacity to present endogenous class II epitopes.
Task7 : Evaluation of the anti-tumor potential of MELOE-1 SLP vaccination in HLA-A2/DR1 mice.
Despite the fact that the sarcoma cell lines were prepared, and despite the fact that we have data showing that the optimized SLP do enhance immunogenicity compared to natural sequence, we did not have time to perform anti-tumor experiments in mice.

Publications and patents
We are in the process of filing a patent on the optimized linkers (INSERM transfert). This patent will get integrated in the portfolio of patents hold by our team leader Dr. Labarrière on class I and II epitopes issued from MELOE-1 antigen.
A manuscript describing all the different linkers tested and their relative capacity to stimulate both CD4+ and CD8+ T cells responses in vitro and in vivo is ready to be submitted for publication to Oncoimmunology as soon as the patent is filed.

Collaborations
Partner 2 had an ongoing collaboration with Dr. Piller (CNRS UPR4301, Orléans, France) to produce chimeric NS3-SLP (task 2).
Partner 1 has worked in collaboration with Dr. Olivier Adotevi (Université Bourgogne Franche-Comté, Besançon, France) to evaluate the immunogenicity of SLP in mice.
Partner 1 has also set up a collaboration with Dr. Latouche (CHU Rouen, France) who provided us with the murine fibrosarcoma SARC cell line transfected with the human HLA A*0201 and the human *meloe* cDNA.

Perspectives of clinical or economical valorization

InsermTransfert will hold the patent protecting the optimized linkers, along with other patents led by our team on Meloe-1 antigen. This project is providing a proof of concept and opens the way to vaccination protocols with an optimized SLP in metastatic melanoma patients. The exploitation of the linkers developed here would benefit from collaborations with other teams or companies developing vaccination strategies (vectorization, DC targeting…).

Added value of / for Labex IGO

Being granted through the Labex IGO gave us a good visibility and enabled us to recruit talented students to help us conducting this project. It also gave us the opportunity to develop collaborations through regular meetings and conferences. We hope that this Labex IGO funding and the success of this project will open the way to clinical development of SLP vaccination in melanoma patients.

T7.4. COMBINING ADOPTIVE T CELL TRANSFER OF ENGINEERED PD-1 DEFICIENT SPECIFIC T CELLS WITH A-RADIOIMMUNOTHERAPY FOR MELANOMA TREATMENT

Coordinator: Nathalie Labarrière

Teams involved: CRCINA UMR1232 Team #3: N.Labarriere, Team #1: E. Scotet, Team #13: J. Gaschet, Nantes
CRTI UMR1064, Team: TH. Nguyen, Nantes

Staff funded by Labex IGO: Marisa Fernandes Capitao, PhD student 36 months, Team ≠13
Sylvain Simon, Engineer, 18 months, Team ≠1
Malika Gantier, Engineer, 10 months, Team GenoCell Edit

Initiation of the project: October 2016
End of the project: September 2019

State of the art

Since 5 years, melanoma is at the forefront of the development of innovative therapies with molecular targeted therapies, adoptive cell transfer and immune checkpoint inhibitors as cornerstones of treatment. New therapeutic strategies like immune checkpoint blockers, such as anti-PD-1, proved to prolong patient survival within clinical trials, and this treatment has already entered routine clinical use. However, there is still a significant fraction of patients who do not respond to these treatments. This clearly justifies the development of innovative combined approaches in order to enhance the therapeutic efficacy of melanoma treatments. Therefore, two main issues that aim at improving the clinical outcome of melanoma patients enrolled in immunotherapy protocols have been identified:

1/ The functional properties of tumor-specific T cells infused in Adoptive Cell Transfer (ACT) treatments should be optimized, especially concerning the expression of inhibitory receptors that could impair the anti-tumor activity of specific T cells *in vivo* (such as PD-1)

2/ The anti-tumor activity of infused T cells in ACT needs to be enhanced against established tumors *in vivo*. This could be achieved by prior modifications of tumor microenvironment parameters.

In this project, we propose to achieve these goals by combining ACT with highly reactive melanoma specific $CD8^+$ ab T cells inactivated for PD-1 gene expression, and a-Radioimmunotherapy (a-RIT), in a mouse melanoma model.

Objectives

The overall objective of this research program is to assess, through a set of complementary *in vitro* and *in vivo* experimental approaches (eg, NSG mice engrafted with PDL-1 expressing human melanoma tumors), the anti-tumor efficacy of an innovative treatment combining ACT, with high avidity $PD\text{-}1^{ko}$ melanoma specific T lymphocytes, and α-RIT. This will be achieved through the implementation and the completion of the following milestones:

1/ **Silencing of PDCD1 gene in high-avidity human melanoma-specific CD8+ αβ T lymphocytes**. This step will be achieved by transfection or transduction of T lymphocytes with PDCD1-specific gRNA and Cas9. The design and validation of these tools will rely on the expertise of the local GenoCell Edit facility (TH. Nguyen), specialized in this technology. The selection, amplification and in vitro validation of PD-1 inactivated melanoma-specific CD8+ αβ T cells will be performed by Team #3.

2/ **Anti-tumor efficiency of engineered CD8+ αβ T cells *in vivo***. The efficiency of engineered $PD\text{-}1^{neg}$ melanoma specific T cells to control the growth of human PDL-1 expressing melanoma tumors will be measured in NSG mice, and compared to that obtained with *wild-type* counterpart T cells (Team #3 and Team #1).

3/ **Development of radiolabeled anti-PDL-1 mAb**. A commercially available anti-human PDL-1 specific mAb will be conjugated and labeled with alpha (for therapy) or beta (+) (for molecular imaging) radionuclides (Team #13).
4/ **Anti-tumor efficiency of PDL-1-specific α-RIT *in vivo***. The objective is to document in NSG mice engrafted with human PDL-1 expressing melanoma tumors the efficiency of α-RIT targeting PDL-1 on tumor growth and to characterize the expression of PDL-1 on remaining or resistant melanoma cells (Team #1 and Team #13).
5/ **Anti-tumor efficiency of combined ACT and α-RIT strategies *in vivo***. The impact of α-RIT immunotherapy targeting PDL-1 molecules expressed on melanoma cells, prior infusion of PD-1^{ko} specific T cells will be assessed in NSG mice carrying human melanoma tumor grafts (Team #3, Team #1 and Team #13).

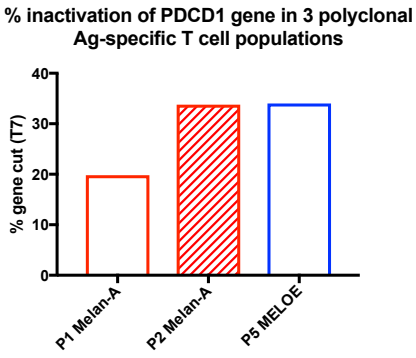
Project progression / Results
The research program achieved the following results :
Objective 1 of the program
Silencing of *PDCD1* gene in high-avidity human melanoma-specific CD8$^+$ T lymphocytes.
1) We selected a sgRNA for PDCD1 inactivation (**Figure 1**) and succeeded to inactivate *PDCD1* gene in effector-memory antigen-specific T lymphocytes upon transfection of a complex CAS9/sgRNA RNP, a technology set up with partner ≠4.

Figure 1 : PDCD1-P sgRNA, located in exon 1 of PDCD1 gene

PD1 Ex 1 : ACAGTTTCCCTTCCGCTCACCTCCGCCTGAGCAGTGGAGAAGGCGGCACTCTGGTGGGGCGCTCCAGGCATTGCA
GATCCCACAGGCGCCCTGGCCAGTCGTCTGGGCGGTGCTACAACTGGGCTGGCGGCCAGGATGGTTCTTAG
sg PD-1.9 *ACAGGCGCCCTGGCCAGTCG*

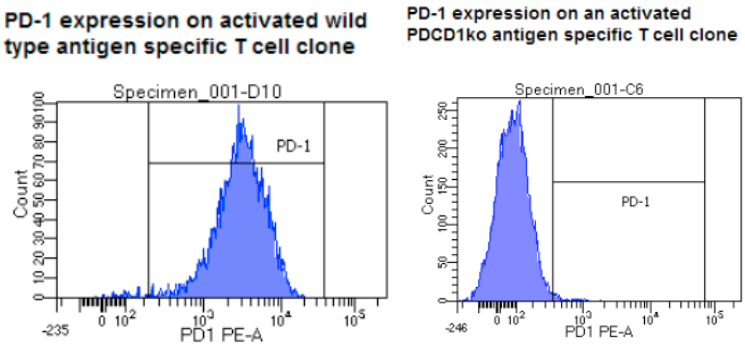
2) We started with polyclonal T cell populations, specific for Melan-A/A2 epitope and with high functional avidity. After transfection of CAS9RNP, we obtained between 20% and 30% of gene inactivation, measured by endonuclease T7 assay (**Figure 2**).

Figure 2:



3) In order to work with homogeneous T cells inactivated for PD-1 expression, we cloned these populations by limiting dilution. We derived at least 10 PD-1 negative T cell clones from the first population, and the other populations will be cloned in the next months.
We selected T cells clones that did not expressed PD-1 upon activation (assessed by Flow cytometry), and wild-type T cell clones highly expressing PD-1 upon activation (**Figure 3**). The inactivation of PDCD1 gene in these T cell clones was also confirmed in a T7 endonuclease assay.

Figure 3 : PD-1 expression on a wt and a PD-1 inactivated T cell clone



We are currently testing the reactivity of these T cell clones on melanoma cell lines expressing PD-L1 and Melan-A antigen, and their relative functional avidities on the cognate antigen. PDCD1 gene will be sequenced in all these T cell clones in order to assess whether the inactivation of *PDCD1* occurs on both alleles or not. Finally, partner ≠4 identified *in silico* nine potential off-target genes for the chosen sgRNA and documented in bulk populations that none of these genes were affected by *PDCD1* inactivation, through a T7 endonuclease assay.

We will now derive additional PD-1^{ko} and wild-type specific T cell clones from the other specific T cell populations, and we will compare their transcriptomic profile, along with their phenotypical and functional features *in vitro* and *in vivo*.

Objectives 3 and 4 of the program:

1) Efficient coupling and radiolabeling of anti-human PD-L1 mAb with alpha-radionuclides: astatine-211 and bismuth-213, have been set up.

2) Dose escalation studies have been performed with the anti-human PD-L1 mAb radiolabeled with astatine-211 and bismuth-213 on tumor free NSG mice. As a result we determine the maximum tolerated doses (MTD) for each radionuclide. The dose limiting toxicity (DLT) inducing major hematologic toxicity were also defined. Animal serum and organs were collected to analyze bone marrow toxicity as well as liver and kidney toxicity. Based on these samples no major toxicity to the bone marrow, liver or kidney was observed at the defined MTD.

3) Biodistribution study has been done with astatine-211-anti-human PD-L1 mAb in NSG mice engrafted with PD-L1 (+) M113 melanoma. We did not observe any major binding of the radiolabeled mAb in the organs except lungs and spleen. In the tumor, binding increased over time after radiolabeled mAb injection attesting of specific tumor targeting.

4) Therapy studies have been initiated at first with an immunotherapy experiment using non radiolabeled anti-human PD-L1 mAb to assess the antibody itself could impact tumor development. 7 days after subcutaneous engrafment with PD-L1 (+) M113 tumor, NSG mice have been treated with either 20 or 100 µg of anti-human PD-L1 mAb. Results were similar in both treated groups compared to control group injected with PBS. Tumor volume increased progressively until reaching limiting volume leading to the sacrifice of the mice. This experiment demonstrates that in this preclinical model, non radiolabeled anti-PD-L1 mAb : i) has no therapeutic effect on PD-L1 (+) M113 melanoma tumor, ii) doesn't induce any short term toxicity. Therapy studies have been recently initiated with astatine-211 and bismuth-213 radiolabeled anti-human PD-L1 mAb. Treatment efficacy will be assessed in the next few months in terms of tumor growth inhibition, animal survival and treatment toxicity.

5)In order to analyze tumor and tumor microenvironment after α-RIT and after α-RIT and adoptive T-cell transfer, tumor collection and immunofluorescence staining have been optimized.

6) Development of *in vivo* imaging has been delayed for a few months waiting for our new imaging platform, CIMA, that gather a small animal PET-CT and a new PET-MR, get open at the nearby University Hospital of Nantes (CHU).

Publications
No publication
Oral presentation (Workshop Canceropole GO, 15-16 March 2018)
Lucine Marotte (PhD student in Team ≠3): "Inactivation of PDCD1 gene in melanoma specific CTL clones

Collaborations
This joint research program strengthened the collaborations between Teams #1, Team #13 (supervision of the PhD) and Team #3 from CRCINA. *In vivo* experiments first relied on interactions with Dr B. Vanhove (INSERM UMR 1064 ITUN). A collaboration with the group of Dr L. Dubreil (Oniris, Nantes) is currently established (bi-photon microscopy) to analyze both *in vivo* localization of injected human effector cells, the impact of radio-therapeutic lines linked to the efficacy of optimized T cell effectors.

Perspectives of clinical or economical valorization
The project aims to provide key insights into the improved clinical outcome of melanoma patients enrolled in immunotherapeutic lines. The functional properties of tumor-specific T cells infused in Adoptive Cell Transfer (ACT) treatments is expected to be optimized, especially concerning the expression of inhibitory receptors that could impair the anti-tumor activity of specific T cells *in vivo* (e.g., PD-1). Moreover, this research program should help improving the anti-tumor activity of infused T cells in ACT that needs to be enhanced against established tumors *in vivo*.

Added value of / for Labex IGO
The efficient and rapid development of animal models of human tumor xenografts and cellular immunotherapies, crucially supported by the Labex IGO, has led to the establishment of recent collaborations with local (e.g., Teams #3 and #8 from CRCINA, INSERM U957 Nantes) and international teams (e.g., M. Eberl, Cardiff University, UK).

APPENDICES

Appendix 1: Scientific partners

Appendix 2: Research staff dedicated to Labex IGO

Appendix 3: Support staff dedicated to Labex IGO

Appendix 4: Finances

Appendix 5: Publications

Appendix 6: Patents

Appendix 7: Summary of thesis defended by PhD students funded by Labex IGO

APPENDIX 1 : SCIENTIFIC PARTNERS

In 2018 Labex IGO gathers 13 teams (all positively evaluated by the HCERES in 2016) belonging to 4 research units located in the western part of France (Nantes, Rennes, Angers and Brest). They have an excellent track record in terms of publications, technology transfer and translational immunology.

ORGANIZATION	RESEARCH UNIT	RESEARCH UNIT FULL NAME	TEAMS INVOLVED IN LABEX IGO	TOPIC
INSERM-Université de Nantes	UMR1232	Center for Research in Cancerology and Immunology Nantes/Angers (CRCINA)	7 teams: Team 1. "Immunobiology of Human Alpha Beta and Gamma Delta T cells & Immunotherapeutic Applications" (E. Scotet) Team 2. "Clinical and translational research in skin cancers" (B. Dréno) Team 3. "Antitumor immunosurveillance and immunotherapy " (N. Labarrière) Team 4. "Immunogenic cellular death and mesothelioma therapy" (M. Grégoire) Team 7. **"**Innate immunity and Immunotherapy**"** (Y. Delneste) Team 9. "Apoptosis and tumor progression" (F. Vallette) Team 13. **"**Nuclear oncology**"** (F. Kraeber-Bodéré / M. Chérel)	Immuno-oncology Oncogenesis and nuclear oncology
INSERM-Université de Nantes	UMR1064	Center for Research in Transplantation and Immunology (CRTI)	4 teams : Team 1. **"**Dendritic cells and immunoregulation in transplantation and immunopathology **"** (MC. Cuturi / R. Josien) Team 2. **"**Genetic and cellular engineering in immunology and regenerative medicine**"** (I. Anegon / C. Guillonneau) Team 3. **"**Immunotherapy in transplantation and autoimmunity**"** (G. Blancho/B. Vanhove) Team 4. **"**Immunoregulation and immunointervention in transplantation and autoimmunity**"** (S. Brouard / D. Laplaud)	Immuno-transplantation and autoimmunity
INSERM-Université de Rennes I	UMR1236	Microenvironment, Cell Differentiation, Immunology and Cancer (MICMAC)	1 team (K. Tarte)	Immuno-oncology
Université de Bretagne Occidentale (UBO)	UMR1227	B Lymphocytes and autoimmunity (LBAI)	1 team (JO. Pers)	Immuno-transplantation and autoimmunity

APPENDIX 2 : RESEARCH STAFF DEDICATED TO LABEX IGO

Since October 2012, 44 people (representing 44 FTE) have been recruited to work on Labex IGO research projects:

- 16 PhD students (14 are beneficiaries of grants funded by Labex IGO (> 50%) and 2 are beneficiaries of grants funded by INSERM and regional councils (6 contracts are still ongoing)
- 7 post-doctoral research assistants (1 contract is still ongoing)
- 21 research staff (5 contracts are still ongoing) *(IR : ingénieur de recherche; IE: ingénieur d'étude; AI : assistant ingénieur; technicien; ADT: adjoint technique)*

Overall 12 contracts are still ongoing (● ended contract / ○ ongoing contract)

POSITION	NAME	RESEARCH UNIT / TEAM	PROJECT	CONTRACT DURATION	START	END	CONTRACT STATUS	GRANT
PhD Student								
	Q. Simon	UMR1227 (Brest)	T1.1.2	3 years	October 2012	Thesis defence : 13/11/2015	●	50% Labex IGO 50% MENR (UBO)
	R. Pacaud	UMR1232-E9 (Nantes)	T6.1	3 years	October 2012	Thesis defence : 26/02/2016	●	50% Labex IGO 50% MENR (UN)
	CM. Peigne	UMR1232-E1 (Nantes)	T4.1.2	3 years	October 2012	Thesis defence : 24/02/2016	●	50% INSERM 50% Région PDL
	H. Symington	UMR1236 (Rennes)	T1.1.2	3 years	October 2012	Thesis defence : 11/03/2016	●	50% INSERM 50% Région Bretagne
	S. Simon	UMR1232-E3 (Nantes)	T4.1	3 years	October 2013	Thesis defence : 16/12/2016	●	100% Labex IGO
	A. Sardaro	UMR1102 (Nantes)	T7.2	14 months	November 2013	January 2015 (dropping out)	●	100% Labex IGO
	A. Mohr	UMR1227 (Brest)	T1.1.2	3 years	November 2013	Thesis defence : 17/10/2016	●	50% Labex IGO 50% Région Bretagne
	J. Gergen	UMR1064-E1 (Nantes)	T1.4	3 years	October 2014	Thesis defence : 15/12/2017	●	50% Labex IGO 50% MENR (UN)
	C. Chauvin	UMR1232-E1 (Nantes)	T4.3	3 years	October 2014	Thesis defence : 19/09/2017	●	100% Labex IGO
	L. Le Lann	UMR1227 (Brest)	T1.1.2	3 years	December 2014	*February 2018*	●	50% Labex IGO 50% Région Bretagne
	N. Hipp	UMR1236 (Rennes)	T1.1.2	3 years	October 2014	*September 2018*	○	50% Labex IGO 50% Région

								Bretagne
	L. Verdière	UMR1236 (Rennes)	T1.1.2	3 years	October 2016	*September 2019*	○	50% Labex IGO 50% Région Bretagne
	A. Freuchet	UMR1064-E2 (Nantes)	T1.7	3 years	October 2016	*September 2019*	○	100% Labex IGO
	TM. Doan Ngoc	UMR1232-E9 (Nantes) UMR1064-E4 (Nantes)	T1.8	3 years	October 2016	*September 2019*	○	100% Labex IGO
	M. Fernandes Capitao	UMR1232-E13 (Nantes)	T7.4	3 years	October 2016	*September 2019*	○	50% Labex IGO 50% MENR (UN)
	M. Boudigou	UMR1227 (Brest)	T1.1.2	3 years	October 2017	*September 2020*	○	50% Labex IGO 50% Région Bretagne
Post-Doc								
	M. Hill	UMR1064-E1 (Nantes)	T4.2.	6 months	November 2012	May 2013	●	100% Labex IGO
	N. Fichou	UMR1232-E13 (Nantes)	T7.1	2.5 years	January 2013	June 2015	●	100% Labex IGO
	N. Fichou	UMR1232-E13 (Nantes)	T5.1	9 months	Sept 2015	May 2016	●	100% Labex IGO
	U. Jarry	UMR1232-E1 (Nantes)	T4.1.2.	2 years	March 2013	February 2015	●	100% Labex IGO
	C. Louvet	UMR1064-E1 (Nantes)	T4.2	19 months	January 2014	July 2015	●	100% Labex IGO
	N. Petit	UMR1232-E3 (Nantes) UMR1064-E5 (Nantes)	T1.5	15 months	January 2016	March 2017	●	100% Labex IGO
	Y. Hamon	UMR1232-E3 (Nantes) UMR1064-E5 (Nantes)	T1.5	1 year	November 2017	*October 2018*	○	100% Labex IGO
Technical staff								
Research Assistant (IE)	S. Bézie	UMR1064-E2 (Nantes)	T1.1.1.	2 years	January 2013	December 2014	●	100% Labex IGO

Research Assistant (IE)	E. Garo	UMR1232-E7 (Angers)	T1.1.3.	6 months	March 2013	August 2013	●	100% Labex IGO
Technician Assistant (ADT)	C. Dumet	PF Humanized Rodents (Nantes)	T2.1	2.5 years	June 2013	December 2015	●	100% Labex IGO
Research Assistant (IE)	K. Duperrier	PF Immunomonitoring (Nantes)	T3.2	20.5 months	July 2013	March 2015	●	100% Labex IGO
Research Assistant (AI)	A. Lemoine	UMR1064-E1 (Nantes)	T1.1.3	2 years	April 2014	March 2016	●	100% Labex IGO
Research Assistant (AI)	L. Florenceau	UMR1232-E3 (Nantes)	T7.3	2 years and 10 months	September 2014	June 2017	●	100% Labex IGO
Technician	M. Maquigneau	UMR1064-E2 (Nantes)	T1.6	1.5 year	November 2014	April 2016	●	100% Labex IGO
Technician	N. Vimond	UMR1232-E4 (Nantes)	T1.6	11 months	March 2015	February 2016	●	100% Labex IGO
Technician	P. Aumond	UMR1102 (Nantes)	T7.2	10 months	March 2015	July 2016	●	100% Labex IGO
Technician	P. Aumond	UMR1232-E3 (Nantes)	T7.3	7 months	August 2016	December 2017	●	100% Labex IGO
Research Assistant (IE)	N. Salabert	PF Immunomonitoring (Nantes)	T3.2	1 year (renewable up to 2019)	April 2015	*December 2017*	○	100% Labex IGO
Research Assistant (IE)	N. Marec	UMR1232-E3 (Nantes)	T3.2	19 months	August 2015	September 2017	●	100% Labex IGO
Technician Assistant (ADT)	M. Jaouachi	PF Humanized Rodents (Nantes)	T2.1	19 months	January 2016	August 2017	●	100% Labex IGO
Research Assistant (IE)	S. Bézie	UMR1064-E2 (Nantes)	T1.1.1	15 months	March 2016	May 2017	●	100% Labex IGO
Technician	S. Salle	UMR1232-E7 (Angers)	T7.3	1 year	January 2016	December 2016	●	100% Labex IGO
Research Assistant (AI)	L. Gueno	UMR1064-E1 (Nantes)	T4.5	1 year	November 2016	October 2017	●	100% Labex IGO

Technician	N. Vimond	UMR1064-E2 (Nantes)	T4.4.1	1 year	December 2016	November 2017	●	100% Labex IGO
Technician	V. Huchet	UMR1064-E3 (Nantes)	T1.8	1.5 year	June 2017	*November 2018*	○	100% Labex IGO
Research Assistant (IR)	S. Simon	UMR1232-E3 (Nantes)	T7.4.1	1.5 year	December 2016	*May 2018*	○	100% Labex IGO
Research Assistant (AI)	M. Gantier	UMR1064-E2 (Nantes)	T7.4.4	11 months	July 2017	*May 2018*	○	100% Labex IGO
Technician Assistant (ADT)	M. Dugué	UMR1064-E3 (Nantes)	T2.1	1 year	September 2017	*August 2018*	○	100% Labex IGO

APPENDIX 3 : SUPPORT STAFF DEDICATED TO LABEX IGO

Currently, **3 people (2.2FTE)** are working as support staff on Labex IGO.

ACTIVITY	NAME	FTE	CONTRACT DURATION	START	END	GRANT
Administrative assistant	**V. Pecqueret**	**0.5**	permanent position	September 2012	*December 2019*	100% INSERM
Financial assistant	D. Merouani *replaced by* **G. Espejo**	0.5 **1**	1 year *(renewable)*	January 2013 February 2015	December 2014 *December 2019*	100% PRES-L'UNAM 100% UNIV NANTES
Delegate	L. Salaün *replaced by* **L. Wolff**	0.5 **0.7**	1 year *(renewable)* 3 years *(renewable)*	February 2013 August 2016	August 2016 *December 2019*	100% PRES-L'UNAM *replaced in 2015 by* 100% UNIV NANTES 100% UNIV NANTES

APPENDIX 4: FINANCES

OVERALL BUDGET

Labex IGO will receive from the French Government via ANR (Agence Nationale de la Recherche) 5,5 million euros. Funds must be used between March 1st 2012 and December 31st 2022.

(in euros)

Budget distribution	2012	2013	2014	2015	2016	2017	2018	2019-2020	balance	Total
Research	466 538	478 962	879 727	666 471	492 494	517 932	465 337	335 252		4387244
Training	32 692	104 236	67 546	38 262	57 767	38 758	58 273	39 046		445508
Dissemination	9 615	74 593	35 250	33 944	33 944	33 944	33 944	33 944		295709
Gouvernance	20 000	19 231	20 000	19 259	19 259	15 556	15 556	17 050		160000
Management cost (4% then 8% since 2015)	21 155	27 081	40 101	60 635	48 277	48 495	45 849	34 023		211539
Total	550 000	704 102	1 042 624	818 572	651 742	654 686	618 959	459 315		**5 500 000**
Payment by ANR	550 000	704 102	1 031 126	830 111	443 077	439 604	439 604	750 990	311 386	**5 500 000**
Balance	0	0	-11 498	11 539	-208 665	-215 082	-179 355	291 675	311 386	0

BUDGET ALLOCATED TO RESEARCH PROJECTS

Research projects (2012-2016)

1 396 k€ have been allocated to 11 research projects (8 tasks and 3 sub-projects). These research projects have up to 60k€ for 3 years for operating costs (consumables, reagents, etc…) and up to 96k€ for 3 years to recruit research staff (PhD, post-doc, research assistants, …).
These projects were initiated from October 2012 to January 2013.

Research projects awarded on Call 2014 (2014-2017)

930 k€ have been allocated to 6 research projects. Each of them gets up to 75k€ for 3 years for operating costs and up to 96k€ for 3 years to recruit research staff.
Among these 6 research projects, 4 have started in Autumn 2014 and 2 have started in Spring 2015.

Research projects awarded on Call 2016 (2016-2019)

1 111 k€ have been allocated to 5 new research projects. The amount awarded per project have been adjusted in order to meet the objectives of the project and the number of partners involved in the project (37.5k€ for 3 years for operating costs and 48k€ for 3 years to recruit research staff per team).
These projects have started in Autumn 2016.

Research projects awarded on Call 2018 (2018-2021)

336 k€ will be allocated to new research projects that will be selected on Call 2018. 4 projects will be awarded with 84 k€.
These projects will run from June 2018 to December 2021.

APPENDIX 5 : PUBLICATIONS

To date, Labex IGO projects led to 188 publications.

2012 (2 publications)		
Corvaisier M, Delneste Y, Jeanvoine H, Preisser L, Blanchard S, Garo E, Hoppe E, Barré B, Audran M, Bouvard B, Saint-André JP, Jeannin P. IL-26 is overexpressed in rheumatoid arthritis and induces proinflammatory cytokine production and Th17 cell generation. PLoS Biol. 2012;10(9):e1001395.	U892 Angers	WP1 T113
Poirier N, Mary C, Dilek N, Hervouet J, Minault D, Blancho G, Vanhove B. Preclinical efficacy and immunological safety of FR104, an antagonist anti-CD28 monovalent Fab' antibody. Am J Transplant. 2012 Oct;12(10):2630-40.	U1064 Nantes	WP1 T21
2013 (7 publications)		
Cheray M, Pacaud R, Nadaradjane A, Vallette FM, Cartron PF. Specific inhibition of one DNMT1-including complex influences tumor initiation and progression. Clin Epigenetics. 2013 Jun 28;5(1):9.	U892 Nantes	WP3 T61
Chesneau M, Michel L, Degauque N, Brouard S. Regulatory B Cells and Tolerance in Transplantation: From Animal Models to Human. Front Immunol. 2013 Dec 31;4:497.	U1064 Nantes	WP1 T112
Foucher ED, Blanchard S, Preisser L, Garo E, Ifrah N, Guardiola P, Delneste Y, Jeannin P. IL-34 induces the differentiation of human monocytes into immunosuppressive macrophages. antagonistic effects of GM-CSF and IFNγ. PLoS One. 2013;8(2):e56045.	U892 Angers	WP1 T113
Labarrière N, Fortun A, Bellec A, Khammari A, Dreno B, Saïagh S, Lang F. A full GMP process to select and amplify epitope-specific T lymphocytes for adoptive immunotherapy of metastatic melanoma. Clin Dev Immunol. 2013;2013:932318.	U892 Nantes	WP2 T411
Ménoret S, Fontanière S, Jantz D, Tesson L, Thinard R, Rémy S, Usal C, Ouisse LH, Fraichard A, Anegon I. Generation of Rag1-knockout immunodeficient rats and mice using engineered meganucleases. FASEB J. 2013 Feb;27(2):703-11.	U1064 Nantes	WP1 T111
Santolaria T, Robard M, Léger A, Catros V, Bonneville M and Scotet E. Repeated Systemic Administrations of Both Aminobisphosphonates and Human Vg9Vd2 T Cells Efficiently Control Tumor Development In Vivo. J Immunol 2013, 191:1993-2000.	U892 Nantes	WP2 T412
Tonnerre P, Gérard N, Chatelais M, Poli C, Allard S, Cury S, Bressollette C, Cesbron-Gautier A, Charreau B. MICA variant promotes allosensitization after kidney transplantation. J Am Soc Nephrol. 2013 May;24(6):954-66.	U892 Nantes	WP1 T15
2014 (21 publications)		
Allard M, Oger R, Benlalam H, Florenceau L, Echasserieau K,	U892	WP2

Bernardeau K, Labarrière N, Lang F and Gervois N. Soluble HLA-I/peptide monomers mediate antigen-specific CD8 T cell activation through passive peptide exchange with cell-bound HLA-I molecules. J Immunol, 2014; 192:5090-5097.	Nantes	T411 T12 T13
Braza F, Chesne J, Castagnet S, Magnan A, Brouard S. Regulatory functions of B cells in allergic diseases. Allergy. 2014 Nov;69(11):1454-63.	U1064 Nantes	WP1 T112
Chauveau A, Tonnerre P, Pabois A, Gavlovsky PJ, Chatelais M, Coupel S, Charreau B. Endothelial cell activation and proliferation modulate NKG2D activity by regulating MICA expression and shedding. J *Innate Immun.* 2014;6(1):89-104.	U892 Nantes	WP1 T15
Chesneau M, Pallier A, Braza F, Lacombe G, Le Gallou S, Baron D, Giral M, Danger R, Guerif P, Aubert-Wastiaux H, Néel A, Michel L, Laplaud DA, Degauque N, Soulillou JP, Tarte K, Brouard S. Unique B cell differentiation profile in tolerant kidney transplant patients. Am J Transplant. 2014 Jan;14(1):144-55.	U1064 Nantes	WP1 T112
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APPENDIX 6 : PATENTS

To date, Labex IGO projects led to 17 patents.

2012		
EP12.305.823.2, 10/07/2102 "Methods and kits for determining whether a cytomegalovirus infection in a transplanted patient is likely to Induce allograft rejection" (Gervois Nadine, Allard Mathilde and Charreau Beatrice, INSERM) ➔ PCT/EP13739172.8-1405 13/02/2015	U892 Nantes	WP1 T12
2014		
EP14.163.081.4, 01/04/2014 "An isolated donor MHC-derived peptide and uses thereof" (Guillonneau C., Anegon I., Picarda E.) ➔ PCT/EP2015/057257 01/04/2015 ➔ WO 2015/150491	U1064 Nantes	WP1 T111
EP14.163.072.3, 01/04/2014 "An isolated donor MHC-derived peptide and uses thereof" (Guillonneau C., Anegon I., Picarda E.) ➔ PCT/EP2015/057258 01/04/2015 ➔ WO 2015/150492	U1064 Nantes	WP1 T111
EP14 306 165.3, 17/07/2014 "An isolated interleukin-34 polypeptide for use in preventing transplant rejection and treating autoimmune diseases" (Guillonneau C., Anegon I., Bézie S.) ➔ WO 2016/009041	U1064 Nantes	WP1 T111
EP14 306 231.3, 01/08/2014 "A CD45RC+ T cell depleting antibody for use in preventing transplant rejection and treating autoimmune diseases". (Guillonneau C., Anegon I.) ➔ WO 2016/016442	U1064 Nantes	WP1 T111
EP14 305 609.1, 24/04/2014 "Soluble HLA-I/peptide monomers and applications as therapeutic treatments in cancer" (Gervois Nadine, Allard Mathilde, Oger Romain and Lang François, INSERM)	U892 Nantes	WP1 T12
EP 143.067.23.9, 28/10/2014 « Composition and methods for antigen-specific tolerance » (Anegon I., Blancou P., Simon T., Pogu J.) ➔ International extension in september 2015 ➔ WO 2016/066618	U1064 Nantes	WP1 T16
2015		
EP 15 305 886.2 (10 juin 2015) « Methods and compositions for RNA-guided treatment of human cytomegalovirus (HCMV) infection » (Haspot F., Gergen J., Coulon F., Halary F.) ➔ 15 décembre 2015 International application	U1064 Nantes	WP1 T14

EP 15.305.797.1 « New method to produce T cells » (Simon S., Lang F., Labarrière N., INSERM) ➔ PCT/EP2016/061941 (25 mai 2016)	U892 Nantes	WP2 T411
EP15.305.715.3, 12/05/2015 «Methods and kits for labeling, detecting and isolating Foxp3+ regulatory T cells, isolated population of Foxp3+ Treg cells thus obtained and uses thereof» (C. Guillonneau I. Anegon S. Bézie)	U1064 Nantes	WP1 T111
EP 15.306.092.6, 03/07/2015 « Methods for obtaining regulatory T cells and uses thereof » (C. Guillonneau I. Anegon S. Bézie) ➔ 2016 : Licensed to TxCell	U1064 Nantes	WP1 T111
EP 15.306.366.4, 07/09/2015 « A new subpopulation of human CD8+CD45RClow Tregs and uses thereof » (C. Guillonneau I. Anegon S. Bézie) ➔ 2016 : Licensed to TxCell	U1064 Nantes	WP1 T111
2016		
EP 16306588.1 « Methods and compositions for RNA-guided treatment of human BK polyoma virus (BK-PyV) infection » (Haspot F., Gergen J., Coulon F., Sikorski M., Bressollette C., Halary F.)	U1064 Nantes	WP1 T14
2017		
EP 17306097.1, 25/08/2017 « Method for monitoring the efficacy of a cancer treatment » (Simon S., Labarrière N.)	U1232 Nantes	WP2 T411
EP 17306783.6. 15/12/17 « Treatment of monogenic diseases with an anti-CD45RC antibody » (I. Anegon, C. Guillonneau)	U1064 Nantes	WP1 T111
EP17305939.5, 13/07/2017 « Methods for increasing expansion and immunosuppressive capacity of a population of CD8+CD45RClow/- Tregs » (C. Guillonneau, I. Anegon, S. Bézie)	U1064 Nantes	WP1 T13
PCT/EP2017/076911 24/07/2017 « Methods for promoting T cells response » (E. Chiffoleau, B. Vanhove, N. Poirier, G. Teppaz, V. Gauttier)	U1064 Nantes	WP1 T13

APPENDIX 7 : LABEX IGO THESIS FUNDED BY LABEX-IGO (>50%) (SINCE APRIL 2016)

PhD student	**Sylvain SIMON**
Start	**01/10/2013**
End (Thesis defence)	**16/12/2016**
Team	UMR 892 – Team 3, Nantes
PhD director	Nathalie LABARRIERE
Funding	100% Labex IGO
University (PhD)	Université de Nantes
University (Master)	Université de Nantes
Thesis title	Regulation of PD-1 expression on melanoma-specific T lymphocytes and immune follow-up of patients treated by anti-PD-1 immunotherapy
Abstract	While having considerably modified melanoma-patients' management, a large fraction of patients remains refractory to the immunotherapies targeting PD-1 axis. The understanding of immunological mechanisms involved in clinical efficacy or tumor resistance is crucial to further improve therapeutic efficiency. PD-1 has been largely documented as a major regulator of anti-tumor T cell responses, but it also identifies melanoma-reactive T lymphocytes. We have demonstrated that PD-1 positive melanoma-specific T cell clones exhibit higher functional avidity than T cell clones unable to induce PD-1 through epigenetic mechanisms. Furthermore, the *in vitro* PD-1 blockade during the generation of melanoma-specific T cells for adoptive cell transfer allows the production of T effectors with optimized functions. The immune follow-up of anti-PD-1 treated melanoma patients demonstrated substantial changes, in all patients, within the Melan-A specific T cell repertoire. We observed the emergence of high functional avidity clonotypes' exhibiting PD-1 and TIGIT co-expression in responding patients. These studies demonstrated that PD-1 is associated with melanoma-specific T cells functional avidity. In addition, therapeutic efficacy of anti-PD-1 treatments is correlated to that property as it allows the emergence of clonotypes exhibiting high functional avidity and reactivity to tumor cells. These T lymphocytes co-express PD-1 and TIGIT, which could therefore represent suitable targets for further combined therapies.
Keywords	Immunology-Immunotherapy-Melanoma-CD8 T lymphocytes-PD-1-Adoptive T cell transfer
Current position	Research assistant (IR) funded by Labex IGO, UMR1232 Team 3, Nantes

PhD student	**Audrey MOHR**
Start	**01/11/2013**
End (Thesis defence)	**17/10/2016**
Team	UMR1227, Brest
PhD director	Christophe JAMIN
Funding	50% Labex IGO, 50% Région Bretagne
University (PhD)	Université de Bretagne Occidentale (UBO)
University (Master)	Université de Strasbourg
Thesis title	Characterization of human regulatory B cells in Chronic Lymphocytic Leukemia
Abstract	Chronic lymphocytic leukemia (CLL) is characterized by expansion of CD5+ B cells associated with disruption of immune responses, contributing to the immunodeficiency and the disease progression. Regulatory B (Breg)

	cells may control the anti-tumor responses favoring tumor escape. Intriguingly, CLL B cells share phenotypical characteristics with these cells. The main focus of this project is to evaluate the regulatory function of CLL B cells, aiming to estimate their influence on the lack of anti-tumor responses mediated by T cells. In vitro models of co-cultures between T and B cells are used to appraise the regulatory capacity of CLL B cells on T cell proliferation. We determined a defective spontaneous regulatory function for CLL B cells. Two groups of patients have been identified following CpG-ODN stimulation. The first group presents defective regulatory B cell functions compared with control B cells. In the second group, no inhibitory activity is detected. TLR-9 gene expression analysis highlighted differential gene expression between controls and the two groups of CLL patients. Moreover,ours observations indicate that patients with low Breg activity have more aggressive disease. These results suggest alteration of the TLR-9 pathway in CLL B cells. To go further, it will be of interest to identify the molecular mechanisms da maging the TLR-9 pathway. These results would contribute to clarify the lack of anti -tumor immune response found in the CLL patients.
Keywords	Regulatory B cells, TLR-9, CLL
Current position	Post-Doctoral research assistant : CIMI Inserm U1135, Paris

PhD student	**Janina GERGEN**
Start	**01/10/2014**
End (Thesis defence)	**15/12/2017**
Team	UMR1064 – Team 1, Nantes
PhD director	Franck HALARY et Fabienne HASPOT
Funding	50% Labex IGO, 50% Université de Nantes (« bourse du Président ») + 3 months Labex IGO
University (PhD)	Université de Nantes
University (Master)	Philipps-Universität Marburg (Allemagne)
Thesis title	The CRISPR/Cas9 system as an anti-viral strategy against the human Cytomegalovirus
Abstract	The human cytomegalovirus (HCMV) primary infection is usually asymptomatic but leads to latent infection of blood progenitor cells. Immunocompromised patients are at high risks of HCMV reactivation, which is associated with severe end organ diseases and increased mortality in transplant patients. Standard anti-viral treatments based on nucleotide analogues decreased the occurrence of HCMV reactivation and diseases, but induce side effects and drug-resistant viral strains. In this thesis, we introduced new anti-viral approaches based on the CRISPR/Cas9 gene editing tool. Two strategies are designed to target the UL122/123 gene of HCMV encoding the immediate early proteins, essential for lytic viral replication and reactivation from latency. We validated that the disruption of the UL122/123 gene by the CRISPR/Cas9 system to abrogate viral replication. The multiplex CRISPR/Cas9 system (three gRNA) was much more efficient than the singleplex approach targeting the same gene. Target gene expression, concomitant genome replication and virion release were significantly impaired by the multiplex strategy. A further anti-HCMV CRISPR/Cas9 system was developed specifically to target the HCMV genome during latency. Two gRNAs target the viral genome at three target sites: LUNA, essential for reactivation, and the two homolog TR regions. We verified this duplex strategy on the lytic replicating virus and

	detected mutations at the target site as well as the reduction of viral genome copy number. In conclusion, the anti-HCMV strategies based on two or three gRNAs efficiently blocked viral replication. This provides the basis for the development of an anti-HCMV CRISPR/Cas9 therapy.
Keywords	Human cytomegalovirus, CRISPR/Cas9, Immediate Early, Multiplex, Anti-viral therapy, Latency, LUNA, TR region
Current position	Apply at Bayer and Charles River

PhD student	**Cynthia CHAUVIN**
Start	**01/10/2014**
End (Thesis defence)	**19/09/2017**
Team	UMR892 – Equipe 1, Nantes
PhD director	Emmanuel SCOTET et Claire PECQUEUR
Funding	100% Labex IGO
University (PhD)	Université de Nantes
University (Master)	Université de Nantes
Thesis title	Adoptive cell therapy for glioblastoma multiforme: tumor target cells analysis and recognition by V!9V"2 T lymphocytes
Abstract	Glioblastoma multiform (GBM) is the most frequent and aggressive primary brain tumor in adults, with a dismal prognosis and few therapeutic advances made over the last decade. Cellular immunotherapies are currently being explored to eliminate highly invasive/resistant GBM cells likely involved in tumor relapse. Nonalloreactive human V!9V"2 T lymphocytes are able to kill a wide range of human tumor cells and display effector functions setting them up as promising cell candidates for efficient immunotherapies. The work described in this thesis aimed at characterizing primary GBM (pGBM) cultures, through phenotypic, metabolic and transcriptomic analyses and at investigating their recognition and killing by V!9V"2 T cells both in vitro and in vivo. We showed that immunodeficient NSG mice carrying orthotopic human pGBM xenografts recapitulate tumor development in patients. Furthermore, we demonstrated that V!9V"2 T cells can survive and patrol within the brain following adoptive transfer and successfully eliminate infiltrative pGBM cells orthotopic zoledronate treatment. In order to bypass this sensitization, several human V!9V"2 T cells have been isolated and amplified from PBMCs of healthy donors and screened for their ability to naturally and specifically react against pGBM cells. These results evidence that allogeneic V!9V"2 T lymphocytes efficiently and preferentially eliminate mesenchymal pGBM cells through !"-TCR- and NKG2D-dependent pathways. Taken together, these results provide an important proof-of-concept for optimized targeted immunotherapies of brain tumors and identify pathways that need to be explored before considering human V!9V"2 T cells in clinical approaches.
Keywords	Glioblastoma multiform, immunotherapy, gd T lymphocytes, adoptive transfert, intracranial xenografts
Current position	Post-doctoral research assistant: Baylor College of Medicine, Houston, Texas (Dr Nabil Ahmed)