ESMO Research Research Fellowship

(September 2018 – September 2020)

Elisa Gobbini

FINAL REPORT

Host Institute: **Cancer Research Center of Lyon**
Mentor: **Jenny Valladeau**
Project title: **Role of XCR1+ (BDCA3high) dendritic cells in anti-tumour immune responses**
Home Institute: **Azienda Ospedaliera Universitaria San Luigi Gonzaga, Orbassano, Italy**

Introduction

Immunotherapy is becoming increasingly important in clinical practice being the immune-checkpoint inhibitors the first-line treatment for melanoma and lung cancer. These drugs (anti-CTLA4 and anti-PD-1/PD-L1) are able to reactivate the host T lymphocytes against the tumor providing an extremely durable clinical response. However, many challenges remain and it seems crucial to further understand the mechanisms that support the establishment of the specific anti-tumor immune response, as well as its positive or negative regulation mechanisms.

Rationale and Aim

It is now clearly established that the induction of cytotoxic CD8 T lymphocytes (LT) allows specific recognition and destruction of transformed cells. For the CD8 LT activation, dendritic cells (DCs) are required. There are several DCs sub-populations, each one characterized by specific features. This variability makes the immune system able of responding to a very wide dangers diversity to which an organism may be exposed.
XCR1+ DCs are a particular DC subset characterized by the unique expression of the Clec9a endocytic receptor, the Pattern Recognition Receptor (PRR) TLR3 and the XCR1 chemokine receptor. This population appears to be more effective than other subpopulations of DCs in the cellular antigen cross-presentation, that represents the key mechanism of the immunological anti-tumor response mediated by CD8+ T lymphocytes (Robbins et al., Genome Biol 2008, Cohn et al J Med 2013). Several recent reports have actually shown that XCR1+ DC play a crucial role in tumor rejection in mice (Hildner et al., Science 2008). Finally, it has been shown that a high XCR1+ DCs gene signature is significantly associated with a better control of tumor progression (Broz et al., Cancer Cell 2014). Only one experience has been conducted until now exploring the predictive role in humans (Barry et al, Nat Med 2018). This work recently demonstrated that the proportion of tumor-associated XCR1+ DCs (determined by FACS) is greater in patients with metastatic melanoma responding to anti-PD-1 than in non-responders. These data do not make it possible to assert that XCR1+ DCs are necessary for the response to the immune checkpoint inhibitors (ICI) in humans, but provides a first lead that raises the interest of analyzing the functional properties of this population.
Few data are available about the role of XCR1+ DCs in anti-tumor immunity in humans, mainly due to the lack of *in situ* methods working on paraffin-embedded samples. It seems thus urgent to find a method to identify XCR1+ DCs *in situ* in human cancers leading then to explore their prognostic impact and their predictive role in ICI response. In parallel, the exploitation of large cohorts of human may allow the in silico confirmation of prognostic impact of this particular DC subset.

Experimental design

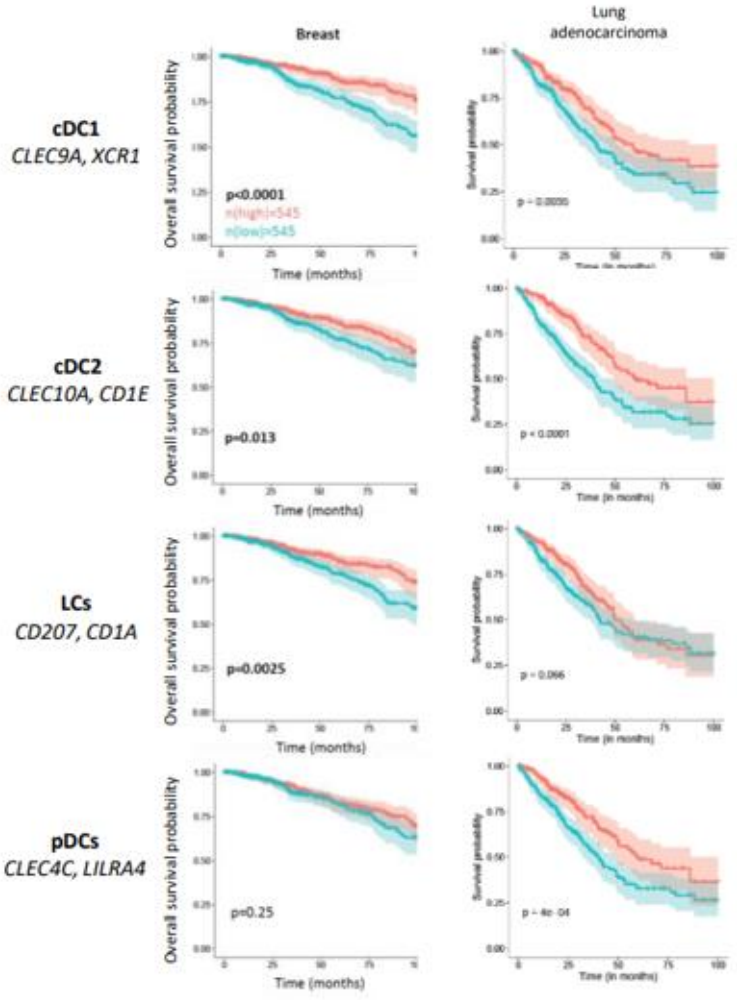
The proposal had been scheduled in four tasks:

- Task 1.a: *In silico* analysis about the prognostic impact of XCR1+ DC in breast and lung cancer based on their specific signatures
- Task 1.b: Establish overall survival correlation with XCR1+ DC infiltration by *in situ* analysis of human breast cancer
- Task 2.a*: In silico* analysis about predictive role of XCR1+ DCs in cancer treatments response in lung cancer
- Task 2.b: Establish correlation between XCR1+ DC infiltration and response to antiPD1/PDL1 immune checkpoint blockers in lung cancer patients by *in situ analysis*

Results, Conclusions and Future Perspectives

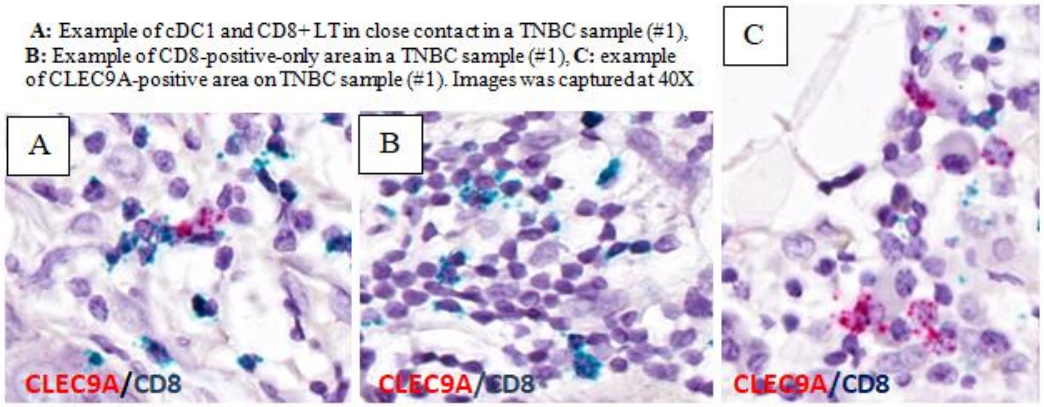
- Task 1.a: In silico analysis about the prognostic impact of XCR1+ DC in breast and lung cancer based on their specific signatures

We have shown the positive prognostic impact of Tumor-Associated-XCR1+ DC in overall survival across different cancer subtypes including breast and lung cancer. These data were calculated by the MCP-counter algorithm using TCGA data sets of 14 types of human cancers. The analysis was conducted on n=1090 breast cancer and n=505 lung cancer (adenocarcinoma) patients. Tumor-Associated-XCR1+ DC provided a positive prognostic impact in overall survival across different cancer subtypes including breast and lung cancer. Notably, in breast and lung cancer, Tumor-Associated-XCR1+ DC presented the higher prognostic impact compared with the other DCs subset, suggesting their central role in the anti-tumor immunity (Hubert M. et al. Science Immunology 2020).



Task 1.b: Establish overall survival correlation with XCR1+ DC infiltration by *in situ* analysis of human breast cancer

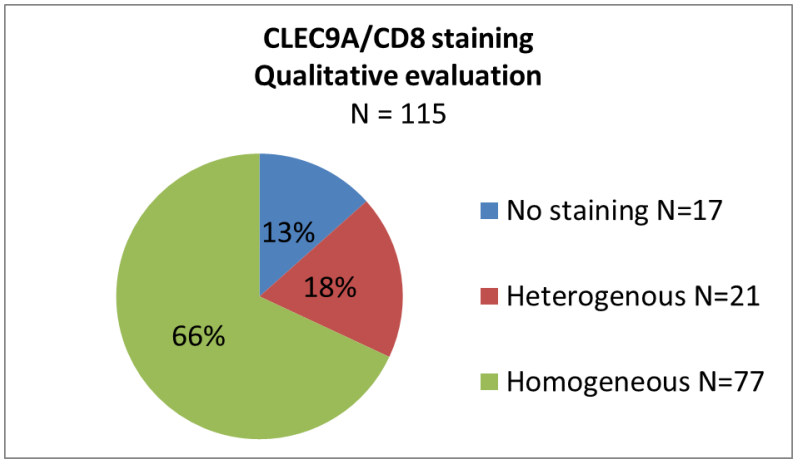
We developed an *in situ* mRNA hybridization protocol (RNAscope) to detect CLEC9A mRNA, which is specific for XCR1+ DC cells, allowing its localization on FFPE sections. This proposal will attempt to correlate patients' overall survival with Tumor-Associated XCR1+ DCs infiltrate.



A: Example of cDC1 and CD8+ LT in close contact in a TNBC sample (#1), **B:** Example of CD8-positive-only area in a TNBC sample (#1), **C:** example of CLEC9A-positive area on TNBC sample (#1). Images was captured at 40X

A cohort of 115 triple negative breast cancers (TNBC) with more than 10 years of clinical follow-up is under evaluation to reach this aim.

To date, I analyzed the entire cohort for XCR1+ DC infiltration by CLEC9A mRNA hybridization, but also for the CD8+ LT infiltration using the Leica Bond RX automated RNAscope assay (assuring a better reliability compared to the manual test). Unfortunately, 17 of them were not evaluable due to technical issue or RNA degradation. Among the others, staining was really homogenous on the infiltrating zones in 77 cases, while it was only partially represented in 21 cases.



Clinical feature n(%)	Whole cohort N =115	Homogeneous N = 77
Median age [range]	57. 5 [19- 96]	57 [34-91]
Histology		
Ductal carcinoma	96 (83%)	65 (84%)
Grade		
III	98 (85%)	66 (85%)
Neoadjuvant CT	4 (3%)	4 (5%)
Adjuvant CT	83 (72%)	58 (75%)
Relapsed	31 (27%)	22 (28%)
Disease free	84 (73%)	55 (71%)

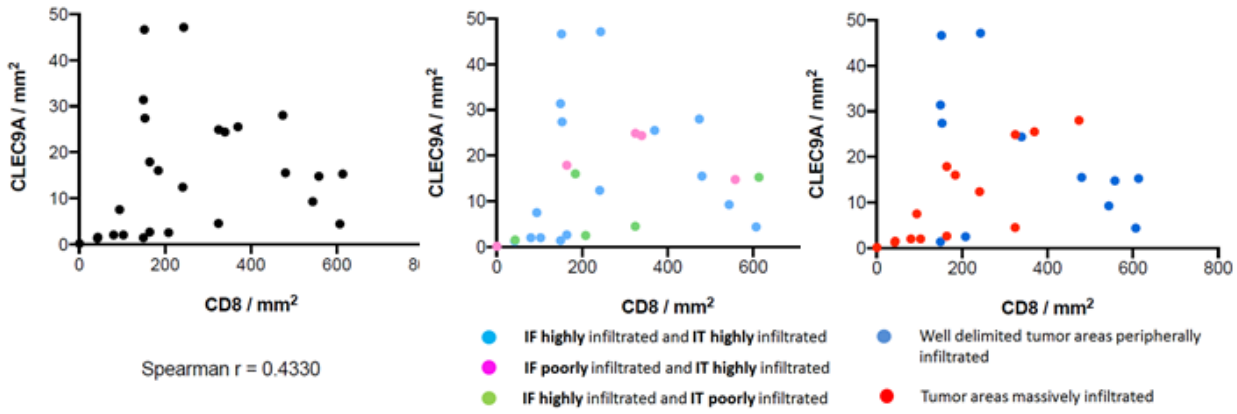
First, we focused on 77 cases presenting a well-represented staining (so called "homogeneous"). Their clinical characteristics were comparable with the general population. Twenty-two patients (28%) relapsed after the surgical intervention while fifty-five patients (71%) were still free from disease at time of analysis.

We performed a qualitative anatomopathological evaluation based on the counterstaining. That allowed us to classified tumors according to immune infiltrate aboundance at invasion front and within the intra-tumoral stroma, the presence or scarring stroma and the principal pattern of tumor tissue organization. The Homogenous group was enriched in tumors with both an invasion front and an intra-tumoral stroma highly infiltrated

Anatomopathological description	Whole cohort N = 115	Homogeneous N = 77
IF highly infiltrated and IT highly infiltrated	63 (54%)	50 (64%)
IF poorly infiltrated and IT poorly infiltrated	15 (13%)	5 (6%)
IF highly infiltrated and IT poorly infiltrated	18 (15%)	12 (15%)
IF poorly infiltrated and IT highly infiltrated	17 (14%)	9 (11%)
PP: well delimited tumor areas peripherally infiltrated	42 (36%)	23 (32%)
PP: tumor areas massively infiltrated	72 (62%)	52 (67%)
Scarring stroma high	47 (40%)	27 (35%)
Scarring stroma poor	66 (57%)	49 (63%)

IF: invasion front, IT: intra-tumoral stroma, PP: Principal pattern

compared to the general population (64% versus 54%).
Due to technical issues (e.g. Weak counterstaining, target staining huge variability, deposits huge variability…) the automatic counting by the HALO™ image analysis software was challenging and most of the time not feasible. Consequently, we started a manual counting, randomly selecting 8 field of 1mm2 each on the homogeneous cases. To date 26 of the 77 cases were analyzed reporting the counting field position (invasion front of intra-tumoral area), the CD8+ cells and the CLEC9A+ cells density along with the percentage of CLEC9A+ and CD8+ cells that are in close contact with CD8+ cells. A strong correlation between CD8+ cell/mm2 and CLEC9A+ cell/mm2 was found (r correlation 0.4330). Interestingly, tumors with different anatomopathological characteristics seemed to have a slightly different correlation pattern. Consequently, these features have to be take into account in the final analysis. Indeed, in tumors with massive infiltrated the correlation is stronger than in tumor not infiltrated.



We launched an automated counting by the HALO™ image analysis software of CD3+ cells stained by immunofluorescence on a separate and sequentially cut slide for each patient. This information will allow us to normalize the CD8 counting on the selected fields and to get more robust the anatomopathogical classification. Finally, 45% of cDC1 were found in close contact with CD8+ cells, suggesting a direct interaction of these two immune populations.

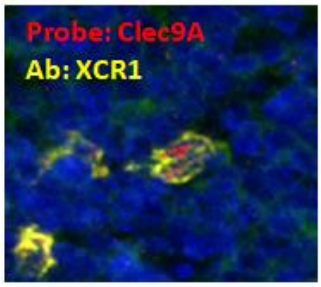
Task 2.a: In silico analysis about predictive role of XCR1+ DCs in cancer treatments response in lung cancer

After an accurate literature revision, no public RNAseq data coupled to clinical information were found so far for immune checkpoint treated lung cancer patients.
To explore the XCR1+ DC predictive impact, we decided to launch a retrospective trial called LUNG-PREDICT [NCT04069442] to form a cohort of NSCLC patients treated with immune checkpoint inhibitors (n=60) or chemotherapy (n=30) in first-line. Patients meeting inclusion criteria were selected in 6 oncologic centers and their samples availability is under evaluation by local pathologists. Samples will be used for *in situ* and

RNAseq analysis providing an exhausting multiparametric portrait to correlate with patients' outcome. The chemotherapy group will be used as reference for outcome comparison. This study will allow us to explore XCR1+ DC prognostic and predictive role in NSCLC taking into account other immune infiltrate and tumor characteristics that have already be showed having a prognostic or predictive impact on human cancers.
Moreover, we will validate the results on a larger cohort in collaboration with the Curie Institute (Paris). The Curie Institute, is actually collecting three prospective cohorts of advanced NSCLC patients (ALCINA [target = 120 patients], PLASTICITY [target = 60 patients] and ONCOSPINE [target= 180 patients]) treated with ICP during their history of disease (mainly in second line). These patients are characterized by RNAseq and WES (Whole Exome Sequencing) and their genomic and clinical data will be available to reach this aim. The LUNG PREDICT cohort will be however more informative because of homogeneity in term of line of treatment (only fist-line immune checkpoint inhibitors allowed) and sample collection timing (only samples collected within the 6 months before the start of checkpoint inhibitors are allowed and patients do not have received any treatment between the sample collection and the immune checkpoint inhibitor starting). Call for funding was also submitted for RNAseq analysis of the LUNG PREDICT cohort (decision pending).

Task 2.b: Establish correlation between XCR1+ DC infiltration and response to anti-PD1/PDL1 immune checkpoint blockers in lung cancer by *in situ* analysis

The co-localization of CLEC9A (red - RNA in situ hybridization) and XCR1 (yellow - OPAL immunofluorescence) allowed XCR1+ DCs visualization on FFPE tonsil tissue



We will explore the predictive impact of XCR1+ DC infiltrating human cancer through the *in situ* analysis of the LUNG PREDICT cohort. We identified another method to test the XCR1+ DCs *in situ* by IHC assay on FFPE samples. However, combining the anti-XCR1 staining by immunofluorescence and the anti-CLEC9A probe to verify the XCR1+ DC antibody specificity, we found that all CLEC9A+ cells express the XCR1 marker. Thus, XCR1-antibody can be considered as a valid and cheaper alternative to the ISH for XCR1+ DC detection on FFPE samples on future cohorts. This allows us to developed a pan-DC multi-immunofluorescence panel including XCR1, CD1a, BDCA2, DC-Lamp and CD8 on tonsil, lung and breast cancer tissue. The *in situ* analysis on the LUNG PREDICT cohort will also include another multi-immunofluorescence panel with CD4, CD8, CD19, CD56, CD68, FOXP3 and cytokeratin already available at the CRCL.
Slides will be scanned using an automate nanozoomer S360 Digital slide scanner (Hamamatsu Photonic) and quantification analysis will be performed using Halo™ Image Analysis Software (Perkin Elmer). Thanks to this software we will be also able to analyze the distance between XCR1+ DC and other immune cells or tumor cells *in situ* using the nearest neighbor method.
These data, merged with the transcriptome data obtained by RNAseq on the same samples, will provide an exhausting multiparametric portrait. Patients will be classified as XCR1+ DC-positive if infiltrated by XCR1+ DC according to RNAscope test and/or if enriched in XCR1+ DC signature (mscore ≥ 20) according to the RNAseq analysis. Conversely, they will be defined as XCR1+ DC-negative if no XCR1+ DC will be detected on FFPE sample by RNAscope and if they will be not XCR1+ DC enriched according to the transcriptome data. Clinical outcomes of these two groups of patients will be compared to attend primary and secondary objectives of the study as detailed below.

Primary EP	Group comparison	Statistical Method
PFS	XCR1+ DC positive vs XCR1+ DC negative	KM α = 0.05 and COX

Secondary EPs	Group comparison	Statistical Method
TTF and OS	XCR1+ DC positive vs XCR1+ DC negative	KM α = 0.05 and COX
ORR	XCR1+ DC positive vs XCR1+ DC negative	Chi-square
cDC1 IO specific predictive value: PFS, TTF and OS	IO treated XCR1+ DC positive vs IO treated XCR1+ DC negative	KM α = 0.05 and COX
	CT-treated XCR1+ DC positive vs CT-treated XCR1+ DC negative	
cDC1 predictive value compares to other biomarkers	cDC1+ vs cDC1-	COX Multivariate analysis and PCA

This study will allow us to explore XCR1+ DC prognostic and predictive role taking into account immune infiltrate and tumor characteristics . Those data would represent the proof of concept about the XCR1+DC role as biomarker in human cancers driving future developments in this field.

List of Publications and Presentations Resulting from the* Translational *Research Project "Role of XCR1+ (BDCA3high) dendritic cells in anti-tumour immune responses"

- Hubert M., Gobbini E., Couillault C., Vu Manh T.P., Doffin A., Berthet J., Rodriguez C, Ollion V., Kielbassa J., Sajous C, Treilleux I, Tredan O., Dubois B., Dalod M, BendrissVermare N., Caux C., Valladeau-Guilemond J. IFN-III is selectively produced by cDC1 and predicts good clinical outcome 2 in human breast cancer. *Science Immunology* 17 Apr 2020:Vol. 5, Issue 46, eaav3942
- M. Hubert* and E. Gobbini*, N. Bendriss-Vermare, C. Caux and J. Valladeau-Guilemond. Human Tumor-Infiltrating Dendritic Cells: From In Situ Visualization to High-Dimensional Analyses. Cancer 2019, July 30;11(8)
  *Co-first authors

List of Publications and Presentations resulting from other projects during the fellowship period (if applicable)

Full paper publications:

1. Thomas Pierret , Anne-Claire Toffart , Matteo Giaj Levra, Denis Moro-Sibilot, Elisa Gobbini. Advances and Therapeutic Perspectives in Extended-Stage Small-Cell Lung Cancer. Cancers 2020, 12, 3224
2. Elisa Gobbini, Aurélie Swalduz, Matteo Giaj Levra, Sandra Ortiz-Cuaran, Anne-Claire Toffart, Maurice Pérol, Denis Moro-Sibilot and Pierre Saintigny. Implementing ctDNA Analysis in the Clinic:Challenges and Opportunities in Non-Small Cell Lung Cancer. Cancers 2020, 12, 3112;
3. Elisa Gobbini; Julie Charles; Anne-Claire Toffart; Marie-Thérèse Leccia; Denis Moro-Sibilot; Matteo Giaj Levra. Literature meta-analysis about the efficacy of anti-programmed death protein 1 and anti-programmed death ligand 1 re-challenge in cancer patients. Accepted by Bulletin du Cancer
4. E.Gobbini, A. Toffart, M. Pérol, J.B. Assié, M. Durisseaux, D. Coupez, R. Gervais, V. Westeel, M. Delaunay, F. Guisier, R. Veillon, V. Gounant, E. Giroux Leprieur, FR. Vanel, N. Chaabane, E. Dansin, H. Babey, C. Decroisette, F. Barlesi, N. Girard, P. Fournel, L. Mezquita, Y. Oulkhouir, A. Canellas, B. Duchemann, O. Molinier, D. Moro-Sibilot, M. Giaj Levra. Immune checkpoint inhibitors (ICPis) re-

challenge: outcomes analysis in a French National cohort of Non-Small-Cell Lung Cancer (NSCLC) patients. Clin Lung Cancer. 2020 May 8;S1525-7304(20)3013. **Presented as Mini-Oral at the 2019 WCLC.**

5. Elisa Gobbini; Julie Charles; Anne-Claire Toffart; Marie-Therese Leccia; Denis Moro-Sibilot; Matteo Giaj Levra. Current opinions in immune checkpoint inhibitors rechallenge in solid cancers. Critical Reviews in Oncology/Hematology 2019, 144:102816.
6. E. Gobbini, R. Chiari, P. Pizzutillo, P. Bordi, L. Ghilardi, S. Pilotto, G. Osman, F. Cappuzzo, F. Cecere, F. Riccardi, V. Scotti, O. Martelli, G. Borra, E. Maiello, A. Rossi, P. Graziano, V. Gregorc, C. Casartelli, C. Sergi, A. Del Conte, A. Delmonte, C. Bareggi, D. Cortinovis, P. Rizzo, F. Tabbo', G. Rossi, E. Bria, D. Galetta, M. Tiseo, M. Di Maio, S. Novello. Real-world outcomes according to treatment strategies in ALK-rearranged Non-Small-Cell Lung Cancer (NSCLC) patients: an Italian retrospective study. Clinical and Translational Oncology 2019, Oct 19. Presented as Mini-Oral at the 2018 WCLC.
7. Julian Pinsolle, Anne Mcleer-Florin, Matteo Giaj Levra, Florence De Fraipont, Camille Emprou, Elisa Gobbini, Anne-Claire Toffart. Translating Systems Medicine into Clinical Practice: Examples from Pulmonary Medicine with Genetic Disorders, Infections, Inflammations, Cancer Genesis, and Treatment Implication of Molecular Alterations in Non-Small-Cell Lung Cancers and Personalized Medicine. Frontiers in Medicine, 2019 Oct 29;6:233.

Publications under review:

1. E. Gobbini, L Bertolaccini, N Giaj-Levra, J Menis, M Giaj-Levra. Epidemiology of oligometastatic NSCLC. Invited article under review for Translational Lung Cancer Research
2. E.Gobbini, J. Pinsolle, L. Schoutteten, T Pierret, I. Federspiel, D. Moro-Sibilot, M. Giaj Levra, AC Toffart. Immune checkpoint inhibitors and hospitalization at home: a French experience. Under review for Bulletin du Cancer

Selection of Courses and Workshops Attended During the Fellowship

1. Master 2 program in "Biology of Cancer" at Lyon 1 University and Cancer Research Center of Lyon (2019)
2. Multiple Departmental Seminars on various immunology issues at the Cancer Research Center of Lyon
3. Multiple educational French events on oncology field
4. ESMO 2019 and 2020 (Virtually)
5. WCLC 2019 and 2020 (Virtually)
6. AACR 2020 (Virtually)

Acknowledgements

A special thanks to my mentor, Jenny Valladeau, for tracking the project and supporting me into its development. Many thanks to all the research team, mainly to Hubert Margaux (Post-Doc), Anne-Claire Doffin (Technician), Sakref Candice (PhD student), Rocca Yamila (Post-Doc) and Justine Berthet (Technician) for forming me and driving me through the project.

References

Balan E, Ollion V, Colletti N, et al. Human XCR1+ dendritic cells derived in vitro from CD34+ progenitors closely resemble blood dendritic cells, including their adjuvant responsiveness, contrary to monocyte-derived dendritic cells. Journal of immunology (Baltimore, Md. : 1950. 193: 1622–35, 2014.

Barry KC, Hsu J, Broz ML, et al. A natural killer-dendritic cell axis defines checkpoint therapy-responsive tumor microenvironments.Nature medicine. 24:1178–1191, 2018.

Broz: Dissecting the Tumor Myeloid Compartment Reveals Rare Activating Antigen-Presenting Cells Critical for T Cell Immunity. Cancer Cell., in (ed Cancer Cell 26(5), 638-652), 2014

Deauvieau F, Ollion V, Doffin AC, et al. Human natural killer cells promote cross-presentation of tumor cell-derived antigens by dendritic cells. International journal of cancer. 136: 1085–94, 2015.

de Groen RA1, Boltjes A, Hou J et al. IFN-λ-mediated IL-12 production in macrophages induces IFN-γ production in human NK cells. Eur J Immunol.45(1):250-9, 2015.

Fuertes MB, Kacha AK, Kline J, et al.: Host type I IFN signals are required for antitumor CD8+ T cell responses through CD8{alpha}+ dendritic cells. J Exp Med 208:2005-2016, 2011

Hubert M, Gobbini E, Bendriss-Vermare N, et al. Human Tumor-Infiltrating Dendritic Cells: From in Situ Visualization to High-Dimensional Analyses. Cancers. 11: 1082, 2019.

Hubert M, Gobbini E, Couillault C, Vu Manh TP, Doffin AC, Berthet J, Rodriguez C, Ollion V, Kielbassa J, Sajous C, Treilleux I, Tredan O, Dalod M, Bendriss-Vermare N, Caux C, Valladeau-Guilemond J. IFN-III is selectively produced by cDC1 and predicts good clinical outcome in breast cancer. Submitted paper.

Le Mercier, I, Poujol D, Sanlaville A, et al.: Tumor promotion by intratumoral plasmacytoid dendritic cells is reversed by TLR7 ligand treatment. Cancer Res 73:4629-4640, 2013

Robbins SH, Walzer T, Dembele D, et al.: Novel insights into the relationships between dendritic cell subsets in human and mouse revealed by genome-wide expression profiling. Genome Biol 9:R17, 2008

Yoshio S, Kanto T, Kuroda S, et al.: Human blood dendritic cell antigen 3 (BDCA3) dendritic cells are a potent producer of interferon-lambda in response to hepatitis C virus. Hepatology, 2012

SIGNATURES	
Award Recipient full name	***Signature and Date***
Elisa Gobbini	8/10/2020

Research Mentor full name	***Signature and Date***
Jenny Valladeau	8/10/2020

This ESMO Translational Fellowship Research Project was supported by an educational grant from Bristol-Myers Squibb