BELSPO Final Report

Name	Martin Guilliams
Selection	2011
Host institution	Ghent University
Supervisor	Bart Lambrecht
Period covered by this report	from 01/01/2012 to 31/12/2013
Title	**Study of the functional role of the distinct lung and liver antigen presenting cell subsets in vivo.**

1. Objectives of the Fellowship

- Establish a novel line of research on the role of monocyte-derived DCs and MFs and prove my qualities as independent investigator
- Study the development of monocyte-derived DCs and MFs
- Study the cellular Origin of Alveolar MFs
- Generate a novel Kupffer Cell specific mouse cell line
- Generate a novel mouse line that allows to restrict MHCII expression on one single antigen-presenting cell subset at a time

2. Methodology in a nutshell

- Use of state-of-the-art multicolor flow cytometry to properly identify macrophage and dendritic cell subsets.
- Sort of distinct populations of interest to perform gene expression profile to identify genes specific to these populations in order to be able to generate novel knock-in mice
- Study of the gene expression profile to predict the function of the distinct macrophage and dendritic cell subsets.
- Construct novel knock-in mice to study macrophage and dendritic cell subsets in vivo.
- Unravel the role of macrophage and dendritic cell subsets in vivo

3. Results

A. Study the development of monocyte-derived DCs and MFs

We have studied the role of moDCs in the lung immune responses. This project was performed in close collaboration with Maud Plantinga. Timing: By joining forces we were able to study moDCs very efficiently and have been able to publish our findings in the Immunity journal. This was achieved ahead of schedule and yields a high impact paper within the first 13 months of the BELSPO fellowship. In this paper I have demonstrated that while CD11b+ cDCs migrate efficiently to the lymph nodes and are the principal DC subset inducing Th2 immunity, moDCs do not migrate efficiently to the lymph node but orchestrate local allergic inflammation in the lung (Plantinga/Guilliams et al. *Immunity* 2013). I am currently also finalizing a paper on the role of these cells during Flu infection. This will be submitted within the next 6 months.

B. Study the cellular Origin of Alveolar MFs

Although this was originally supposed to present a minor project we have unexpectedly found that Alveolar MFs are long-lived cells that derive mainly from embryonic precursors. The findings were

published in the Journal of Experimental Medicine. In this paper I have demonstrated that AMΦs derive from fetal monocytes that seed the lung before birth, differentiate into AMΦs under influence of GM-CSF, and then self-maintain throughout life. As such I **proposed a novel model for tissue-resident MΦ development** (Guilliams et al. *JEM* 2013). This manuscript represents a major breakthrough for me because: 1) I was sole corresponding author on it, 2) it was massively cited very rapidly, 3) it gave me a lot of visibility in the field of ontogeny of myeloid cells and due to this I have been invited to international meetings as speaker but was also invited to write review articles for prestigious journals like Nature Reviews Immunology and Immunological Reviews. 4) I am now regularly asked as external reviewer for macrophage and DC papers. Note that we also published a small article on our findings in the VIB journal that is read by the broad public.

C. Generate a novel Kupffer Cell specific mouse cell line

By the end of my PhD I had developed a chronic passion for KCs. However, I realized that I was lacking KC-specific tools to properly address the biological function of these cells. **Therefore, I devised a long-term strategy to generate the crucially needed KC-specific tools myself (Figure 1). First, using a micro-array-based systems biology approach, I identified a KC-specific gene that I dubbed the Liver Macrophage Marker (LMM).**

The tools (Figure 1) I have generated through this Belspo project bring a solution to the following needs:

No micro-array of KCs are available to perform *in silico* predictions of KC function

→ *I generated Immgen-compatible KC micro-arrays to be able to identify the genes that are specifically expressed in KCs as compared to other Immgen populations (Figure 2).*

Lack of a commercial antibody for a KC-specific surface marker to identify KCs *ex vivo*

→ *I generated a LMM-specific nanobody, allowing the identification of KCs by flow cytometry (Figure 1) and whole body imaging (Figure 3).*

There is no mouse model available to specifically deplete KCs *in vivo*

→ *I generated a LMM-YFP-DTR KI mouse, allowing not only the identification of KCs by their expression of the Yellow fluorescent Protein (YFP) in flow cytometry (Figure 1) and microscopy, but also allowing the depletion of KCs due to the expression of the Diphtheria Toxin receptor on KCs (Figure 1).*

As such the Kupffer Cell-DTR tools have been successfully generated and I am very proud to announce that these mouse models work perfectly in fact these are yielding results that go far beyond anything I had imagined. We are presenting our data in March at the International Macrophage & DC meeting in Montreal and are convinced this will yield very positive feedback from the community. The project is progressing well and we will be able to submit our manuscript soon.

The Kupffer Cell-CRE mice are now ready. We are going to test them for the first time at the end of this month. Since the construction is very similar to the Kupffer Cell-DTR mice we expect that this mouse model will work equally well.

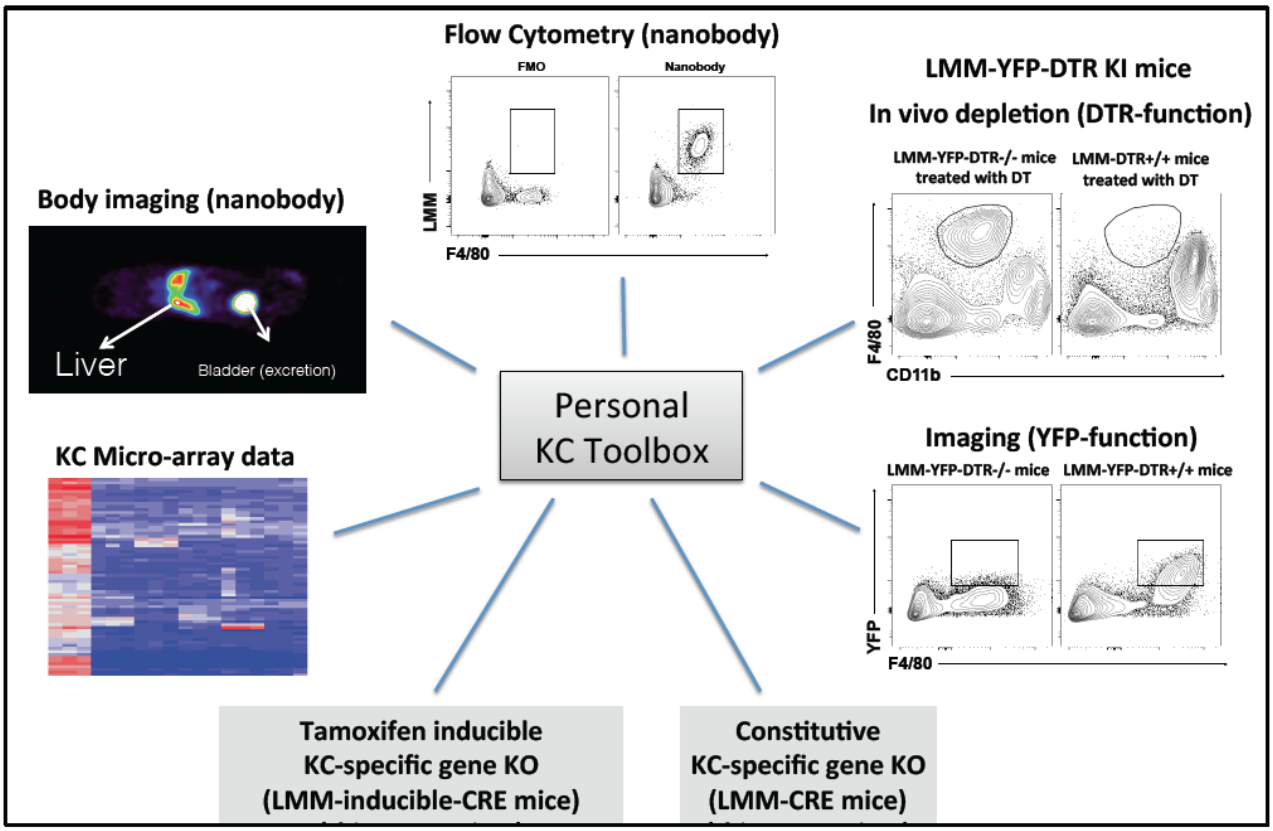


Figure 1: My personal Kupffer Cell Toolbox

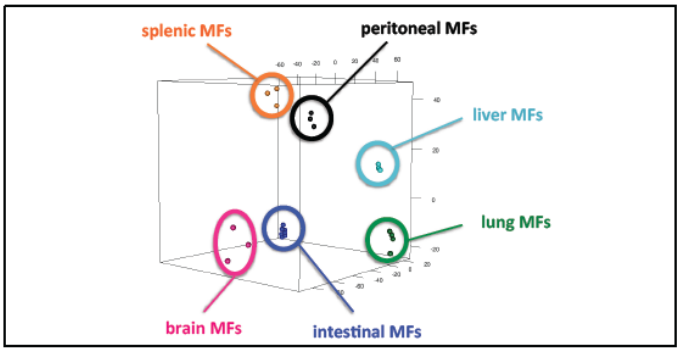
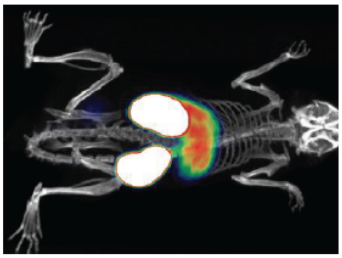


Figure 2: Principal Component analysis of tissue-resident MΦs from distinct organs. Lung MΦs and Liver MΦs (KCs) micro-arrays were generated in-house. Others MΦs were downloaded from www.Immgen.org. All arrays were performed on the same platform to allow direct comparison.



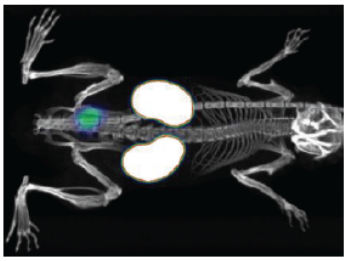


Figure 3: Whole body imaging using radioisotope technetium (^{99m}Tc) labelled LMM-nanobody labeled (above) or a control-nanobody (below). This emonstrates that LMM is only expressed in the liver (all naonobodies are excreted through the kidneys which explains that the kidneys are labelled with both the LMM- and the control-nanobody. We also validated by flow cytometry that the kidney does not contain LMM^{+} cells (not shown). Note that within the liver the only LMM^{+} cells are the F4/80^{hi} cells (see Figure 1). Moreover, within the liver of the LMM-YFP mice, the only YFP^{+} cells are also the F4/80^{hi} cells – i.e. the bona fide KCs.

D. Generate a novel mouse line that allows to restrict MHCII expression on one single antigen-presenting cell subset at a time

This is the only serious set-back we have encountered. The MHCII expression is too low to be functional and we have had to start the construction again. The ES cells are ready and we will generate the mice this year. We are confident the new strategy will work but until we validate is this remains uncertain.

E. Proposition of novel nomenclature for dendritic cells and macrophages

Because I feel that the nomenclature in our MΦ and DC field is leading to a lot of confusion, I have personally contacted the younger scientists that have, together with me, been the main drivers in the current conceptual MPS revolution and have invited them to join me in my effort to **define ontogeny as the main basis for determining the nomenclature of cells of the MPS** (authors are Florent Ginhoux, Shalin Naik, Barbara Schraml, Elodie Segura, Simon Yona, Claudia Jakubzick, Roxane Tussiwand and Nobuyuki Onai). The novel nomenclature has been published in Nature Reviews Immunology and can be summarized as shown in Figure 4.

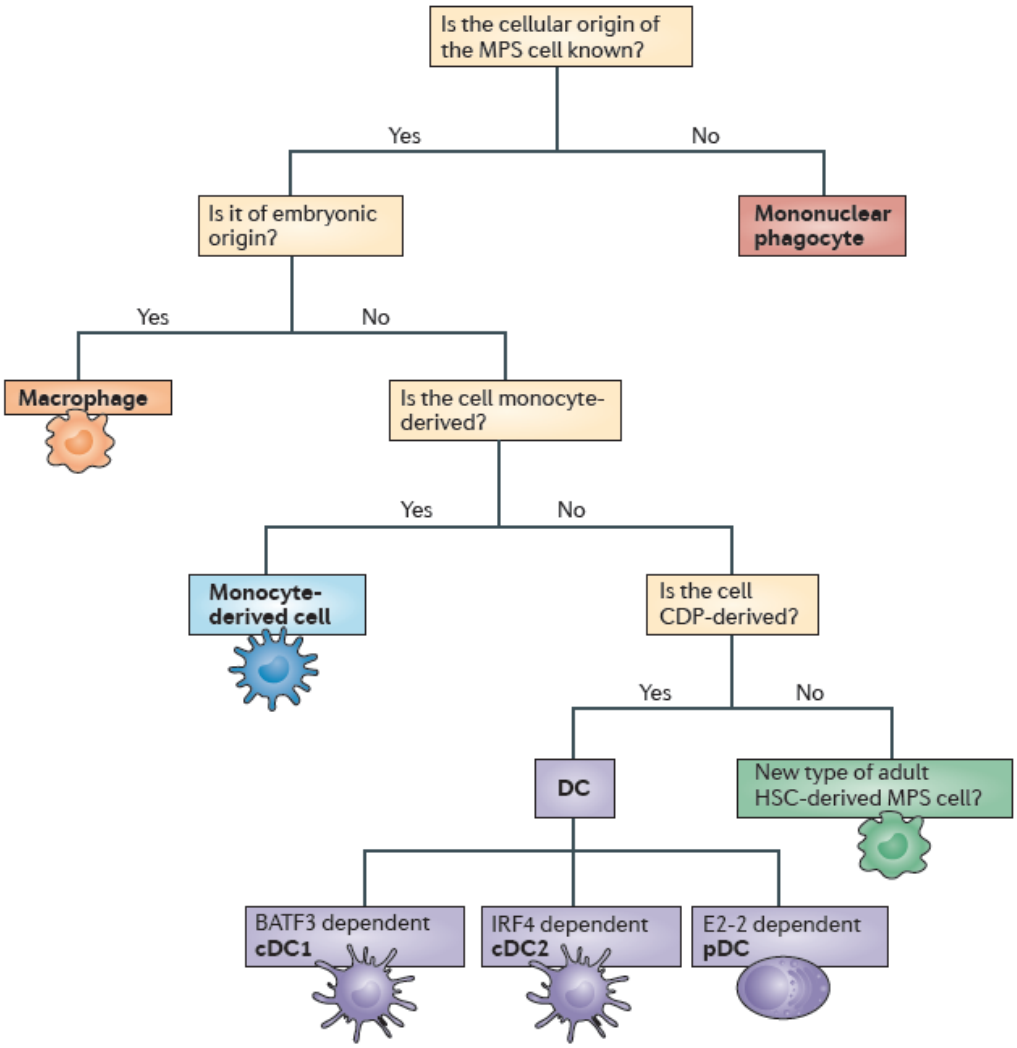


Figure 4: Proposed novel nomenclature based on Ontogeny rather than functional specialization (Guilliams et al. Nature Reviews Immunology 2014).

4. Perspectives for future collaboration between units

The generation of these macrophage specific tools will allow future collaborations on the study of Kupffer Cells. In fact we are collaborating with IMI in Charleroi for the role of Kupffer cells in tumor metastasis to the liver. As our tools allow to specifically eliminate Kupffer Cells from the very first experiment our collaborators in charleroi could demonstrate in 5 fold increase of the number of metastasis in the liver in absence of Kupffer Cells. This identifies the Kupffer Cells as a first line of defence against circulating tumor cells. We also soon launch a collaboration on the role of Kupffer Cells in iron metabolism with one of the best iron metabolism labs on the planet, the lab of Guenter **Weiss** in Innsbruck.

Closer to home we will initiate a collaboration with Ruth De Bruyne at the Ghent University Hospital to study human Kupffer Cells to be able to compare the gene expression profile of human Kupffer Cells with the once of mouse Kupffer Cells. It is important to realize that the functional specialization and the ontogeny of human Kupffer Cells is totally unknown. In fact, there are currently no Kupffer Cell specific markers to correctly distinguish Kupffer Cells from any liver infiltrating inflammatory macrophage.

Now that the KC-CRE tools are ready we will launch a collaboration within our institute: As macrophage and DC activation and function crucially depends on the NF-kB signaling pathway it will be most exciting to develop a collaboration with the Group of Dr. Rudy Beyaert. The generation of the Kupffer Cell-CRE KI mouse model will allow to directly study the role of IKKa, IKKb, A20 or JIK mediated regulation of NF-kB signaling within Kupffer Cells using the IKKa and IKKb floxed mice (both mouse strains are available at the CIML), the A20 floxed mice (available in the Beyaert Laboratory) or the JIK floxed mice (EUCOMM consortium) crossed on our Kupffer Cell-CRE KI mice.

5. Valorisation/Diffusion

Scientific Articles:

Articles as corresponding and/or last author:

Scott CL, Henri S, **Guilliams M[#].** Mononuclear phagocytes of the intestine, the skin and the lung. *Immunological Reviews*. 2014 Nov;262(1):9-24. **Special Issue on Macrophages. [#] = Corresponding/Last author.**

Guilliams M[#], Ginhoux F, Jakubzick C, Naik SH, Onai N, Schraml BU, Segura E, Tussiwand R, Yona S. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nature Reviews Immunology*. 2014 Aug;14(8):571-8. **[#] = Corresponding/Last author.**

Guilliams M[#], Bruhns P, Saeys Y, Hammad H and Lambrecht BN. The function of Fcγ receptors in dendritic cells and macrophages. *Nature Reviews Immunology*. 2014 Feb;14(2):94-108. **[#] = Corresponding/Last author. Recommended by "Immune Regulation News".**

Guilliams M*,[#], De Kleer I*, Henri S*, Post S, Vanhoutte L, De Prijck S, Deswarte K, Malissen B, Hammad H, Lambrecht BN. Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. *J Exp Med*. 2013 Sep 23;210(10):1977-92. *** = Equal contribution, [#] = Corresponding/Last author.**

Guilliams M[#], Lambrecht BN and Hamida Hammad. Division of labor between lung dendritic cells and macrophages in the defense against pulmonary infections. *Mucosal Immunol*. 2013 May;6(3):464-73. **[#] = Corresponding/Last author. Recommended by "Immune Regulation News".**

Lambrecht B, **Guilliams M[#]**. Monocytes find a new place to dwell in the niche of heartbreak hotel. *J Exp Med.* 2014 Oct 20;211(11):2136. Insight paper on Molawi et al. *J Exp Med* 2014. **[#] = Corresponding/Last author.**

Articles as First Author:

Tamoutounour S*, Guilliams M*, Montanana Sanchis F, Liu H, Terhorst D, Malosse C, Pollet E, Ardouin L, Luche H, Sanchez C, Dalod M, Malissen B, Henri S. Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin. *Immunity.* 2013 Nov 14;39(5):925-38. *** = Equal contribution. Recommended by "Immune Regulation News".**

Plantinga M*, Guilliams M*, Vanheerswynghels M, Deswarte K, Branco-Madeira F, Toussaint W, Vanhoutte L, Neyt K, Killeen N, Malissen B, Hammad H, Lambrecht BN. Conventional and Monocyte-Derived CD11b^{+} Dendritic Cells Initiate and Maintain T Helper 2 Cell-Mediated Immunity to House Dust Mite Allergen. *Immunity* 2013; 38(2):322-35. *** = Equal contribution. Recommended by "Faculty of 1000".**

Scientific Meetings:

- Annual Meeting of the *Société Française d'Allergologie* 2015 – Paris, France – **Invited Speaker**
- CIIT Science Day 2015 – Innsbruck, Austria 2015 – **Invited Speaker**
- Mini-Symposium 'Immune-mediated Inflammatory Diseases' – Ghent, Belgium – **Invited Speaker**
- International DC Symposium DC2014 – Tours, France 2014 – **Invited Organizer Nomenclature Round Table**
- Annual French Immunology Society Meeting, Lille, France 2014 – **Invited Speaker**
- Annual Belgian Dermatology Meeting, Brussels, Belgium 2014 – **Invited Speaker**
- Keystone Meeting on Macrophages – Santa Fe, USA 2013 – Poster Presentation
- Annual Meeting of European MF & DC Society – Erlangen, Germany 2013 – **Oral presentation**
- Keystone Meeting on Dendritic Cells – Keystone, USA 2013 – Poster Presentation
- EMBO Meeting on Macrophages – Marseille, France 2013 – Poster Presentation
- Annual Meeting of European MF & DC Society – Brussels, Belgium 2012 – **Oral presentation**
- Annual Meeting of Scandinavian Society for Immunology – Geilo, Norway 2011 – **Invited Speaker**

Seminars in Foreign Research Institutes:

- Hosted by Dr. Allen & Dr. Pollard – Edinburgh University, UK 2013
- Hosted by Dr. Mowat – Glasgow University, UK 2013
- Hosted by Dr. Woltman – Erasmus MC, The Netherlands 2012

International Visibility:

Recently, I have started to gain international visibility in the Marcophage and Dendritic Cell field as evidenced by the fact that I have been directly contacted as reviewer for journals. Moreover, I was **selected as Invited Speaker** for the Annual Meeting of Scandinavian Society for Immunology in 2011 and for the Joined Meeting of the French and the Belgian Immunology Societies that will be held in Lille in 2014. I am currently **writing an opinion paper for Nature Reviews Immunology on the nomenclature of Dendritic Cells and MΦs** (deadline for submission = April 2014) and was invited as **Organizer for a Round Table** on this subject at the DC2014 Symposium in Tour. Moreover, I was invited to write a **review paper for a special issue on Monocytes and MΦs in Immunological Reviews**. Furthermore, I was asked to **join the editorial board of the Cellular Immunology journal.**

6. Skills/Added value transferred to home institution abroad

I have transferred a couple of essential techniques and skills to the VIB institute. This includes mainly:

- Use of state-of-the-art multicolor flow cytometry to properly identify immune cells
- Use of excellent protocols to perform gene expression profile of cells of interest
- Construction of state-of-the art knock-in mice

I would like to illustrate this through the following. The VIB has a Nucleomics facility where they perform micro-arrays for all the VIB teams. We had performed a screen for the gene-expression profile of many macrophage and dendritic cell subsets, both in the steady-state and during inflammation and we have asked them to perform a micro-array analysis on more than 150 samples. Once they received our material they contacted me to say that in 15 years they had never received samples of that quality since every single sample had extremely high RNA quality. As a result we were asked to provide a state-of-the-art protocol to

perform these type of experiments for the whole VIB institute (an institute with more than 1000 scientists in total in Leuven, Ghent, Antwerp and Brussels.

7. Additional information

Thanks to the support of Dr. Bart Lambrecht I could successfully launch my own line of research within the laboratory. In addition to the Belspo grant I have also applied for a Odysseus Fellowship, FWO Project grants and a Maie-Curie Career Integration Grant (CIG) on the role of monocyte-derived DCs and MFs and obtained almost 1.500.000€ through both fellowships and assurance for 5 years of work. This has allowed me to be ambitious and launch several high risk/high gain projects. In the mean time I have set-up my own small team (called the ONSET team for ONtogeny and Specialization of myeloid cell subsETs) am applying for 2 Professorship position at the UGent. I am in the final round for both calls and this is due to the fact that I could launch my own line of research thanks to the Belspo and the other grants and prove my quality as independent investigator.

Funding:

- Belspo Return Grant — 25.000€ for 2 years, covering research costs, 2012-2013
- Marie-Curie Career Integration Grant — 100.000€ for 4 years, covering research costs, 2012-2015
- Odysseus Return Grant — 980.000€ for 5 years, covering research costs, 2013-2017
- FWO Research Project I — 80.000€ for 5 years, covering research costs, 2014-2018
- FWO Research Project II — 300.000€ for 5 years, covering research costs, 2015-2019

TOTAL = 1.485.000€ for the period from 2012 to 2018

External Reviewer for:

- Mucosal Immunology
- European Journal of Immunology
- Critical Reviews in Microbiology
- Journal of Innate Immunity
- Expert Review of Vaccines
- Cellular Immunology
- Scientific Reports

The ONSET team members:

• Sofie De Prijck: Technician 2013 – 2017
My role: PI, salaried on my personal Odysseus Grant
• Charlotte Scott: Post-Doc who will study the role of KCs in Oral Tolerance 2013 – 2017
My role: PI, salaried on my personal Odysseus Grant
• Dorine Sichien: PhD Student 2012 – 2016
My role: PI, Co-promotor, **salaried on FWO Fellowship written by me**
• Liesbet Martens: Bachelor in Bio-informatics in 2013, now part of the VIB Bio-IT Core 2012 – 2013
My role: PI, Co-promotor for her Bachelor
• Lianne van de Laar: Post-Doc 2012 – 2015

My role: PI, salaried on her personal EMBO fellowship

8. My project in 5 keywords:

Dendritic Cell, Macrophage, Antigen-presentation, Ontogeny, Immunity

9. Summary:

During this Belspo research project I have studied the cellular origin and functional specialization of liver and lung myeloid cells. Myeloid cells are primarily composed of dendritic cells, macrophages and monocytes. First, we have found that conventional dendritic cells migrate efficiently to the lymph nodes and are the principal DC subset inducing Th2 immunity during a model of allergic asthma. In contrast we found that monocyte-derived cells do not migrate efficiently to the lymph node but orchestrate local allergic inflammation in the lung (Plantinga/Guilliams et al. *Immunity* 2013). Second, we have studied the cellular origin of macrophages in the lung and found that Alveolar lung Macrophages derive from fetal monocytes that seed the lung before birth, differentiate into AMΦs under influence of GM-CSF, and then self-maintain throughout life. As such we **proposed a novel model for tissue-resident MΦ development** (Guilliams et al. *JEM* 2013). This paper is part of a conceptual revolution in the macrophage field as it induced the collapse of the mononuclear phagocyte system (MPS) dogma originally proposed by van Furth almost 50 years ago. The MPS dogma states that all MΦs constantly derive from circulating monocytes. However, two landmark papers, one of which I contributed to, demonstrated that most tissue-resident MΦs do not derive from circulating monocytes (Schulz et al. *Science* 2012, Yona et al. *Immunity* 2013) but instead develop before birth and then self-maintain throughout adult life. Our study further confirmed these studies for the Alveolar Macrophages. In fact, because our new data did not fit the current nomenclature of macrophages and dendritic cells, I have personally contacted the younger scientists that have, together with me, been the main drivers in the current conceptual revolution in the field and have invited them to join me in my effort to **define ontogeny as the main basis for determining the nomenclature of macrophages and dendritic cells** (Guilliams et al. Nature Reviews Immunology 2014).

This BELSPO project also had as aim to build tools to be able to unravel the accessory function of the most abundant MΦ in the body, the liver resident Kupffer Cell (KC). KCs are one of the oldest immune cells known to man and are considered as the prototypical example of tissue-resident MΦs. Paradoxically, very little is known about the function of these cells. Therefore during this project we generated the first Kupffer Cell specific knock-in mice and constructed Kupffer Cell-YFP-DTR mice that allow to (i) visualize KCs by flow cytometry and intravital imaging due the expression of the Yellow Fluorescent Protein (YFP) in these mice and (ii) specifically deplete KCs *in vivo* via injection of diphtheria toxin (DT) since KCs are the only cells

to express the human diphtheriatoxin-receptor (DTR) in these mice. These LMM-YFP-DTR mice are now ready and function perfectly and form the basis of my future research strategy.