In September 2018, I visited the Department of Applied Physics at KTH Royal Institute of Technology in Stockholm, Sweden for two weeks. I learned a microchip-based technique to perform single cell screening and time-lapse experiments, developed by Björn Önfelt's research group. The aim of the work visit was to elucidate the serial killing properties of our CD34+ progenitor-derived Natural Killer (NK) cells and to start up a collaboration with Björn Önfelt's group.

My project at the Radboudumc in Nijmegen focuses on maximizing immunotherapy for ovarian cancer using highly active NK cells generated ex vivo from CD34+ progenitor cells from cord blood. One of the goals of my project is to unravel the serial killing properties of these CD34+ progenitor-derived NK cells, in order to identify and apply ways to expand the serial killers in our method for superior efficacy of our NK cell product in cancer. In initial 24h time-lapse bright field microscopy experiments in Nijmegen using a 3D collagen-matrix model we found that our NK cells serially kill up to 3 SKOV-3 (ovarian cancer cell line) cells. Since the number of wells is limited in this experimental set up (only 6 wells of a 96 wells plate can be used per experiment) and analysis has to be performed manually, it is highly time-consuming to analyze numerous NK cells. Furthermore, it is hardly possible to track and analyze serial killing properties in wells seeded with higher densities of tumor cells. As sensing of a neighboring viable tumor cell, after giving the lethal hit and detachment of the previous killed tumor cell, is necessary for NK cells to perform subsequent killing it is very difficult to analyze serial killers in this model. Hence, we started a collaboration with Björn Önfelt who has developed a unique microwell system to study serial killing, allowing analyses of thousands of wells containing one or few NK cells per well. Moreover, they have developed software to perform automated analysis of the screening experiments. As they have optimized this technique using a leukemia cell line, we will first use these cells for the experiments in Stockholm.

In Stockholm, I have performed screen-like experiments where we studied single NK cells and their individual cytotoxic potential against K562 and THP-1 (both leukemia) cells in the presence of supporting growth factors IL-2, IL-15 or a super IL-15 agonist complex. These experiments are being analyzed by an automatic counting program. Furthermore, we have performed time-lapse experiments with a similar set-up to analyze sequential killing behavior of NK cells in the presence of IL-2, IL-15 or IL-15 super agonist. Analysis of these experiments will require manual work and still has to been done using Fiji software analysis for which I am currently following a course in Nijmegen.
We have used NK cells from two different donors in order to perform statistics. Cells of the first donor were used to optimize the experiments with our NK cell product: we tested various media and staining procedures with fluorescent dyes to visualize the NK cells, leukemia cells and dead cells after a lethal hit. With the optimal medium and staining procedure, I have performed the first real screening experiment with K562 and NK cells from the first donor. In the second week, I could successfully perform all experiments with the second NK cell donor. A post-doc from the Önfelt group will repeat these experiments for 2 more donors. In the meantime, we are analyzing all data which will be incorporated in an important figure in one of my manuscripts in preparation.

Next to all the experiments that I performed, I gave a presentation of my PhD project for Björn Önfelt's group. As all of the people in his group work on NK cells and most of the people have a background in biotechnology or physics, this led to interesting discussions and useful suggestions for future research, including performing single-cell RNA sequencing on serial killer NK cells. Moreover, I attended a Biophysics seminar, with presentations from 4 different departments, ranging from NK cell research to discovering the crystal structure of a lactose transporter.

It was a great experience to do this work visit abroad, not only for my scientific progress, but also for my personal development and social skills. Last but not least, Stockholm is a beautiful city with colorful buildings and a lot of interesting museums visited in the weekend, for me the ABBA museum was one of the highlights☺. I would like to thank the Dutch Society for Immunology for their financial support for this work visit.


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