

In Vitro NK Cell Transdifferentiation from Murine Myeloid Progenitors

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Part 1: Research Report

Introduction

Natural Killer (NK) cells are specialised white blood cells that are part of the innate immune system. The system is present from birth, acting as the body's first line of defence to all bodily intrusion. It responds in a non-specific way with general effectiveness.¹ As opposed to the adaptive immune system, the innate immune system requires no previous sensitisation allowing it to rapidly respond (within just minutes to hours).²

NK cells are primarily responsible for immunosurveillance, where they work to identify and destroy cells of potential harm. This includes virus-infected cells and tumorigenic cells - abnormal cells that have the potential to divide uncontrollably, forming malignancies. The NK's role is to lyse harmful and cancerous cells before the body is damaged. They identify cells by their abnormal surface, before releasing a cytotoxin attack that damages the membrane, causing lysis.³

Due to the destructive properties of NK, scientists are studying them as a novel therapy for cancer, looking to exploit their anti-tumoral properties. NK cell-based immunotherapies offer a promising cancer treatment following high success rates and low side effects during clinical studies.⁴ NK cells are proliferated and/ or enhanced before being reinfused into patient's bloodstream, heightened their immune response.⁵

However, there are limitations when investigating immune-cell differentiation and function, due to insufficient experimental systems available. For this reason, Hoxb8 - a hematopoietic progenitor cell line (HPC) from murine bone marrow - is being

¹ Aristizábal, B. and González, Á. 2013. Innate immune system [Online]. El Rosario University Press. [Accessed 24 July 2026]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK459455/>.

² Informed Health 2023. The innate and adaptive immune systems [Online]. Institute for Quality and Efficiency in Health Care (IQWiG). [Accessed 31 July 2026]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK279396/>.

³ Thermo Fisher Scientific 2016. Natural killer cells overview | Thermo Fisher Scientific - US. Thermofisher.com. [Online]. [Accessed 24 July 2026]. Available from: <https://www.thermofisher.com/hk/en/home/life-science/cell-analysis/cell-analysis-learning-center/immunology-at-work/natural-killer-cell-overview.html#mediated-killing>.

⁴ Ma, S., Yu, J. and Caligiuri, M.A. 2025. Natural killer cell-based immunotherapy for cancer. The Journal of Immunology. **214**(7). [Accessed 31 July 2026]. Available from: <https://pubmed.ncbi.nlm.nih.gov/40246292/>

⁵ ASIA IMMUNOTHERAPY 2026. Natural killer cell (NK) therapy | asia immunotherapy | authority on immune cell therapy - Dr. yoichi kato. Asiaimmune.com. [Online]. [Accessed 24 July 2026]. Available from: <https://www.asiaimmune.com/en-hk/immunotherapy/nk-therapy>.

investigated as a robust source of progenitor cells. The Hoxb8 gene is regulated by oestrogen and the cell line can be expanded in FLT3 to conditionally immortalise early HPCs. This cell line is known as Hoxb8-FL, and has the potential to differentiate into myeloid and lymphoid cells.^{6,7} While human equivalents (CD34+ HPCs) have successfully transdifferentiated into CD56+ cells (NK1.1+ human equivalent), a murine version has yet been developed.⁷ This finding suggest although *differentiation* was thought to be exclusively lymphoid when achieving NK, there's potential for *transdifferentiation* using the myeloid lineage.⁷

Research Objectives

During research, my aim was to develop a protocol that would allow the Hoxb8 cell line to transdifferentiate into NK cells; this uses a myeloid progenitor cell to try to transdifferentiate along the lymphoid lineage. A combination of cytokine addition, and estrogen and FLT3L removal was tested to see if the fate of Hoxb8-FL cell line could be altered.

My primary objective was to obtain **NK cells** from the murine myeloid progenitor cell line, Hoxb8-FL. The aim was to achieve a small percentage increase in **NK1.1+** (marker) during flow cytometry.

My secondary objective was to expand the myeloid progenitors following their natural lineage, culturing **macrophage** immune cells. This would allow a comparison between the two fates of Hoxb8-FL.

Research Method

First a stable source of Wild Type (WT) Hoxb8-FL cells was required. Prior to my arrival, Hoxb8 cells with derived and cultured in FLT3-L, to conditionally immortalise the protein-coding gene.

The cell line required passaging frequently using a standard culture medium, to prevent cell death from malnutrition and overcrowding. To prepare this, Advanced RPMI 1640 Medium (Gibco, Cat. No. 12633012) was supplemented with 10% Foetal Bovine Serum (FBS), 1% Penicillin-Streptomycin-Glutamine (PSQ), 0.1% β -mercaptoethanol, β -

⁶ Redecke, V., Wu, R., Zhou, J., Finkelstein, D., Chaturvedi, V., High, A.A. and Häcker, H. 2013. Hematopoietic Progenitor Cell Lines with Myeloid and Lymphoid Potential. *Nature Methods*. 10(8), pp.795–803. [Accessed 31 July 2026]. Available from: <https://www.nature.com/articles/nmeth.2510>.

⁷ Grzywacz, B., Kataria, N., Kataria, N., Blazar, B.R., Miller, J.S. and Verneris, M.R. 2011. Natural killer–cell differentiation by myeloid progenitors. *Blood*. 117(13), pp.3548–3558. [Accessed 31 July 2026]. Available from: <https://www.nature.com/articles/nmeth.2510>.

estradiol (272 ng/mL) and 70ng/mL Fms-like tyrosine kinase 3 ligand (FLT3L) in a 50mL conical tube.

Normal Medium for culture (half FLT3)					
Reagent	Required Conc (ng/ml)	Stock conc (ng/ml)	Volume (ul)	Total Volume (ul):	50,000
RPMI	-		44,400		50mL
FBS	10%		5000		
PSQ	1%		500		
b-mercap	0.10%		50		
B-estradiol	272.38	50,000,000	0.27238		
FLT3L	70	70000	50		

For cell passaging, cells were collected and centrifugated at 12,000 rpm, 300g for 3 minutes. Supernatant was removed and the pellet was resuspended in medium, then transferred to a 6 well plate using 1:50 and 1:20 ratios. Cells were incubated in 37°C atmosphere containing 5% CO₂.

Cytokine Preparation

The powdered cytokine stock was centrifuged at 300g for 3 mins, then dissolved in a buffer solution of 0.2% Bovine Serum Albumin (BSA) in Phosphate-Buffered Saline (PBS). The correct volume of buffer was added to the stock mass of each cytokine to achieve the desired concentration needed for the culture mediums. Solutions were aliquoted into 1.5mL vials of 50µL each and placed into -25°C freezer.

Control Mediums (CM)

Control cultures were required for comparative purposes against differentiated cells. This allowed the effects of the cytokines and changed conditions to be analysed. The first control culture came from the maintained WT Hoxb8 cell line, as described above (CM 1). CM 2 included no cytokines, standard FLT3L concentration (70ng/mL) and no β - estradiol.

Control Medium 2

Transdifferentiation CONTROL					
Reagent	Required Conc (ng/ml)	Stock conc (ng/ml)	Volume (ul)	Total Volume (ul):	15,000
RPMI	-		74		15mL
FBS	10%		1500		
PSQ	1%		150		
b-mercap	0.10%		15		
FLT3L	70	70000	15		

Lastly, CM 3 encouraged macrophage differentiation, allowing a comparison between the cells of the culture mediums. This was to see if NK had been achieved during *transdifferentiation* or macrophage during *differentiation* of the Hoxb8-FL.

Control Medium 3

Transdifferentiation to macrophage CONTROL					
Reagent	Required conc (ng/ml)	Stock conc (ng/ml)	Volume	Total volume (ul):	15,000
RPMI			13,305		15mL
FBS	10%		1500		
PSQ	1%		150		
b-mercap	0.10%		15		
FLT3L	70	70,000	15		
M-CSF	50	50,000	15		

Culture Mediums

A variety of culture mediums were prepared throughout the project, changing concentrations and reagents, to see their effects on NK transdifferentiation.

Medium 1 – All Cytokines

The initial differentiation medium experiment was an alteration of the previously discussed study, where human equivalent HPCs and NK markers were used (CD34+ myeloid HPCs were transdifferentiated into CD56+ NK cells).⁷ Here the standard culture medium ('Normal Medium') had β -estradiol (272 ng/mL) removed and Flt3L reduced to 10ng/mL. A combination of cytokines were added: IL-3 (5ng/mL), IL-7 (20ng/mL), IL-15 (10ng/mL) and rhSCF (20ng/mL). Cells were washed in 2mL PBS following centrifugation to remove old medium, before being resuspended.

Transdifferentiation to NK medium					
Reagent	Required Conc (ng/ml)	Stock conc (ng/ml)	Volume (ul)	Total Volume (ul):	50,000
RPMI	-		44140		50mL
FBS	10%		5000		
PSQ	1%		500		
b-mercap	0.10%		50		
FLT3L	10	5000	100		
IL-3	5	25,000	10		
IL-7	20	20000	50		
IL-15	10	10000	50		
rhSCF (only human avail	20	10000	100		

Medium 2

During round one of culturing, Hoxb8 were also cultured in a standard medium with only IL-2 (10ng/mL). IL-2 is secreted by T-cells which activate NK function.³ This medium hoped to replicate this natural process, initiating NK transdifferentiation.

Transdifferentiation with IL2 only					
Reagent	Required Conc (ng/ml)	Stock conc (ng/ml)	Volume (ul)	Total Volume (ul):	50,000
RPMI	-		44250		50mL
FBS	10%		5000		
PSQ	1%		500		
b-mercap	0.10%		50		
FLT3L	10	5000	100		
IL-2	10	5,000	100		

Medium 3

A culture medium like that of Medium 1 was prepared, however FLT3L concentration was increased from 10ng/mL to 70ng/mL.

Transdifferentiation to NK medium (more FLT3L)					
Reagent	Required Conc (ng/ml)	Stock conc (ng/ml)	Volume (ul)	Total Volume (ul):	50,000
RPMI	-		44190		50mL
FBS	10%		5000		
PSQ	1%		500		
b-mercap	0.10%		50		
FLT3L	70	70000	50		
IL-3	5	25,000	10		
IL-7	20	20000	50		
IL-15	10	10000	50		
rhSCF (only human avail	20	10000	100		

Medium 4

Cells were cultured in mediums that kept β -estradiol (present in the standard medium) yet at decreased concentrations. A high concentration of 100ng/mL was prepared. All cytokines (IL-3, 7, 15 and SCF) were present to keep conditions constant.

Transdifferentiation to NK medium inc. high B-ESTRADIOL					
Reagent	Required Conc (ng/ml)	Stock conc (ng/ml)	Volume (ul)	Total Volume (ul):	15,000
RPMI	-		13220		15mL
FBS	10%		1500		
PSQ	1%		150		
b-mercap	0.10%		15		
B-estradiol	100	40,860	37		
FLT3L	70	70000	15		
IL-3	5	25,000	3		
IL-7	20	20000	15		
IL-15	10	10000	15		
rhSCF (only human avail	20	10000	30		

Medium 5

A medium of low β -estradiol concentration (30ng/mL) was also prepared. Again, all cytokines (IL-3, 7, 15 and SCF) were present.

Transdifferentiation to NK medium inc. small B-ESTRADIOL					
Reagent	Required Conc (ng/ml)	Stock conc (ng/ml)	Volume (ul)	Total Volume (ul):	15,000
RPMI	-		13246		15mL
FBS	10%		1500		
PSQ	1%		150		
b-mercap	0.10%		15		
B-estradiol	30	40,860	11		
FLT3L	70	70000	15		
IL-3	5	25,000	3		
IL-7	20	20000	15		
IL-15	10	10000	15		
rhSCF (only human avail	20	10000	30		

Cell Analysis

Cells were culture for 7 days each and passaged frequently throughout the week for a steady supply of nutrients. Following 7 days of culture, cells were harvested and prepared for flow cytometry.

Cell Counting

10 μ L of resuspended cells were counted using 0.4% Trypan Blue Solution (10 μ L) to test for cell health prior to cytometry. A hemacytometer was loaded with 10 μ L of 1:1 dye to cell solution and live cells were counted under a microscope. Average cell count was calculated. If viable cell count fell between 10⁵-10⁶ flow cytometry could proceed.

Cell Collection

Suspension was collected, centrifugated and supernatant was removed into a 5mL rounded bottom tube. Wells were washed with 1 mL PBS, then PBS was removed. To dissociate adherent cells, 2mL TrypLE was added to each well, then incubated at 37°C for 5 mins. Suspension was neutralised with 2mL of the corresponding culture medium.

Flow Cytometry Preparations

Cell suspensions, collection suspensions (previously discussed) and control mediums were all prepared for analysis. Cell pellets were collected (centrifugation at 300g for 3 mins, supernatant removed) and resuspended in 5% FBS in PBS (100 μ L). Marker antibodies were added to each sample: 1 μ L at 0.2mg/mL of PE anti-mouse NK-1.1 Antibody (BioLegend Cat. No. 108707) and 1 μ L at 0.2 mg/mL of APC anti-mouse F4/80 Antibody (BioLegend Cat. No. 123116). Samples incubated in dark environment at 4°C for 30 mins. Suspensions were washed with PBS (3mL) and centrifuged. DAPI in PBS (1:1000) was added to each sample (200 μ L/ tube).

Flow Cytometry

Using BD FACSymphony A1, samples were analysed to produce analytical drop plots. Plots were analysed using FlowJo, Version 10.8.1.

Macrophage Staining

Adhesive cells were collected as previously described (Cell Collection section) before being centrifugated, supernatant removed and resuspended. Cells were **counted** and **seeded** at 3x10⁴ per well in a μ -Slide 8 Well high chamber. Cells were **fixed** with 4% PFA for 15 mins at room temperature, then washed 3 times with PBS. Cells were **permeabilised** with 0.1% Triton X-100 in PBS (incubated 15 mins at room temperature) and washed twice in PBS. Cells were **blocked** with 5% BSA in PBS for 1 hour at room temperature (RT) before solution was removed. **Primary antibody** (F4/80) was diluted in 1% BSA in PBS in 1:100 ratio. 120 μ L was added to each well and incubated at RT for 2 hours. Cells were washed in PBS 3 times for 5 mins each. **Secondary antibody** staining used Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647 (Cat. No. A-21247) in 1:1000 dilution with 1% BSA in PBS (incubated in dark RT environment for 2 hours then washed using same 3x5min protocol). Cells were stained with **DAPI** in PBS (1:1000) for 15 mins in dark, RT conditions. Again, washed using 3x5min protocol. Mounting medium was added and cells were visualised under confocal microscope.

Method Limitations

As shown by Grzywacz et al. (2011)⁷, 28 days allowed for the highest proportion of cell differentiation, with each extra week (days 21 and 28) of culturing providing significantly higher NK rates. During my 6 weeks research period however, there was not enough time to culture the cell lines for this amount of time. For this reason, the highest possible proportion of NK differentiation could not be achieved. For future research, I will culture my cells for longer periods, analysing each week to compare how these rates change.

Results

Control Medium 2 and Culture Medium 2 could not be analysed due to cell death. However, every other sample was analysed using both microscopy imaging and flow cytometry. Macrophages (Control Medium 3) were also stained using immunocytochemistry staining.

Morphology – Microscopy Images And Staining

Cells were imaged at 40x and 20x and processed using Fiji (ImageJ2) Version 2.16.0. Images had their colour balance adjusted, were scaled using known pixel size and had scale bars added.

Control Culture Experiments

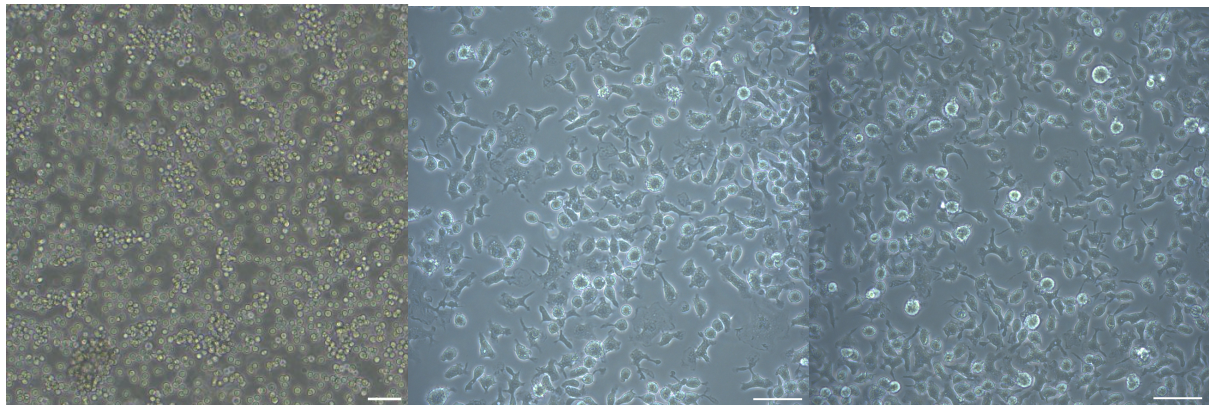


Figure 1 – Control-culture microscopy images. Left to right - LEFT: WT Hoxb8 cell line – day 3 (scale bar = 200µm) CENTRE and RIGHT: Macrophage culture – day 7 (scale bar = 50µm)

A stable and healthy population of WT Hoxb8 is shown in the left-hand image. A high proportion of macrophage can be seen using Control Culture 3 - images centre and right.

Cytokine Experiments – Culture Mediums 1 & 2

Using FLT3 and IL2 experimentally

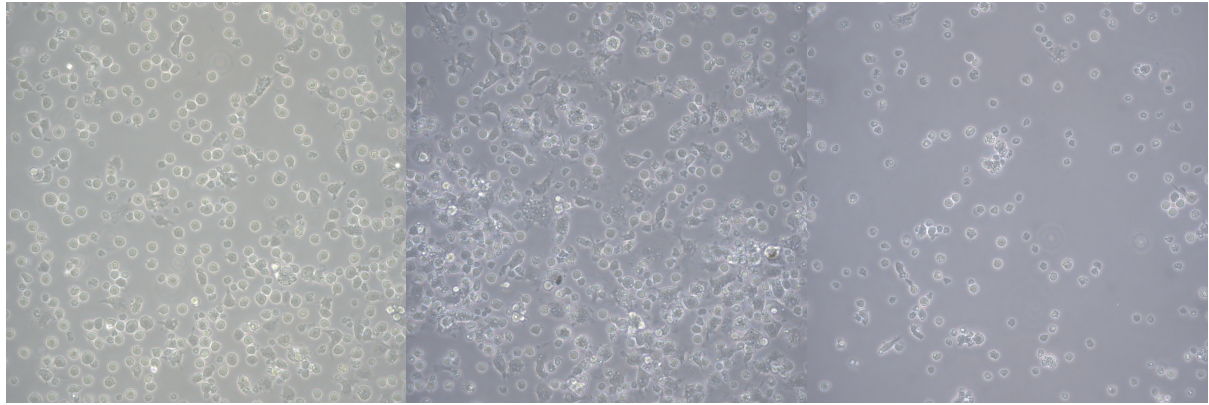


Figure 2 – Cytokine culture experiments (mediums 1 & 2) microscopy images. LEFT: medium 1– day 4 CENTRE: medium 1 – day 5 RIGHT: medium 2 – day 4

Between the left and central photo, a large difference can be seen in macrophage populations, only one day apart. Medium 2 (right photo) shows a decreased cell density.

Cytokines Experiments – Culture Mediums 4 & 5

Using β -estradiol experimentally

Day 5

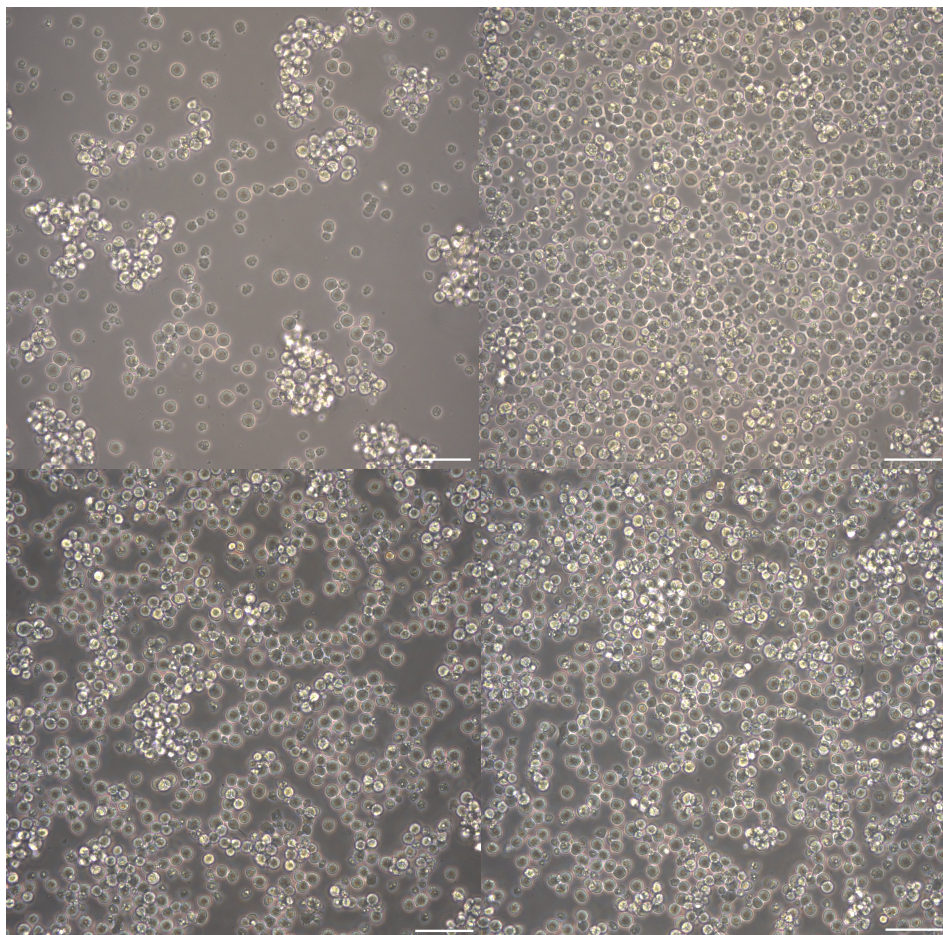


Figure 3 - Cytokines and β -estradiol culture experiments (mediums 4 & 5) microscopy images. TOP ROW: 100ng/mL β -estradiol culture (4) – day 5 (scale bar = 50 μ m). BOTTOM ROW: TOP ROW: 30ng/mL β -estradiol culture (5) – day 5 (scale bar = 50 μ m)

Day 11

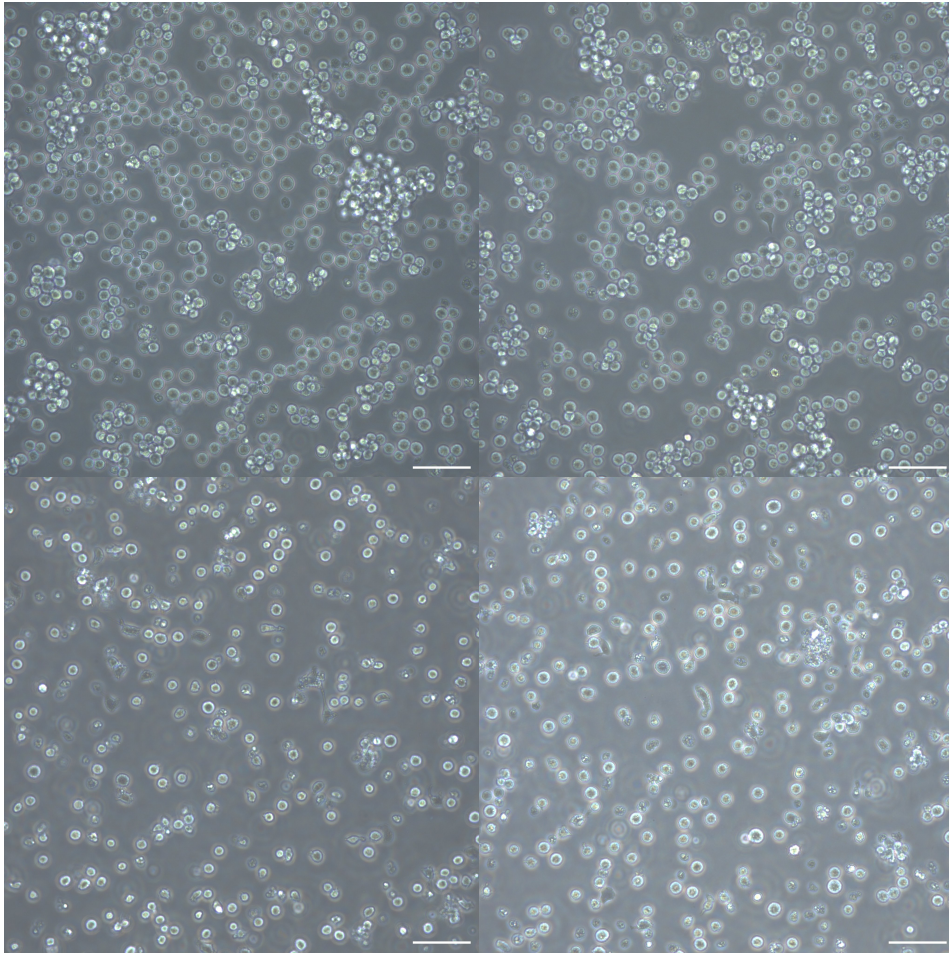


Figure 4 - Cytokines and β -estradiol culture experiments (mediums 4 & 5) microscopy images. TOP ROW: 100ng/mL β -estradiol culture (4) – day 11 (scale bar = 50 μ m). BOTTOM ROW: TOP ROW: 30ng/mL β -estradiol culture (5) – day 11 (scale bar = 50 μ m).

Macrophage Staining Images

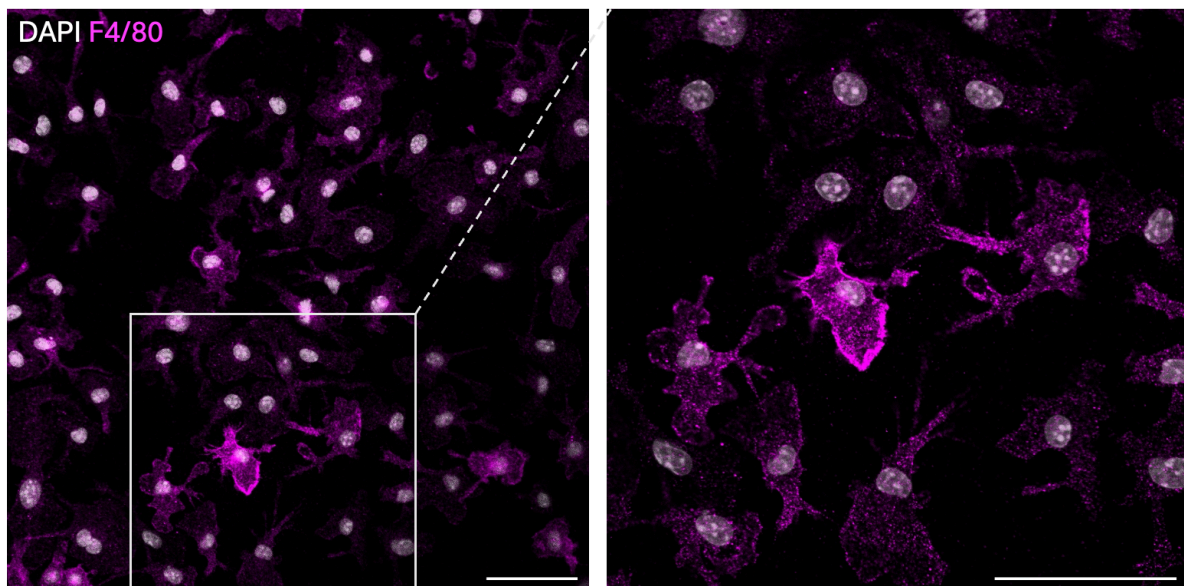


Figure 5 - Immunofluorescence staining of Hoxb8 cells after 7 days of differentiation with M-CSF. Scale bar = 50 μ m.

Macrophage cells were stained for F4/80 (magenta), using APC anti-mouse F4/80 Antibody, which is a cell surface protein marker for mouse macrophages. DAPI was used to stain the nucleus of cells (white).

Flow Cytometry

To measure the difference between basal level NK count in WT Hoxb8-FL and transdifferentiated NK cell count, flow cytometry was used as an NK cell assay. Cells were initially gated for **lymphocytes** using SSC-A and FSC-A, then **single cells** using FSC-H to remove doublets. As mentioned previously, **dead cells** were stained using DAPI and live cells were gated using BV421-A.

First Round

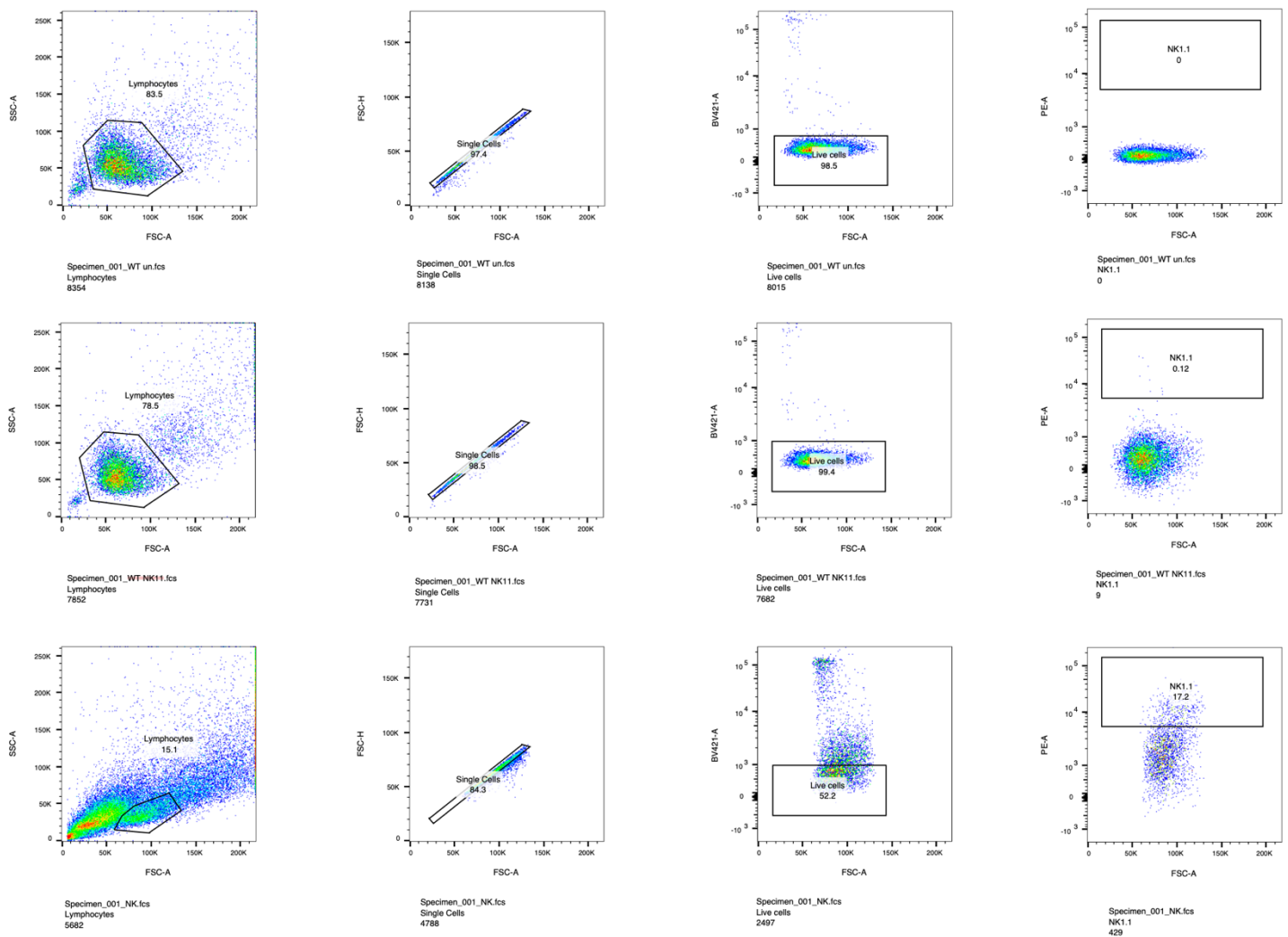


Figure 6 – Flow Cytometry of Control and Culture Mediums stained with NK1.1 Antibody TOP ROW: WT Hoxb8 unstained cells MIDDLE ROW: WT Hoxb8 stained BOTTOM ROW: Culture Medium 1

The control-stained WT Hoxb8 cell line shows a high percentage of **lymphocytes** recorded of 78.6% compared to the bottom row (Culture medium 1 – all cytokines and low FLT3L) having only 15.1%. **Live** cell count was 99.4% of WT Hoxb8 control when stained with DAPI, compared to 52.2% of Culture Medium 1. However, NK1.1 positive cells were much higher in Culture Medium 1 (17.2%) compared to 0.12% of WT Hoxb8.

Second Round

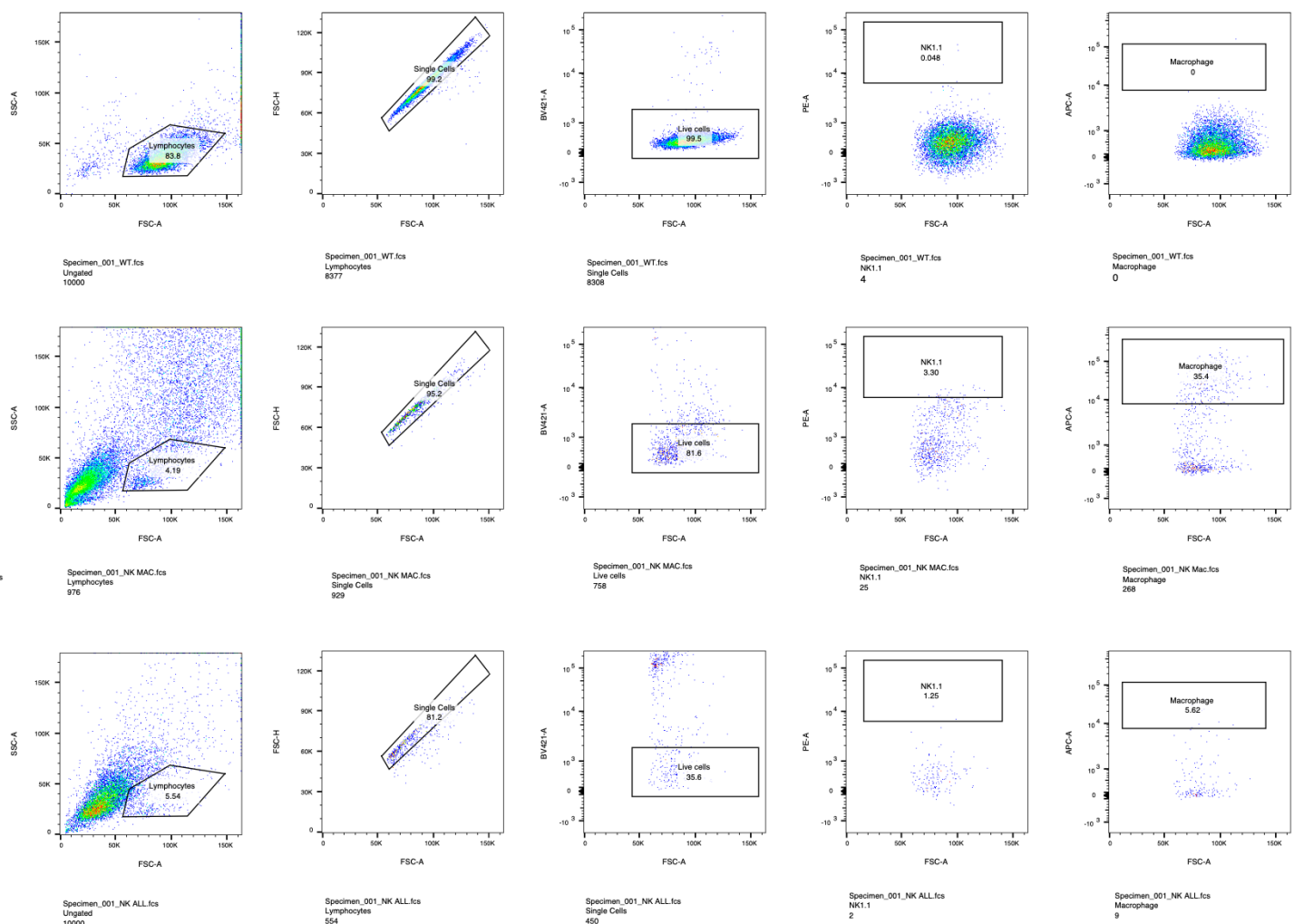


Figure 7 – Flow Cytometry of Control and Culture Mediums stained with NK1.1 Antibody AND F4/80 Antibody TOP ROW: WT Hoxb8 cells stained (Control Medium 1) MIDDLE ROW: Control Medium 2 BOTTOM ROW: Culture Medium 3

On the second round of cytometry, again PE anti-mouse NK-1.1 Antibody was used to stain NK1.1+ cells (seen on the fourth column of graphs with y-axis PE-A). APC anti-mouse F4/80 Antibody (final column of cells with y-axis APC-A) was added to stain for macrophage. The first row shows WT Hoxb8 cell line (CM 1) that have been stained for both antibodies. The second row shows Control Medium 2, allowing the

transdifferentiation to macrophage. The final row shows Culture Medium 3 – all cytokines and more FLT3L.

Third Round

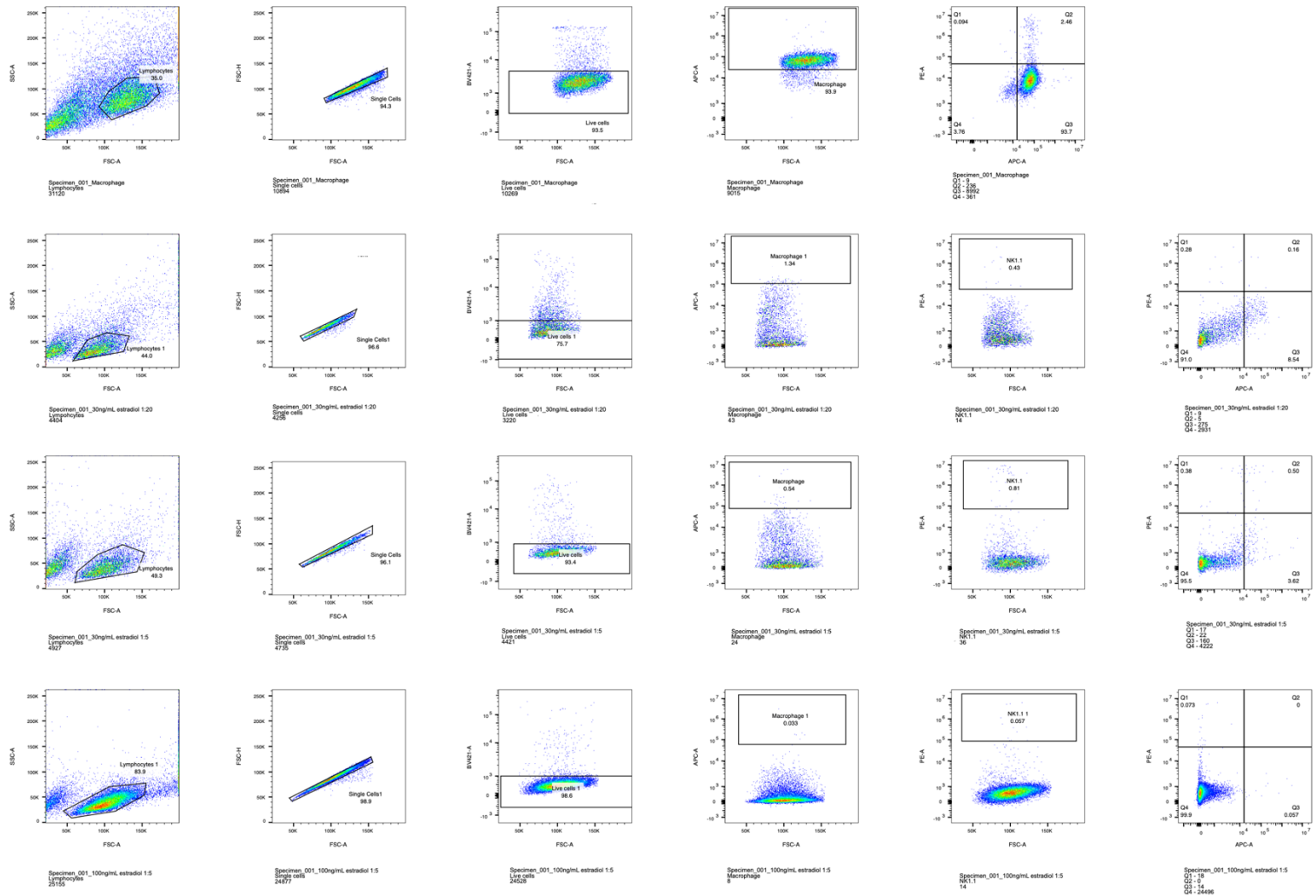


Figure 8 – Flow Cytometry of Control and Culture Mediums stained with NK1.1 Antibody AND F4/80 Antibody TOP ROW: Control Medium 3 SECOND AND THIRD ROW: Culture Medium 5 BOTTOM ROW: Culture Medium 4

The third and final round of flow cytometry, used both PE anti-mouse NK-1.1 Antibody and APC anti-mouse F4/80 Antibody like the second. The first row shows Control Medium 3, hence 93.9% macrophage were achieved. The second and third row show Culture Medium 5, from two different wells. And final row shows Culture Medium 4.

0.81% of Culture Medium 5 (30ng/mL β -estradiol) cells visualised NK1.1 marker compared to Control Medium 1 (WT Hoxb8-FL) of 0.048% (Figure 8). This time a quadrant gate was also visualised to make clear those cells that were NK, those that were macrophage and those that were invalid as presented both antibodies. We can see

NK cell count of 0.81% for 1:5 30ng/mL and 0.057% for 1:5 100ng/mL, compared to basal WT Hoxb8 (Control Medium 1) of 0.048%.

Discussion

Initially FLT3L was decreased to prevent the Hoxb8 continued proliferation, however this led to high cell deaths of Culture Medium 1 and 2. Only when FLT3L was return to 70ng/mL were death rates decreased. This was seen through the microscopy images, visualising a large proportion of cell lysis. Therefore, its concluded FLT3L is essential for cell survival and growth.

Equally β -estradiol was initially removed, as it is responsible for activating and immortalising the Hoxb8 gene. In removing this, it was predicted it would aid differentiation; however it too led to high cell death as shown in Figure 6 final row with 52.2% live cell rate and Figure 7 final row 35.5% live cell rate. This is compared to 75.5% of 30ng/mL β -estradiol (Medium 5) and 98.6% of 100ng/mL β -estradiol (Medium 4). It can be concluded, β -estradiol is therefore required to maintain the WT Hoxb8 in order to give them time to transdifferentiate.

While the percentage increase in NK1.1 marker was small, this was expected due to the difficulty of transdifferentiating Hoxb8 against their natural myeloid lineage. Macrophage were easy to culture, achieving 93.9% with F4/80 marker, due to the natural progression down the myeloid lineage. Cells tended to differentiate to macrophage yet Culture Mediums 4 and 5, that maintained Hoxb8 survival reagents and added cytokines, saw the best NK1.1+ cell count. This suggests cells need to survive long enough for transdifferentiation to occur.

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Part 2: Reflections on my Research

Personal Impacts

During my time researching in Sugimura's lab and living abroad for the first time in Hong Kong, I learnt an immense range of skills - both scientific and personal. Prior to the project, I felt uncertain about the research aspect, as I had minimal knowledge of the topic area. The most worrying part of moving to Hong Kong was the idea I would be surrounded by experts of immunology, only having first-year undergraduate knowledge. At first, I was overwhelmed by the amount of new content, however during the 6 weeks these worries eased. I found maintaining an openness when learning new things was crucial even if I felt apprehensive. I learnt holding a growth mindset when challenged by tasks allowed me to bounce back from mistakes quickly. I gained confidence from overcoming these challenges in I hadn't thought I could accomplish in the short time I was there. I quickly realised it was not about knowing everything to start but about holding a mindset that allowed me to learn novel and challenging ideas, remaining adaptable and resilient if I faced issues. This confidence was the biggest development in my character, which I believe is a vital attribute for leadership.

During my time in Hong Kong my resilience massively grew. From learning to live alone in a foreign country to the unpredictable results in the lab, I found it challenging at times to remain positive. While I had anticipated issues arising, there were some situations I hadn't prepared for, and these were the most challenging. Throughout my project and time abroad, things didn't go as planned which I found uncomfortable. I found the best way to cope with these issues was looking for ways I could control the situation and letting things out of my control wash over me. For example, when one of my samples died, I wanted to learn from my mistakes to prevent it from happening with other samples. I linked it to the lack of β -estradiol which I increased for my next experiments. Although I was disheartened my sample I had worked on for over a week had died, I acknowledged this was part of the research process. Recognising these feelings but remaining resilient allowed me to look forward and progress my project from the difficult times. Learning to staying positive and flexible meant I wasn't defeated when things didn't go to plan.

Leadership Skills

Initially I was unsure how I could show leadership during my project, as immunology and labs were entirely new to me. During my first few days, I observed Chloe (my mentor) closely to learn the basic lab skills including sterile techniques and pipetting. From shadowing and practicing, these techniques quickly got easier allowing me to direct my project. Along with independent research and peer guidance, I explored different avenues my project could take before constructing a clear plan.

Courage was a key skill needed to make decisions in an unfamiliar field. Due to the unpredictable nature of biological molecules and trying to find novel protocols, many of my plans were uncertain and dependent on results to come. For example, when deciding on components for my mediums, I needed to weigh all accumulated facts to arrive at a decision. I learnt to be calculated in the risks I took, knowing when to be bold or cautious. For example, macrophage were more robust cells than NK cells, so I could experiment with more extreme changes to medium. I learnt to make confident decisions for the fate of my cell's even if I was uncertain of the results. Equally, only changing one condition at a time meant not risking all my cells; sometimes I had to delay or not conduct experiments if this meant prioritising the health of my cells. Learning when to be brave and when to be cautious was vital when making decisions, which I now understand is an important quality of a leader who is responsible for goal.

A key part of my project was problem-solving as I needed to understand why some cells differentiated, some died and some remained Hoxb8. I found being organised throughout the 6 weeks was crucial in figuring out why these differences occurred. Early on many cells lysed compared to the Hoxb8 cell line. I looked at which components had been added and subtracted, conducted smaller scale experiments and measured cell populations, to find the FLT3-L concentration was too small to keep the cells alive. I learnt 70ng/ml was critical to maintain cell's, hence I adjusted all my existing mediums. Keeping an organised log of all my protocols and medium formulas was essential when solving why cell death was occurring.

Before my project, I believed you could only be a leader in areas you had expertise in. However, I have now learnt how you prepare and respond to issues and challenges is an essential aspect of leadership, and one which can be developed through experience and reflection. This allows you to become an effective leader who delivers impactful projects.

Dissemination of Work

Working in a lab with other interns gave me the opportunity to present my findings and teach others. Halfway through my time, two new interns arrived and I shared my findings and taught them the techniques I had learnt in my first few weeks. Racheal planned on conducting a derivation of my research, examining the same Hoxb8 cell line and macrophage differentiation I had been conducting. I spent additional time teaching her basic principles and culture techniques, including issues I had faced and how I overcame them. For example, I found 10ng/mL FLT3L was insufficient in maintaining macrophage and recommended she increased the concentration of the original medium. I also guided her in culturing her own cell lines so she too could experiment further. Sharing the knowledge I had previously learnt, felt rewarding as I could be a part of another's learning process.

During my supervisor meeting, I presented my findings in front of my lab members and Professor Sugimura. I clearly outlined the experiments I conducted, their results and future plans for my project. I had the opportunity to gain valuable advice from my peers

and supervisor on further experiments to overcome issues I faced. For example, I found my WT Hoxb8 cell line tended to differentiate down its natural lineage towards macrophage, preventing NK cells. By presenting clearly what I had already tried, it allowed my peers and supervisor to understand where issues were arising and suggest alterations. Chloe recommended I maintain β -estradiol, which I had previously removed, and Rio suggested back-gating the flow-cytometry results to prevent macrophage detection which warped my results. I found by clearly presenting my findings, using a PowerPoint presentation, I could receive more directed feedback, valuable to advancing my research.

I attended multiple lab meetings at Hong Kong Science and Technology Park (HKSTP) where lab members shared their PhD progress and the latest studies published. Watching others present, taught me how to clearly convey findings in a way people with no prior knowledge could understand. For example, protocols were explained step by step with simple images to guide the listener, and complex terms and concepts were built upon throughout the presentation. As someone with less experience in the field, I found these presentations easier to learn from and therefore more rewarding. I hope to use these same presentation skills at the Laidlaw Conference in London this Autumn, to share my findings further with other scholars.

Future Plans

Having hands-on experience in a lab setting, has shown me the vast possibilities available in the research field. Having the opportunity to conduct independent research in this environment, not possible from my undergraduate degree alone, has helped to shape my future plans - both my degree and career. It has confirmed my plan for a year in industry where I hope to further develop my research skills and gain more lab experience. Equally I really enjoyed learning deeply about one specific area of biology, such as NK cells. I am still keen to do a master's degree where I can develop knowledge in a specialised topic.

My time in the lab also allowed me to build valuable connections with people equally interested in scientific research. I hope these friendships will remain and will allow for collaboration and support in future projects.

Acknowledgements

I would like to thank Professor Rio Sugimura for opening his lab to me and providing me with the opportunity to contribute to his immunology work. Providing me with lab facilities allowed me to have first-hand experience of biomedical research and has shown me the research possibilities available. I am especially grateful to Chloe Kan who has been incredibly generous with both her time and knowledge, teaching me the basics of working with cells, and supporting me throughout my research. Lastly, I am extremely grateful to the Laidlaw Foundation for offering me this incredible opportunity

to research in an academic setting. I will truly treasure all the experiences, knowledge and memories made possible by the programme.

Part 3: Supervisor Reflective Report

Laidlaw Research Reflective Report – Supervisor Comments

Scholar Name: Claudia Minor

Supervisor Name: Rio Sugimura

Strengths:

- **Quick learner:** During her one-month internship, Claudia demonstrated a strong ability to learn quickly and adapt to a new environment. She showed enthusiasm when learning new techniques, recording experimental information, and organising research notes.
- **Independent:** After receiving appropriate instructions and supervision, Claudia was able to organise her tasks and follow established protocols to complete her assigned work. She also demonstrated good initiative in clarifying uncertainties.
- **Open to feedback:** Claudia responded positively to guidance, incorporated suggestions into her project and worked to improve her technical approach and understanding.

Leadership Attributes Shown:

- **Critical thinking and reflection:** Claudia showed her ability to think critically about experimental procedures and observations. She reflected on how her experimental outcomes could inform her future work.
- **Collaborative mindset:** She worked cooperatively with her direct supervisor and other interns in the laboratory. She was willing to seek assistance when necessary and contributed to the team environment.
- **Design thinking and problem-solving:** When she encountered challenges, Claudia was willing to consider alternative explanations, identify possible sources of difficulty and discuss appropriate next steps.

Areas for Improvement:

- Claudia would benefit from further developing her confidence and familiarity with laboratory techniques, such as cell culture. More practice would help her improve her technical consistency and efficiency.
- By continuing to strengthen her experimental planning and record-keeping skills, she will be able to maintain reproducibility and quality of her data. Claudia can further develop skills in anticipating challenges and maintaining structure in research to manage a project more efficiently.

Overall, Claudia made a positive contribution during her internship at our lab and demonstrated qualities of a quick learner who is able to work independently. With

continued practice and development in her project management skills, she would be able to make further significant contributions to the research environment.

Signature of Scholar: CMinor

Date: 31/08/2026

Signature of Project Leader:

A handwritten signature in black ink, consisting of several loops and a trailing line.

Date: 07/Aug/2026