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CREATE CHANGE

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Re: Supervisor's Review for Matus Otruba

To whom it may concern,

I supervised Mr Matus Otruba in my lab in Australia from February until June 2023 and then provided off-site guidance as he wrote his master's thesis from November 2023– March 2024. I did not provide guidance for the resubmission of his Master's thesis in August 2024, but I approved his resubmission.

This project was a collaboration between my lab and my colleague, Dr Samantha Stehbens. Dr Stehbens discovered that metastasising tumour cells can squeeze through confined tissue spaces of less than 3 microns in width. She found that if the microtubule stabiliser 'Clasp2' is depleted, these tumour cells can no longer squeeze through the spaces and instead explode. This suggests that targeting Clasp2 during the cancer metastasis stage may prevent tumour metastasis and cancer spread.

My lab works on innate immune sensing of infection and damage, including how innate immune cells such as macrophages sense dying cells. We, therefore, wanted to ask how neighbouring immune cells sense Clasp2-depleted tumour cells that die as they attempt to squeeze through confined tissue spaces (simulated by compressing the tumour cells). This project had several key aims:

1) Determine if human macrophages (Thp1 cells) responded to supernatants from compressed, Clasp2-depleted tumour cells (but not WT compressed tumour cells or non-compressed cells).
2) Determine if human macrophages (Thp1 cells) responded to tumour cells killed by current chemotherapeutic drugs (e.g. Braf inhibitors) – to determine if there was an advantage of depleting Clasp over standard of care
3) To determine how human macrophages sense a dead, Clasp2-depleted cell (e.g. sensing cellular DNA, RNA)
4) To determine if other migrating cells (e.g. immune cells themselves) express Clasp. If they do, this might indicate there would be unwanted off-target effects.

Matus took on this project in the lab. Matus was very enthusiastic student and keen to learn. When we first interviewed Matus for the placement, we understood that he had more practical laboratory experience and that he didn't need much hands-on supervision. This meant we took him on despite not having dedicated students or laboratory staff who could teach him the basics (due to our own time constraints. Unfortunately, it turned out he did need far more basic training than we anticipated, and had we known that, we wouldn't have taken him on without having more support available.

Nevertheless, Matus mastered multiple techniques including RNA extractions and qPCRs, cell culture of multiple cell types (human Thp1 cells, Melanoma cells), ELISAs (CXCL10 and IL-6), dose-response titrations for cell death assays (LDH or ATPlite). He kept decent records and we met for regular meetings. Matus was not afraid to ask questions or to ask for help and was very hard-working and enthusiastic. However, Matus was severely limited by his short time in my lab and his other commitments (e.g. work to support himself and other classes), which meant he was not able to troubleshoot some of the technical problems, nor attend our regular lab meetings and benefit from the peer support of the other students in the lab. Due to Matus's inexperience and his time constraints, it meant we had to co-ordinate experiments between two labs, making it difficult to successfully complete the project. Had Matus been able to work full-time in the lab for more months, this would not have been an issue.

It took a lot of meetings with him to explain the results, help him interpret the data, and design new experiments. It was not always clear that he understood the aims of the project, the full literature, and how his results fit in with the project. This is relatively common with students of Matus' experience, and I believe it would improve over time with meetings. Dr Stehbens and I also had lengthy Zoom meetings with him and read over his drafts of his master's thesis multiple times between November 2023 and March 2024. In my opinion, Matus was not ready for a PhD when he left my lab in June 2023 (both technically but also in project planning and literature reviewing, experiment planning and data interpretation). However, given his enthusiasm and his dedication to continuing the project despite his time constraints and technical setbacks, I feel that Matus produced a significant amount of quality data.

Due to the time constraints, and issues with the content of his thesis, I did not pick up on the errors of statistical presentation, that were highlighted and improved in the second submission by his defense opponent. These issues are highly important to me, and we are grateful that they have been rectified in this submission.

Please contact me if you have any further queries,

Yours sincerely

Dr Larisa Labzin
Lab head, Viruses and Innate Immunity Lab.