Sabra Salim Brain Tumour Foundation of Canada Final Progress Report

Since the summer of 2018, I have been continuously working with members of the Singh Lab to assess the efficacy of our CD133 CAR-T modality (CART133). While initially we proposed assess the use of an inducible safety switch in CAR-Ts, we were adamant on developing a more thorough characterization of this modality before optimizing a suicide-system. This work has now been submitted to *Cell Stem Cell* and is currently under review for publication.

Previous findings from Dr. Singh's lab implicated CD133, a pentaspan glycoprotein, in brain tumour-initiating cells. CD133 has been correlated with clinical outcomes including poor patient prognosis, metastases, relapse and poor overall survival. At a functional level, CD133+ cells have been shown to exhibit chemo- and radioresistance. These cells may ultimately evade therapy to initiate disease relapse despite aggressive treatment. These findings ultimately highlight the need to target CD133+ BTIC populations through a targeted approach. Thus, during the summer of 2018, I assisted in the *in vitro* functional validation of our CART133. This included performing cytotoxicity, activation and proliferation assays. After optimizing a luciferase-based cytotoxicity assay, we found that CART133 had a potent anti-tumour effect against CD133-expressing patient-derived lines at low effector:target (E:T) ratios. We also assessed the specific activation of these CAR-T cells in the presence or absence of CD133-expressing cells. Using flow cytometry, we found that CART133 expressed the activation markers CD25 and CD69 only when exposed to CD133-expressing cells. These cells also specifically proliferated and released pro-inflammatory cytokines such as interferon-gamma and tumour necrosis factor-alpha when co-cultured with CD133-expressing cells. *In vivo* treatment of CART133 also significantly reduced tumour burden and provided a survival advantage in orthotopic human xenografts.

While we showed pre-clinical efficacy *in vivo* and *in vitro*, the expression of CD133 on healthy tissues needed to be considered before moving into the clinic. In particular, CD133 is expressed on hematopoietic stem and progenitor cells (HSPCs) that undergo hematopoiesis to produce many types of blood cells. While we give our treatment through an intracranial delivery, it was necessary to establish whether CART133 could leak into the peripheral system where CD133-expressing HSPCs reside. Ultimately, we found that although leakage of cells was small, it was highest one week after the end of treatment. Thus in collaboration with the Hope Lab at McMaster during the summer of 2019, we generated a humanized mouse model to assess toxicity to HSPCs from these leaking cells. This involved irradiating mice and transplanted human cord blood cells to reconstitute a human hematopoietic system. Once engrafted, we treated with our CD133 CAR-T intravenously at increasing doses and assessed changes in the graft and HSPC compartment one week later. We ultimately found CD133-targeting CAR T-cell treatment did not significantly reduce the number of human HSPCs or impair hematopoiesis. This may be due to lower copy number of CD133 in normal tissues or the plasticity of the human hematopoietic system in regenerating CD133+ cells through changes in cell state/asymmetric division. Ultimately, these findings demonstrate the first use of a humanized mouse model to test the toxicity of anti-CD133 immunotherapies against a human hematopoietic stem cell niche.

Over the course of the past two years, I have also had the opportunity to present some of this work at a conference. This past October, I was invited for an oral presentation at the BioCanRx

Summit for Cancer Immunotherapy where I was awarded Runner-Up for best Oral presentation. I was also fortunate to work with Brain Tumour Foundation of Canada on numerous occasions. I have had the honour to be invited to present on updates of this project from the summer of 2018 at the Research Symposium where I also competed at the Pam and Rolando Del Maestro competition. Here, student teams across Canadian institutions created and presented an innovative proposal based on the case study given, which was judged by a panel of physicians and researchers. In 2018, I presented alongside a fellow undergraduate student on novel agents targeting therapy-resistant stem cells of diffuse astrocytoma. Having come third place in this competition, we were also given the opportunity to present at the Join the Movement to End Brain Tumours National Conference, along with a monetary award. Ultimately, I would like to thank Brain Tumour Foundation of Canada and the Taite Boomer Foundation for this award. While I am grateful that this award has allowed me to further my scientific career, it has been equally an honour to join this passionate community of scientists, donors, families and patients in the fight against brain cancer.

Published Papers

Vora P, Seyfrid M, Venugopal C, Qazi MA, **Salim SK**, Isserlin R, Subapanditha M, O'Farrell E, Mahendram S, Singh M, Bakhshiniyan D, Chokshi C, McFarlane N, Dvorkin-Gheva A, Brown KR, Murty N, Moffat J, Bader GD, Singh SK. (2019). Bmi1 regulates human glioblastoma stem cells through activation of differential gene networks in CD133+ brain tumor initiating cells. Journal of Neurooncology. 143(3): 417-428.

Papers Under Review

Vora P, Venugopal C, **Salim SK**, Tatari N, Singh M, Seyfrid M, Upreti D, Rentas S, Wong N, Williams R, Qazi MA, Chokshi C, Ding A, Subapanditha MK, Savage N, Mahendram S, Ford E, Bakhshiniyan D, Adile AA, McFarlane N, Pan J, Bramson J, Hope K, Moffat J, Singh SK. The rational development of CD133- targeting immunotherapies for glioblastoma. Cell Stem Cell.

Adile AA, Kameda-Smith M, Bakhshinyan D, Banfield L, **Salim SK**, Farrokhyar F, Fleming A. (2019). Salvage therapy for progressive, treatment refractory or recurrent pediatric medulloblastoma: a systematic review protocol. Systematic Review.

Book Chapters

Kameda-Smith M, Subapanditha MK**, Salim SK**, Venugopal C, Singh SK. (2019). Differentiation of Brain Tumor Initiating Cells. Methods in Molecular Biology. : 85-91.
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Published