

PhD Project

Elimination of cancer stem cells using chimeric antigen receptor T cells

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Background:

Chimeric antigen receptor (CAR-) T cells are designed to exploit the intrinsic cytotoxic function of T cells whilst manipulating specificity by expressing an antigen-specific receptor and cytoplasmic activation domain. Such cells have recently revealed remarkable clinical success with multiple studies reporting the ability to unburden CD19⁺ malignancies (1). However, transitioning this technology to the treatment of solid tumours is a recognised hurdle in the field (2,3). Additionally, disease relapse has been observed in a limited number of haematological malignancies suggesting that the antigen targeted by these CAR-T cells is not expressed on the small subset of cells known as cancer initiating cells or cancer stem cells (CSCs).

Currently, two models of carcinogenesis have been proposed (**Figure 1**). The Clonal Evolution model suggests all cells have the potential to generate cancer growth (4). In contrast, the Hierarchical Model attributes tumorigenic capacity to a small subset of cells from which the tumour bulk is derived; a thesis which is consistent with the presence of CSCs (5,6). CSCs are a highly tumorigenic, aggressive sub-population of cancer cells that exhibit progenitor behaviour and give rise to heterogeneous populations. They demonstrate decreased sensitivity to ionising radiation (7) and the cytotoxic effects of chemotherapeutic compounds with evidence showing systemic treatment leads to CSC enrichment (8).

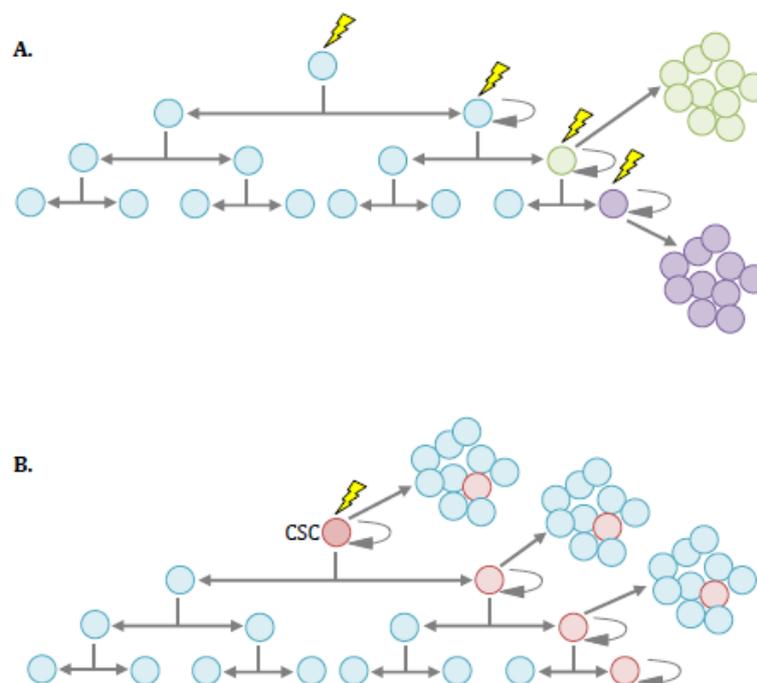


Figure 1: Two proposed models of tumorigenesis

- A. The Clonal Evolution Model depicts all cells with the ability to generate cancer growth.
- B. The Hierarchical Model contributes cancer growth to a small subset of cells from which the tumour bulk is derived. This model is consistent with the presence of cancer stem cells (CSCs).

CSCs have been isolated and characterised in a number of leukemic and solid-carcinomas however, disease relapse resulting in mortality remains a significant clinical problem. Therapeutic methods currently implemented in the treatment of a variety of cancers include surgical resection, chemo- and radiotherapy and hormonal ablation either in isolation or in combination. Current drug-related treatments facilitate the eradication of rapidly proliferating tumour cells ultimately de-bulking the tumour mass, however these approaches invariably leave behind treatment resistant cells (i.e. CSCs). This is because CSCs are commonly characterised as being a slow growing, quiescent cell able to evade therapies that require rapid cellular proliferation to be effective, ultimately resulting in disease recurrence despite a decrease in tumour burden. Although the radio- and chemotherapy resistance of CSCs is in contrast to the initial efficacy observed with these common treatment modalities, CSC-tailored therapies may provide a novel mechanism to eradicate disease.

A logical approach would be to use the sophisticated specificity of immunotherapy and CAR-T cells in particular to target surface membrane antigens present on the CSC. Unlike current therapeutic modalities, this approach negates the need for the cancer cell to be proliferating to efficiently elicit a cytotoxic effect. To date, however, data demonstrating the ability for CAR-T cells to eliminate this important cellular subpopulation are lacking.

Aim:

This project is based on our novel finding that Tag72 is expressed on a number of adenocarcinomas, but intriguingly on cutaneous T cell lymphoma (CTCL). The project will thus explore whether CSC are present in CTCL and if so, do they express Tag72 and can be killed *in vitro* and *in vivo* by our CAR-T cells targeting Tag72. If the CTCL lack Tag 72+ CSC, we will explore other cancers such as ovarian and gastric.

Proposed project outline:

Pre-clinical studies conducted by Cartherics to date have demonstrated that CAR-T cells possess the ability to effectively eliminate cancer cell lines *in vitro*. In addition, these CAR-T cells have the ability to de-bulk tumour mass and significantly delay tumour regression in cell line derived xenograft models *in vivo*. However, cell lines are relatively homogenous and may not accurately represent the heterogeneous cross-section of cells within a tumour and the related microenvironment. It is therefore vital to understand the ability of CAR-T to eliminate various cancer cell subpopulations expressing variable levels of the nominal target antigen(s) with a particular focus on the CSC subpopulation. Embedded within this, there are three main questions to be addressed:

1. *How can we characterise CSCs?*

There is an increasing body of evidence which indicates a suite of surface markers are able to characterise the CSC subpopulation in a number of cancer indications. Initial experiments would involve isolating cells bearing the CSC phenotype and functionally testing their 'stem-like' properties via colony forming assays *in vitro* and tumorigenic assays *in vivo*.

2. *What cellular subpopulations express our cancer antigen of interest?*

CSCs functionally confirmed in Question 1 would be characterised by flow cytometry for the co-expression of the relevant CSC marker in conjunction with expression of our cancer antigen of interest. The non-CSC compartment would also be characterised for cancer antigen expression.

3. *Can CAR-T cells circumvent disease relapse via the elimination of CSCs in vivo?*

It is speculated that CSCs reside within niches formed to large a degree from tumour associated stromal cells, which play a physical and immunosuppressive role in protecting this subpopulation from current therapy modalities. It will therefore be vital to demonstrate that CAR-T mediated elimination of CSCs demonstrated *in vitro* translates to the ability to eliminate CSCs *in vivo* where cells will be protected by these niche components. In addition, we postulate that removal of the CSC compartment will translate to reduced disease recurrence and increased overall survival.

At the conclusion of this project, you will have successfully characterised the CSC subpopulation in select cancer indications and demonstrated the ability or otherwise of CAR-Ts are able to eliminate these cells both *in vitro* and *in vivo*.

References:

1. CAR T cells — what have we learnt? Nat Rev Clin Oncol [Internet]. 2017;15(1):1–1. Available from: <http://www.nature.com/doi/10.1038/nrclinonc.2017.196>
2. Mirzaei HR, Rodriguez A, Shepphird J, Brown CE, Badie B. Chimeric Antigen Receptors T Cell Therapy in Solid Tumor: Challenges and Clinical Applications. Front Immunol [Internet]. 2017;8. Available from: <http://journal.frontiersin.org/article/10.3389/fimmu.2017.01850/full>
3. D'Aloia MM, Zizzari IG, Sacchetti B, Pierelli L, Alimandi M. CAR-T cells: The long and winding road to solid tumors review-article. Cell Death Dis. 2018;9(3).
4. Nowell PC. The clonal evolution of tumor cell populations. Science (80-) [Internet]. 1976 Oct 1;194(4260):23 LP-28. Available from: <http://science.sciencemag.org/content/194/4260/23.abstract>
5. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med [Internet]. 1997 Jul 1;3:730. Available from: <https://doi.org/10.1038/nm0797-730>
6. Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours : accumulating evidence and unresolved questions. 2008;8:755–68.
7. Phillips TM, Mcbride WH, Pajonk F. The Response of CD24 – / low / CD44 + Breast Cancer – Initiating Cells to Radiation. 2006;98(24).
8. Sajithlal GB, Rothermund K, Zhang F, Dabbs DJ, Latimer JJ. Permanently Blocked Stem Cells Derived from Breast Cancer Cell Lines. 2010;(412).

Some additional questions/possible roadblocks:

- 1) What will happen if TAG-72 isn't expressed on CSCs? Will we be able to change the CAR specificity?
- 2) Would we want to look at dual CARs or KO CARs?
- 3) Is there a particular indication that should be the primary focus? We have a number of CRC/gastric cell lines plus the access to organoids through collaboration with Ron so maybe better to focus on this?