Supercharging the attack on ovarian cancer: CAR-Ts, hyaluronidase and hydrogel microspheres

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This goal of this project is to unlock the utility and efficacy of CAR-T immunotherapy treatment of solid tumours. CAR-T cells are revolutionising our ability to fight liquid tumours such as acute lymphoblastic leukaemia (AAL), however the inherent complexities and defence mechanisms associated with solid tumours stifle their clinical application. In order for the immunotherapy to be effective, a sufficient number of CAR-T cells need access and be effective to completely eradicate the cancer. Beyond disguising itself as normal healthy tissue and releasing PD-1 and PD-1L to shut down immuno-attack, solid tumours have the added complexity in that they create a thick external wall of hyaluronic acid that protect access of CAR-T cells into the cancer.

When CAR-T cells are delivered, studies have shown that only 10% of the cells actually reach the cancer. In order to create effective treatments, large doses of CAR-T cells ($1x10^9$ cells) are required which are costly, time consuming to manufacture, and difficult to even create particularly if the patient has been pre-treated with chemotherapy. Furthermore, a side effect of high-dose CAR-T therapy is extreme fever (which can be fatal), associated with a storm of cytokines that are released from the CAR-T cells into the blood stream.

In order to unlock the efficacy of CAR-T attack on solid tumours, we need to (1) have focused delivery of CAR-T cells at the tumour site, (2) break down the defence mechanisms of the cancer prior to release or activity of CAR-T, (3) provide enough CAR-T cells to completely eradicate the cancer (this includes sustained presence in the body), (4) supercharge the killing potency of each CAR-T cell as it interacts with a cancer cell, and (5) reduce the number of CAR-T cells per injection which will reduce the cost per treatment and potential severity of cytokine storm.

This project will develop an injectable, biodegradable micro-carrier particle carrying CAR-T cells, hyaluronidase (HAase) and a specific combination of cytokines that enhance the proliferation, survival and cytotoxic activity of the CAR-T cells within the tumour micro-environment. The action of these micro-carriers will first involve release of HAase that acts to break down the hyaluronic acid protective walls that surround the tumour. During this process CAR-T cells will be stimulated to proliferate and activate their cytotoxic killing machinery. Then as the hydrogel micro-carriers degrade over 1-3weeks,

CAR-Ts are released directly into the weakened solid tumour to enable complete annihilation of the entire cancer- the only way to stop relapse.

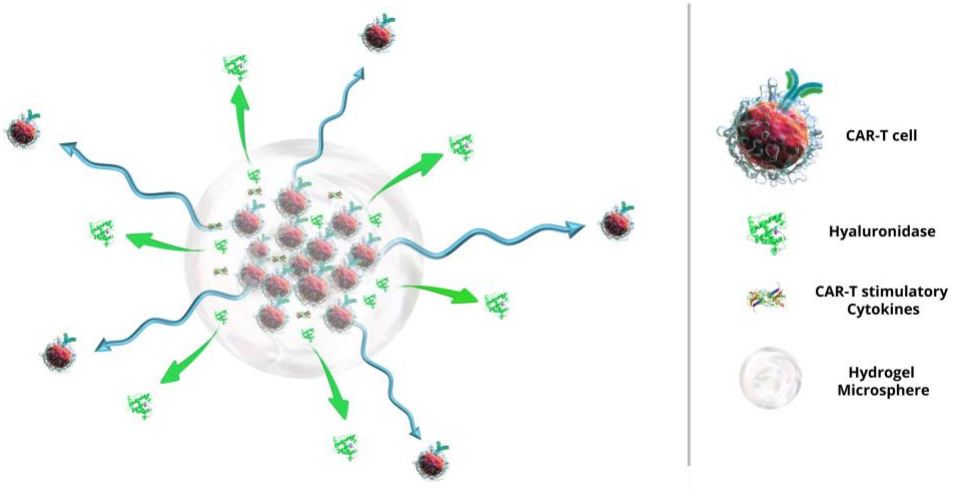

Figure 1: Proposed micro-carrier hydrogel schematic

Aim 1

Encapsulate CAR-T cells in microgels to analyze the viability of T cells within microgels after 7 days.

Aim 2

Optimize concentration of Hyaluronidase capable of matrix breakdown but non-toxic to CAR-T cells.

Aim 3

Phenotypic analysis of CAR-T cells pre and post microgel encapsulation via FLOW cytometry.

Aim 4

Functionality analysis of CAR-T cells retrieved from microgels after 7 days.

1. xCelligence killing assay: Killing ability of microgel recovered CAR-T cells on OVCAR3 monolayer cultures will be analyzed by real time xCelligence killing assay

2. 3D spheroid killing assay: CAR-T cells recovered from microgels killing tumor spheroids will be analyzed by real time video imaging and FLOW cytometry.