Title: Development of Molecular Imaging Technologies for Monitoring the Fate of T Cell Immunotherapies

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Structured Abstract:

Introduction: Chimeric antigen receptor T (CAR-T) cell therapy is an emerging cancer treatment in which a patient’s own T cells are isolated and engineered to express a CAR, which redirects the T cells to bind to a specific cancer antigen and induce cancer cell death. CAR-T cell therapies have shown promising results in patients with hematological malignancies with up to 70% of patients responding to treatment. Despite this success, CAR-T cell therapies can cause severe off-target toxicities and not all patients respond to this treatment (up to 30%). An imaging tool for tracking CAR-T cells could provide important patient-specific data on CAR-T cell fate to inform on potential success or failure of treatment as well as off-target toxicities. Fluorine-19 magnetic resonance imaging (19F MRI) allows for the detection of perfluorocarbon (PFC) labeled cells non-invasively to provide information on cell location(s), cell number, and tumour homing ability. Our goal is to track the fate of PFC-labeled CAR-T cells in a mouse model of leukemia using 19F MRI.

Methods: T cells were engineered to co-express a CD19 targeting CAR and GFP. Engineered cells were evaluated with flow cytometry, expanded, and then labeled with 5 mg/ml PFC overnight. Renilla luciferase-expressing CD19+ leukemia cells (NALM6) were co-cultured with CAR-T cells and bioluminescence imaging (BLI) was performed to evaluate cancer cell cytotoxicity. Pilot experiments were performed in nod-scid-gamma mice (n=2) that received subcutaneous injections of 1 million NALM6 cells followed by weekly BLI to monitor tumour growth. After 2 weeks, 3-5 million PFC labeled CAR-T cells were injected intravenously into each mouse. After 24 hours, 19F MRI was performed on a 3T clinical scanner using a dual tuned surface coil and balanced steady state free precession (bSSFP) sequence. MRI and BLI images were analyzed to evaluate CAR-T cell location and tumour kill, respectively.

Results: Flow cytometry revealed that engineered T cells populations were 75-99% GFP/CAR positive. In vitro BLI cytotoxicity assays show that PFC labeled CAR-T cells showed a trend towards decreased leukemia cell viability. In vivo BLI images show increases in BLI signal day 7 and day 14 post NALM6 leukemia injection. One day after PFC labeled CAR-T cell injection, 19F signal was detected in the abdomen of both mice on the ipsilateral side of NALM6 injection, however direct evidence of 19F signal in tumour regions was lacking.

Discussion: We have shown that 19F MRI is able to detect signal one day post injection of PFC labeled CAR-T cells. Images show that the CAR-T cells either did not home to tumours or were present at cell numbers too low to be visualized. Current work is focused on improving the engineering of CAR-T cells along with extensive characterization of their cytotoxicity in vitro prior to further in vivo work. If successful, these imaging tools may be useful for the evaluation of CAR-T cell therapies in patients.