INTRODUCTION

Cell therapy for cancers has gained great momentum as a promising new treatment approach. In particular, autologous T cells modified with a chimeric antigen receptor (CAR-T) has recently been regulatory-approved for the treatment of patients with CD19+ acute B cell leukemia. However, effective monitoring and characterization of the CAR-T cells after administration, in order to understand the treatment effect and underlying mechanisms, remains an urgent unmet need for CAR-T cell therapy in the clinic. Currently, there are two major methods to monitor the presence of CAR-T cells in patients: 1. Flow cytometry (a single cell based assay recognizing the tag engineered in the CAR molecule), but with extremely poor sensitivity (~1%). 2. Real-Time PCR (amplifying the engineered tag sequences), being more sensitive, but without the ability to identify and characterize individual CAR-T cells. Thus, a method with high sensitivity, and also the ability to characterize individual CAR-T cells, is in high demand.

METHODS

Fig 1. CAR-T sample preparation, staining, and analysis workflow

We are attempting to establish a novel platform, based on rare cell detection in a pathology format, to detect and characterize rare CAR-T cells in patients treated by CAR-T, using RareCyte technology. First, 1×10<sup>6</sup> white blood cells (PBMC from C57BL/6 mice), spiked with the serial dilution of 0.250 human breast cancer MCF-7 cells, were smeared on a pathology slide and subjected to staining by antibodies against human cytokeratin 18 (Abcam, ab181597), human EpCAM (Abcam, ab8666), and mouse CD45 (R&D, AF114), followed by analysis by either flow cytometry or the RareCyte system, in order to test the detection sensitivity and quantification dynamic ranges. Second, purified CD19 CAR-T cells, which were mixed into CD3+ T cells, were analyzed by flow cytometry and the RareCyte system individually, and the detection rate was compared.

Lastly, whole blood obtained from a DLBCL, patient treated with CAR-D CAR-T cells (100mL), was serially diluted with whole blood from healthy donors and then subjected to the same two analyses as detailed above with CAR-T specific staining (CD3-FITC, CD19-CAR-PE, DAPI (nucleus)), as controlled by the whole blood from healthy donors.

RESULTS

Fig 2. Representative staining images of the breast cancer cell line MCF-7 spiked-in mouse WBC

Fig 3. Linear regression and sensitivity analysis

Fig 4. Comparison of detection visibility between RareCyte and flow cytometry with purified CD19 CAR-T cells mixed with CD3+ T cells

Fig 5. CAR-T cell identification with RareCyte and flow cytometry in treated patient blood

SUMMARY

- Preliminary data from the spiked study indicates that our method has high sensitivity down to 2 cells (>99% recovery), and a good linear range of quantification. The sensitivity (<1/1 million) is at least 3-orders of magnitude above that of flow cytometry (~1%).
- Our CAR-T treated clinical data demonstrated the detection and quantification of CAR-T cells in patients, whereas flow cytometry has limitations. In addition, a single CAR-T can be picked up for further characterization, e.g. single cell genomics.
- Our method for detection and monitoring offers a great opportunity for the characterization of CAR-T cells in the clinical setting, in order to monitor patient treatment and to understand treatment mechanisms, that is otherwise unavailable. This could become a useful diagnostic tool to provide guidance on the treatment and prognosis of patients.

REFERENCES