Sensitive Detection and Quantification of CAR-T Cells in Treated Patients

Abstract No.1443

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INTRODUCTION

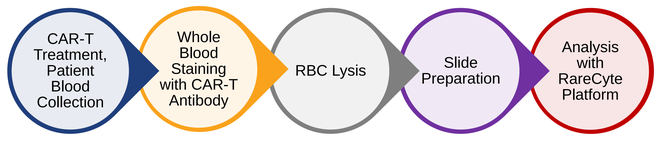
Cell therapy for cancers has gained great momentum as a promising new treatment approach. In particular, autologous T cells modified with a chimeric antigen receptor (CAR-T) has recently been regulatory-approved for the treatment of patients with CD19+ acute B cell leukemia[1,2]. However, effective monitoring and characterization of the CAR-T cells after administration, in order to understand the treatment effect and underlying mechanism, 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[3,4].

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, 1x10^6 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 CD19 CAR-T cells (100μL) was serially diluted with whole blood from heathy 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

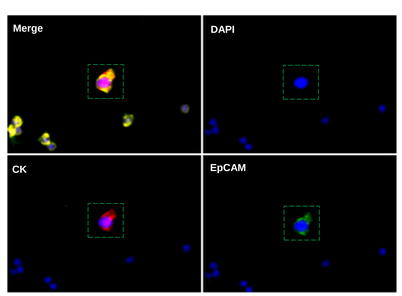


Fig 3. Linear regression and sensitivity analysis

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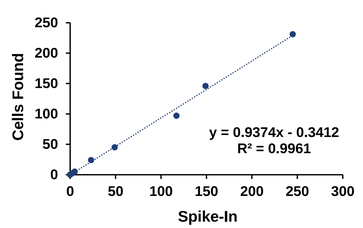

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Test	Linearity						Sensitivity		
	1	2	3	4	5	6	7	8	9
Spike-In	5	23	49	117	149	245	0	0	2
Cells Found	5	24	45	97	146	231	0	0	2

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

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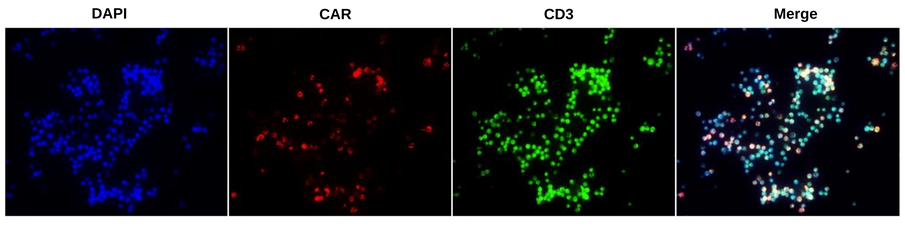

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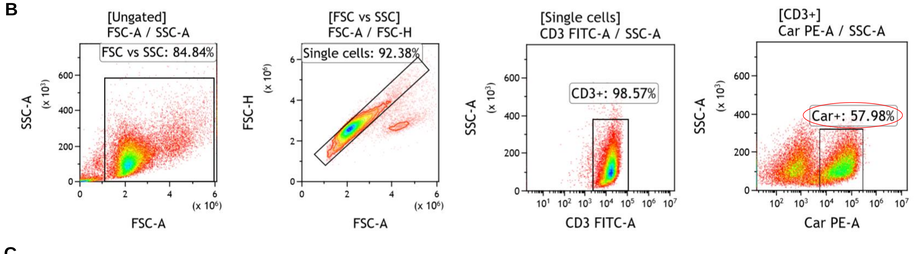

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Cell Type	Field										Average
	1	2	3	4	5	6	7	8	9	10	
CD3+ Cells	433	461	46	117	285	170	122	231	241	346	245.20
CAR-T Cells	251	262	25	72	157	97	61	124	132	197	137.80
%CAR-T Cells/ CD3+ Cells	57.97	56.83	54.35	61.54	55.09	57.06	50	53.68	54.77	56.94	56.20

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

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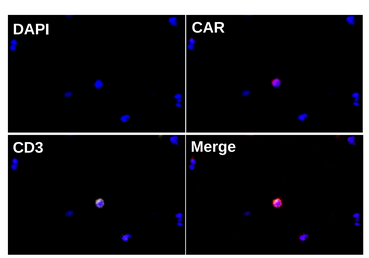

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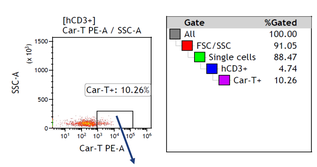

Hard to differentiate real CAR-T cells by flow cytometry when CAR-T cells were low density

C

Detection Platform	CAR-T Treated Patient Blood /Serial Dilution						Healthy Blood (Control)
	Undiluted	1:5	1:25	1:125	1:625	1:3125	
RareCyte (CAR-T+ Cell Number)	141	12	3	0	1	5	0
Flow Cytometry (%CAR-T+ in CD3+ T Cells)	10.26	0.98	0.38	0.27	0.27	0.18	0.21

SUMMARY

- Preliminary data from the spiked study indicates that our method has high sensitivity down to 2 cells (>90% 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 understand treatment mechanisms, that is otherwise unavailable. This could become a useful diagnostic to provide guidance on the treatment and prognosis of patients

REFERENCES

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