Poster #
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ESTABLISHMENT OF QPCR ASSAY FOR DETECTION OF CAR-T CELL PRODUCTS IN HUMAN WHOLE BLOOD

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PURPOSE

Chimeric antigen receptor (CAR) T-cell technology reprograms T cells to engage and eliminate cancer cells, where patients' T cells are transduced in vitro using lentiviral or retroviral vectors containing a CAR transgene. Following infusion, CAR-T cells expand in vivo and may persist in the peripheral blood for years. A sensitive and specific method is required to quantify copies of CAR transgene in vivo to support CAR-T product clinical trials. Herein, we describe development and validation of a qPCR-based assay to detect CAR transgene.

METHOD(S)

A universal method is established using the sequence of CAR's signal transduction domain for primer and probe design. To increase method specificity, Taqman probe approach is used to establish qPCR assay method. In addition, the forward and reverse primers are designed from the sequences encoding two adjacent domains, while the probe is designed using the sequence at the junction of these two domains (Figure 1).

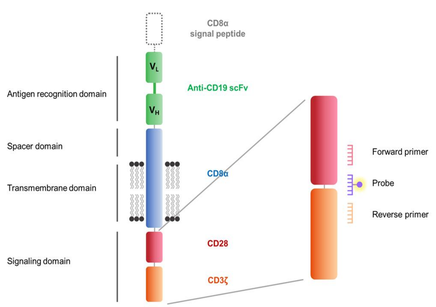

Figure 1 Design of the primers and probe

RESULTS

Using CAR gene-harboring plasmid DNA as calibrator, a representative standard curve for the CAR qPCR assays is shown in Figure 2. The mean R^2 value (0.9998 ± 0.0001) from three independent assay runs indicated a high degree of linearity in the range of 20–10,000,000 transgene DNA copies/400 ng human gDNA. The limit of detection was determined to be 14.52 copies/400 ng human gDNA (Figure 3). Standard curve performance is evaluated with back-calculated values of 7 calibrators (Table 1). Based on 5 levels of quality control samples (i.e., LLOQ, LQC, MQC, HQC, and ULOQ), assay precision and accuracy results are summarized in Table 2. Addition of E. Coli DNA instead of transgene-harboring plasmid DNA resulted in no amplification reaction, demonstrating assay specificity. Results of all test parameters are within the acceptance criteria.

Table 1 Accuracy and precision of calibrators

Parameter	Calibrator (CAR gene copies/400 ng human gDNA)						
	STD1 1E7	STD2 1E6	STD3 1E5	STD4 1E4	STD5 1E3	STD6 **100**	STD7 20
Back-calculated	10679875.7	957733.9	98239.9	9587.1	1017.9	98.5	20.9
Inter-run %CV	4.4	1.5	3.5	2.7	5.1	8.7	7.6
Inter-run %RE	6.8	-4.2	-1.8	-4.1	1.8	-1.5	4.4
n	6	6	6	6	6	6	6

Table 2 Accuracy and precision of QC samples

Parameter	QC (CAR gene copies/400 ng human gDNA)				
	LLOQ (20)	LQC (60)	MQC (3E4)	HQC (7.5E6)	ULOQ (1E7)
Back-calculated	23.2	69.8	32114.0	7293447.9	9883775.3
Inter-run %CV	12.1	11.8	12.6	7.9	7.6
Inter-run %RE	16.2	16.4	7.0	-2.8	-1.2
n	18	18	18	18	18

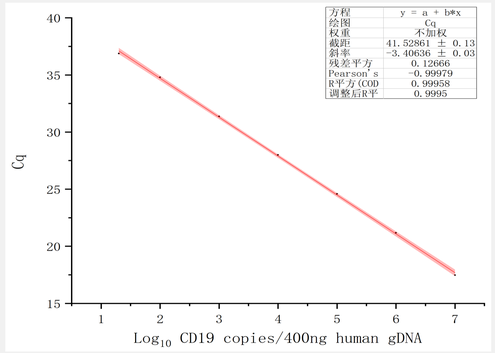

Figure 2 A representative standard curves

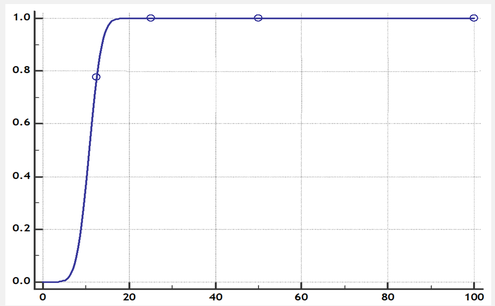

Figure 3 Limit of detection of the qPCR assay

CONCLUSION(S)

A qPCR method has been developed to quantify the copies of CAR transgene in human whole blood specimens. The validation results demonstrated adequate calibration curve performance, assay sensitivity, accuracy, precision, and method specificity. Thus, the assay is adequate for intended purpose.