Utilization of the Tapestri Platform for Quantification of Single Cell Vector Copy Number in CAR T Cell Products

Lindsey Murphy[1], Amanda Winters [1], Saurabh Parikh[2], Khushali Patel[2], Qawer Ayaz[2], Yilong Yang[2], Yue Wang[2], Shu Wang[2], Daniel Mendoza[2], Matt Cato[2], Terry J. Fry[1], Benjamin Schroeder[2], Chieh-Yuan Li[2]

1. University of Colorado and Children's Hospital Colorado, 13123 East 16th Ave, Aurora, CO 80045;
2. Mission Bio. 400 E Jamie Ct, Suite 100, South San Francisco, CA 94080

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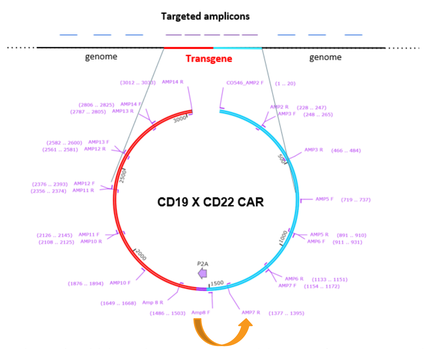
INTRODUCTION

Chimeric antigen receptor (CAR) T cell immunotherapies have been transformative solutions to treat both adult and pediatric patients with a variety of cancers. Given that CAR T cell therapies involve genetic alterations of a patient's T cells by introduction of the CAR into host cells using lentiviral vectors, the quality of CAR T cell therapies are extensively regulated to warrant efficacy and safety. Key safety and efficacy attributes need to be accurately measured prior to reintroducing modified T cells back into patients, including CAR transgene copy number or viral vector copy number (VCN). Yet, conventional methods for measuring gene transfer lack the resolution and representation to truly reflect sample composition and either report a population average (bulk) or involve laborious and time-consuming clonal outgrowth. Mission Bio has developed an end-to-end solution from panel design to data analysis for single-cell targeted DNA sequencing to interrogate transgenes. Here, using the Tapestri platform we demonstrate that single-cell DNA sequencing identifies transduced versus non-transduced cells with exceptional accuracy and precision, as well as measures the single-cell level vector copy number (VCN) for populations of thousands of cells with single nucleotide resolution while reducing sample processing time from weeks to days.

VECTOR BACKGROUND

All validation work was performed on the novel bicistronic CD19xCD22 CAR T cell construct developed in the laboratory of Dr. Terry Fry and currently in Phase I clinical trials for the treatment of relapsed/refractory (R/R) non-Hodgkin lymphomas in the adult population, with Phase I trials opening soon for the treatment of R/R pediatric acute lymphoblastic leukemia.

Figure 1: Lentiviral transduced CAR cell lines and assay design

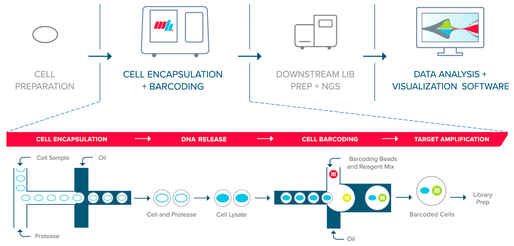


Targeted assays (purple) designed to interrogate CAR and the genome for vector copy number and genotype information (clonal/cell type differentiation) via Tapestri platform. Orange arrow is location of ddPCR amplicon.

METHODS

The Tapestri® Platform utilizes droplet microfluidic technology to rapidly encapsulate, process, and profile up to 10,000 individual cells for multi-analyte detection. The platform is enabled by a novel two-step microfluidic workflow and a high multiplex PCR biochemistry scheme. The two-step microfluidics allows for efficient access to DNA for downstream genomic reactions and provides flexibility to adapt for additional applications and multi-omics. The multiplex PCR chemistry is developed and co-optimized with an AI-powered panel design pipeline and enables direct and efficient amplification of targeted genomic regions within barcoded individual cells. Taken together the platform produces high genomic coverage, low allele dropout rate, highly uniform amplification in thousands of cells from a single run, is compatible with diverse and difficult samples, and is easily deployable for custom content. The final products are sequenced on an Illumina sequencing instrument (Figure 2).

Figure 2: Tapestri workflow



Validated (ddPCR BioRadQx200), lentiviral transduced Jurkat clonal cell lines with bicistronic CD19/CD22 CAR vector copy number of 0 and 2 copies were used in this study. A custom panel of amplicons was designed to enable the vector copy number analysis (Figure 1). Pure clonal cell lines of VCN 0, 2 and two (2) mixtures (50:50 and 25:75 VCN0:VCN2) with non-transduced GM12878 cell (from NIST) spike-in was processed for single-cell sequencing using Mission Bio's Tapestri platform in triplicates. Tapestri data from one of the VCN2 run was used as control reference. All samples were analyzed with a copy number caller to produce single-cell level VCN calls, as well as a population average VCN and a percent transduction for each sample. An average of 6,893 cells were analyzed for each Tapestri sample.

RESULTS

Transduction efficiency can be detected with high sensitivity and specificity

Tapestri provides vector amplicon specific measurements that can be used to determine transduction efficiency. Mixtures of non-transduced GM12878 and vector containing Jurkat cells (VCN2), as well as non-vector containing Jurkat cells (VCN0) were used for this study. Cells were genotyped based SNPs called by the human amplicons. Transduction analysis was characterized based on the presence or absence of vector amplicons in each single-cell. The Tapestri VCN can detect transduction efficiency with >98.8% and >99.2% specificity and sensitivity (Table 1).

Table 1: Transduction efficiency sensitivity and specificity

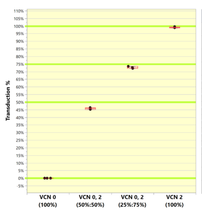
99.2% sensitivity
98.8% specificity

VCN 2 Jurkat | VCN 0 Jurkat | VCN 0 GM12878

	Transduced	Not transduced	Not transduced
Detected Transduction	**20,093** cells	**0** cells	**5** cells
No transduction detected	**164** cells	**9,505** cells	**428** cells

Applying the transduction efficiency caller on Jurkat cells across technical replication of Tapestri runs, one can appreciate the precision of transduction % measurements. Table 2, left graph shows the transduction % called for each sample with different percentage of mixed VCN0 and VCN2 cells. The green line indicates the expected positive % based on cell counter-based measurement. Table 2, right table indicates the range of transduction % called for each mixtures with 95% confidence interval, with %CV ranging from 0.38% to 1.27%.

Table 2: Transduction % of mixtures between replicates

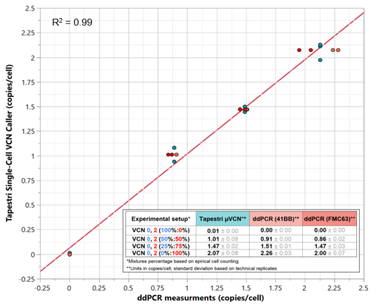


Transduction %			
Experimental Design	lower	median	upper
VCN 0 (100%)	0.00%	0.00%	0.00%
VCN 0 (100%)	0.00%	0.00%	0.00%
VCN 0 (100%)	0.00%	0.00%	0.00%
VCN 0, 2 (50%:50%)	46.06%	45.91%	45.97%
VCN 0, 2 (50%:50%)	45.12%	45.19%	45.28%
VCN 0, 2 (50%:50%)	46.06%	46.34%	46.43%
VCN 0, 2 (25%:75%)	73.26%	73.46%	73.50%
VCN 0, 2 (25%:75%)	71.91%	72.13%	72.31%
VCN 0, 2 (25%:75%)	72.61%	72.74%	72.83%
VCN 2 (100%)	98.66%	98.83%	99.02%
VCN 2 (100%)	99.53%	99.53%	99.53%
VCN 2 (100%)	98.86%	98.94%	98.66%

Tapestri VCN measurements correlate with orthogonal ddPCR technology

Tapestri single-cell VCN assay can measure populational VCN average, as well as gives average VCN value on transduced cells only (due to the ability to call transduced cells). Figure 3 shows the X-Y scatter plot of the average VCN for each sample called using Tapestri VCN caller (based on per cell VCN calls) vs. ddPCR measurement (two separated ddPCR probe assays targeting 41BB and FMC63 region; albumin as reference; BioRad QX200, n=2). The average copy number of the population estimated using Tapestri (µVCN, achieved by averaging each single-cell VCN value) correlates well with ddPCR measurements (R^2= 0.99). The average and standard deviation of replicate measurements (n=2-3) are shown in the bottom right table. Note that the sample average VCN measured by two separate ddPCR assay may not necessarily agree with each other based on assay dependent performance and the degree of optimization.

Figure 3: Tapestri vs. ddPCR VCN measurements



Single-cell level resolution of vector copy number distribution characterization

Besides the measurement of average VCN per sample, Tapestri VCN caller calculates VCN distribution of a particular sample at single-cell level, which provides VCN distribution modality insights. In Figure 4, the pie charts shows the VCN distribution calculated for each of the four mixtures prepared manually at VCN 0 (100%), VCN 0, 2 (50%:50%), VCN 0, 2 (25%:75%) and VCN 2 (100%). The Tapestri VCN caller range is dynamic and can be adjusted; here, in this set of experiments the caller estimates up to VCN of 9 copies per cell.

Figure 4: Tapestri vs. ddPCR VCN measurements

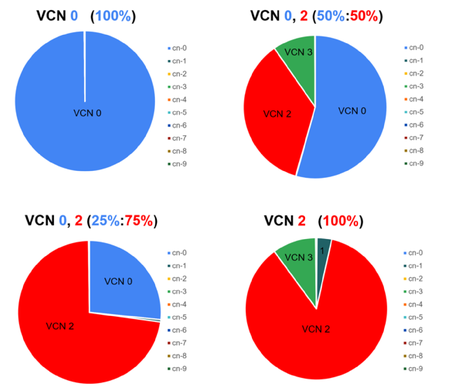


Table 3: Single-cell level VCN distributions

	Copy number distribution									
	cn-0	cn-1	cn-2	cn-3	cn-4	cn-5	cn-6	cn-7	cn-8	cn-9
VCN 0 (100%)	99.82%	0.00%	0.00%	0.00%	0.04%	0.05%	0.05%	0.04%	0.00%	0.00%
VCN 0 (100%)	99.78%	0.00%	0.00%	0.00%	0.05%	0.05%	0.06%	0.05%	0.00%	0.00%
VCN 0 (100%)	99.79%	0.00%	0.00%	0.00%	0.00%	0.07%	0.07%	0.07%	0.00%	0.00%
VCN 0, 2 (50%:50%)	54.95%	0.12%	40.52%	4.37%	0.04%	0.00%	0.00%	0.00%	0.00%	0.00%
VCN 0, 2 (50%:50%)	53.63%	0.08%	31.44%	14.77%	0.08%	0.00%	0.00%	0.00%	0.00%	0.00%
VCN 0, 2 (25%:75%)	25.82%	0.00%	73.90%	0.27%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
VCN 0, 2 (25%:75%)	27.42%	0.04%	72.27%	0.27%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
VCN 0, 2 (25%:75%)	26.64%	1.47%	71.85%	0.04%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
VCN 2 (100%)	0.32%	10.02%	82.62%	6.61%	0.22%	0.05%	0.04%	0.05%	0.05%	0.03%
VCN 2 (100%)	0.00%	0.00%	87.38%	12.61%	0.02%	0.00%	0.00%	0.00%	0.00%	0.00%
VCN 2 (100%)	0.05%	0.00%	89.39%	10.50%	0.05%	0.00%	0.00%	0.00%	0.00%	0.00%

Table 3 shows the percentage of individual VCN 0 to 9 estimated in each sample. Median cells analyzed for each run is 6,893 cells.

Conclusions

Using the Mission Bio Tapestri single-cell DNA sequencing platform, this study shows a consistent and reliable assay for in-depth quantification of cell and gene therapy transduction efficiency, single-cell vector copy number distribution, and populational average. The single-cell level VCN analysis provides unprecedented resolution and insight to assess the potential functional efficacy and safety for CAR-T therapy. Together, these characteristics and data demonstrate the potential to accelerate and streamline both the development and release testing of cell and gene therapy products.