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Track 1 – Advances in Therapeutic Development Across Modalities

(T1130-01-02) Evaluating a Merged Well Analysis Strategy for Validation of a Cellular Kinetics Assay to Quantify an Allogeneic Cell Product in Human Blood by ddPCR

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Purpose: Cancer is a serious public health concern that is a major cause of morbidity and mortality. Treatment of cancers via transfusion with chimeric antigen receptor (CAR) expressing immune cells has proven clinically successful since 2017. Currently approved CAR therapies have modified patient derived cells to produce a population of autologous CAR-expressing T-cells (CAR T). However, the time-consuming process of producing autologous CAR T-cells can lead to delay in initiation of treatment, and CAR T therapies can have severe side effects that include graft-vs-host disease and cytokine release syndrome. Century Therapeutics, Inc. has developed novel ‘off-the-shelf’ product candidates consisting of induced pluripotent stem cells (iPSC)-derived Natural Killer (iNK) cells expressing CAR. The use of this allogeneic CAR iNK therapy may potentially improve cancer patient outcomes by reducing time-to-treatment and avoiding side effects common in other CAR therapies. Clinical monitoring of expansion and persistence of CAR expressing immune cells is frequently performed via PCR (polymerase chain reaction) analysis of nucleic acid extracted from patient-derived PBMCs (peripheral blood mononuclear cells). To achieve optimal sensitivity, such assays must quantify a gene sequence specific to the CAR-expressing cells in a potentially large background of endogenous PBMCs. ddPCR (Digital Droplet PCR) is a novel technology that permits absolute quantification of such gene targets, and offers theoretically superior sensitivity and robustness than comparable qPCR (quantitative PCR) methods. Furthermore, the sensitivity of ddPCR may be theoretically increased via the use of a merged-well analysis technique. Given the novelty of ddPCR, and the lack of clear regulatory guidance regarding validation of ddPCR assays, it is important to understand considerations and best practices for validating these assays. To address some of these challenges, we validated an assay to detect a transgenic sequence unique to Century Therapeutics, Inc.’s CAR iNK in a background of human PBMC genomic DNA via ddPCR. The ddPCR data are herein presented to demonstrate the capabilities of ddPCR, to compare the sensitivity of merged-well and individual-well ddPCR analysis, and to provide insight on the use of ddPCR in clinical monitoring of cellular kinetics.

Methods: A duplexed, florescent, ddPCR assay to detect both a CAR iNK-specific target and a genomic DNA loading control (RPP30, ribonuclease P/MRP subunit p30) was used to quantify

prepared standards and controls of known concentrations to evaluate and validate assay performance. A master mix was prepared with ddPCR Supermix, CAR iNK-specific primers and FAM-labeled probe, RPP30-specific primers and HEX-labeled probe, and restriction endonuclease. The master mix was aliquoted into a 96-well sample plate, and standards and controls were added to appropriate wells. The sample plate was sealed and loaded into a Bio-Rad QX200 automated droplet generator (AutoDG). The AutoDG transferred the contents of the sample plate to a PCR reaction plate while simultaneously partitioning the contents of each sample plate well into ~20K nL individual droplets, each containing all the components necessary for a PCR reaction. The PCR reaction plate was loaded into a thermal cycler and endpoint PCR was performed. The PCR reaction plate was placed into the Bio-Rad QX200 droplet reader to measure the levels of hydrolyzed FAM and HEX probe fluorescence in each droplet. Droplets in which the target was amplified (positive) had higher FAM and/or HEX fluorescence readings whereas droplets without amplification (negative) had little to no FAM or HEX fluorescence. Bio-Rad QuantaSoft software used Poisson distribution statistical analysis to determine the absolute quantity of the target in the sample well based on the total number of positive and negative droplets detected. The samples were analyzed with multiple replicates, using both individual-well and merged-well analysis.

Results: Both individual-well and merged-well analysis produced data that was acceptably accurate and precise (|RE| and %CV ≤ 30%), linear (R^2 ≥ 0.95), and specific (No Template Control, NTC near 0). Using individual-well analysis, the CAR iNK target assay was validated with a lower limit of quantification (LLOQ) and limit of detection (LOD) of 25 and 12 copies/well respectively. Using merged-well analysis, the CAR iNK target assay was validated with a LLOQ and LOD of 10 and 4.4 copies/well respectively.

Conclusion: The validated ddPCR assay offers sufficient performance to detect and quantify circulating CAR iNK cells in human blood regardless of whether merged-well or individual-well analysis is performed. However, merged-well analysis achieved an approximately 60% reduction in both the LLOQ and LOD of the CAR iNK assay. The improved sensitivity offered by merged-well analysis may facilitate CAR iNK detection and quantification in PBMC samples where either the total cell number is low (shortly after lymphodepletion) or the CAR iNK abundance is low relative to the native PBMC population (after recovery from lymphodepletion). Our results demonstrate the suitability of such fit-for-purpose ddPCR data analysis for cell therapy projects in the absence of concrete regulatory guidance.