DEVELOPMENT OF ANALYTICAL ASSAYS FOR THE CHARACTERIZATION OF GENE CIRCUIT ENABLED CELL THERAPIES

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Senti Bio has built a synthetic biology platform to improve next-generation cell and gene therapies with “gene circuits.” Designing gene circuit product candidates requires the use of multicomponent genetic constructs (smart sensors, logic gates, regulator dials, multiple payloads) to reprogram cells with biological logic to sense inputs, compute decisions and respond to their cellular environments. These sophisticated, dynamic, multiple component systems require more advanced analytics to characterize insertion, expression and function of each component. For example, the SENTI-301 product candidate is a CAR-NK cell therapy for the potential treatment of hepatocellular carcinoma that contains 4 genetic components: i) a GPC3 CAR, ii) a novel IL-15, iii) a synthetic transcription factor inducible by a small molecule drug, and iv) secreted IL-12 under the control of iii. Here, we describe the development of novel assays to support this multicomponent product candidate, with focus on methods to characterize transduction (gene modification) and to demonstrate the function of these genetic modifications.

Characterization of transduction relies on the ability to detect genomic modifications in the NK cells, transcription of mRNA, and translation to protein. A key challenge for multicomponent products is developing assays (DNA, RNA, and protein-based) capable of detecting each component in relation to the others within a heterogeneous cell population. Senti Bio is leveraging digital PCR (dPCR) assays to determine gene insertion and mRNA expression of multiple cellular modifications, taking advantage of improved sensitivity and precision compared to traditional qPCR assays. Multiplexed dPCR assays were developed to determine genomic copies and mRNA expression allowing us to look at the relationship of integrated DNA to mRNA expression, for each genetic component. Population analysis (transduction efficiency) of transduced NK cells is achieved by using two flow cytometry methods to detect expression of the GPC3 CAR and the synthetic transcription factor. For the CAR, a recombinant GPC3 protein was identified to detect surface expression on transduced NKs. For the synthetic transcription factor, which is only expressed intracellularly, PrimeFlow™ technology is used, which combines in situ hybridization with single cell resolution by flow cytometry. Expression of the secreted cytokines IL-15 and IL-12 are determined by ELISA. To further characterize these modifications, functional analysis is necessary to show that all components maintain their desired properties in the end product. Function of the regulated expression of IL-12 will be determined by monitoring the activation of the synthetic transcription factor after exposure to a small molecule drug (grazoprevir) by quantifying IL-12 secretion by ELISA. To show GPC3-specific killing of cancer cells, we have implemented real-time electrical impedance detection (xCELLigence) to provide real-time monitoring of killing potential and potency of the NK cells. As we advance our gene circuit technology platform, additional assays will be developed to support potency and to determine critical quality attributes and control of the manufacturing process.