

DEVELOPMENT OF A HIGH-DOSE ENGINEERED TCR T CELL MANUFACTURING PROCESS USING AUTOMATED SEMI-CONTINUOUS PERFUSION BIOREACTORS

Kenny Choi, Kite Pharma
 kchoi@kitepharma.com
 Jacob Okhovat, Kite Pharma
 Marianna Sabatino, Kite Pharma
 Marc Better, Kite Pharma

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Clinical activity with engineered TCR (eTCR) T cell products directed against solid tumor indications may require doses up to two orders of magnitude greater than those investigated during CAR-T studies in hematological malignancies.¹ Novel methods for optimizing productivity and production times are required to progress the industrial feasibility of high-dose cell therapies. We developed an automated semi-continuous perfusion culture method suitable for rapidly generating high T cell densities with the aid of risk-based process models. The result is a robust manufacturing process capable of generating a target dose with high certainty, and minimal operational complexity and variability.

Process design was initiated with the characterization of T cell growth kinetics in suspension culture. An exponential regression model was utilized to calculate the maximum specific growth rate (μ_{max}) during log expansion, and the results were summarized as a probability density function. Measured ranges for specific rates of substrate consumption and byproduct production were defined using confidence limits at $\alpha=0.05$. A first-order differential expression associating the cell growth model and analyte kinetics in the culture medium was solved at steady state, and a process model for calculating a volumetric perfusion rate as a function of process time was

formulated (D = dilution rate, μ_s = limiting specific analyte consumption/production rate, x = modeled cell conc. per unit time, S = substrate conc.).

Optimization studies for culture productivity and process

$$\frac{dS}{dt} = 0 \therefore D = \frac{\mu_s x_0 e^{\mu_{max}(t-t_0)}}{S_0 - S}$$

robustness were designed using iterations of the model,

Equation 1 - Steady-State Solution for Calculating Volumetric Perfusion Rate

and the output was tested with normal donor material on the GE Xuri Cell Expansion System W25.

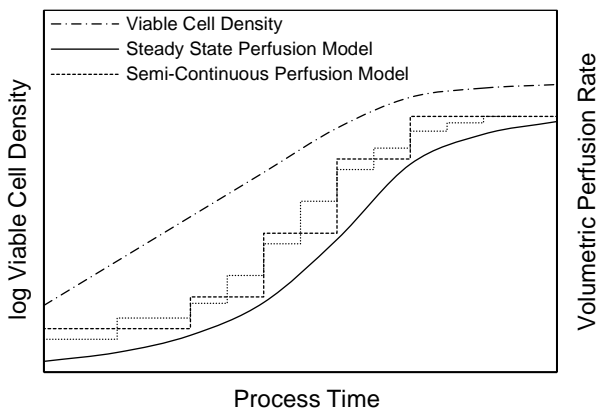


Figure 1 - Modeled Semi-Continuous Perfusion Rate

In preparation for clinical manufacturing, an 8-day eTCR T cell manufacturing process (Day 0 apheresis received) using serum-free cell culture medium has been executed for n=10 development runs. Cell viability (median 96%, range 93-98) and doubling time (median 14.9 hours, range 13.6-16.5) were measured from inoculation to harvest, and exponential growth was maintained at steady state (model $R^2=0.985$). Fold expansion was measured for 5 days of bioreactor processing (median 106 FE, range 81-116), and the optimized perfusion schedule achieved a mean 5.6-fold improvement in productivity from initial developmental conditions (cells generated per vol. of media perfused). With a high retroviral transduction efficiency to introduce the exogenous TCR genes (median 60% eTCR^{pos} T cells, range 48-70), sufficient product T cells were generated to achieve a modeled 99% manufacturing success rate within 8 days (median 2.2 doses, range 1.7-3.0).

Using principles of risk-based process design, a robust and optimized manufacturing method was developed to support the rapid production of high-dose T cell therapy. Performance data generated from normal donor material provide confidence for work with subjects, and this process which was designed to minimize the manufacturing failure rate will be utilized for eTCR T cell studies in solid tumor indications.

Robbins P, et al. A pilot trial using lymphocytes genetically engineered with an NY-ESO-1-reactive T cell receptor: Long term follow up and correlates with response. *Clin Cancer Res.* 2015-3; 21(5): 1019-1027.