Linearity for Tranduction Efficiency Assay Qualification

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1 Introduction

This report documents the statistical analysis performed for the linearity of the Transduction Efficiency (TE) Assay. This document is intended to be read as part of the qualification report for Tranduction Efficiency. In brief, the TE assay employs a flow cytometry method to establish the proportion of transduced cells in the test article (cell suspension). In the manufacturing of the CART cells, B7-H3 and Serpin B9 (CAS) were introduced into the T cells via transduction using a replication-incompetent retrovirus vector. Fluorescent-labeled antibodies targeting the VHH and T2A were used to label the B7-H3.CAR and the transduced Serpin B9 (CAS). The proportion of the positively stained cells (B7-H3 and Serpin B9 (CAS) positive) out of the total amount of cells in the cell suspension is reported as the Transduction Efficiency.

Source: Article Notebook

2 Data generated for Linearity

The IHC harmonized tripartite guideline (Borman & Elder, 2017) defines linearity as:

> **IHC Guidlines**
>
> A linear relationship should be evaluated across the range of the analytical procedure. It may be demonstrated directly on the drug substance (by dilution of a standard stock solution) and/or separate weighings of synthetic mixtures of the drug product components, using the proposed procedure. The latter aspect can be studied during investigation of the range.

In line with this guidance, samples with different transduction efficient were generated using CART cells expressing the B7-H3 CAR antigen (drug substance, DS) are diluted at various known ration (volume per volume) with untransduced T cells that does not express the CAR on the surface, as shown in Table **??**. The expected transduction efficiency was normalized based on the 100%(v/v) and 0 %(v/v). These set of samples were ran by two operators across three days to incorporate inter-operator and inter-day variations.

Source: Article Notebook

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3 Methods

Taking reference to the IHC harmonized tripartite guideline (Borman & Elder, 2017):

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Table 1: Data generated for linearity in qualification

Operator	Day	Observed (%TE)	Expected (%TE)
Operator 1	Day1	0.00	0.00
		16.85	16.91
		33.49	33.82
		52.28	50.73
		69.70	67.64
		83.62	84.55
	Day2	0.00	0.00
		14.81	16.91
		33.33	33.82
		51.54	50.73
		67.39	67.64
		84.16	84.55
	Day3	0.01	0.00
		13.65	16.91
		33.43	33.82
		49.99	50.73
		68.44	67.64
		84.73	84.55
Operator 2	Day1	0.00	0.00
		16.84	16.91
		33.79	33.82
		52.43	50.73
		68.34	67.64
		84.26	84.55
	Day2	0.00	0.00
		14.94	16.91
		32.43	33.82
		50.61	50.73
		68.26	67.64
		83.25	84.55
	Day3	0.01	0.00
		13.70	16.91
		32.38	33.82
		49.11	50.73
		68.48	67.64
		87.28	84.55