Example TORTOISE Test™ Potency Analysis Report

PROJECT ANALYSIS PLAN

Stem Cell Source: Commercially supplied mixed $CD34^+$-selected human umbilical cord blood cells ("**CD34-CBCs"**) purchased from **STEMCELL Technologies (#70008)**

Culture Medium: StemSpan™ SFEM II (StemCell Technologies, Cat# 09655)
Supplemented with StemSpan™ $CD34^+$ Expansion Supplement (StemCell Technologies, Cat# 02691)
1% penicillin/streptomycin

Serial Culture: Performed in triplicate
Input total cell number per culture = 177,000
1:5 spit every 3 days until natural cell culture terminal proliferation arrest

Specific $CD34^+$-selected HSC Potency Factors

The following key potency factors for therapeutic tissue stem cells were defined by the TORTOISE Test™ analysis results that follow.

- HSC-Specific Dosage — 1785 ± 553 per 10^4 MNCs
- Specific Replenishment Rate of Mature Functional Cells — 0.86
- HSC-Specific Death Rate — 0.03
- HSC-Specific Fraction Half-Life in Culture — 1.36*

*Determined by subsequent RABBIT Count™ analysis. Value indicates the number of cumulative populations required to reduce the HSC-specific fraction by 50%.

RESULTS

I. Cumulative Population Doubling (CPD) Data

These basic data (**Fig. 1**) are derived from simple viable cell counts performed at each passage. CPD data are the input for the TORTOISE Test™ software analyses.

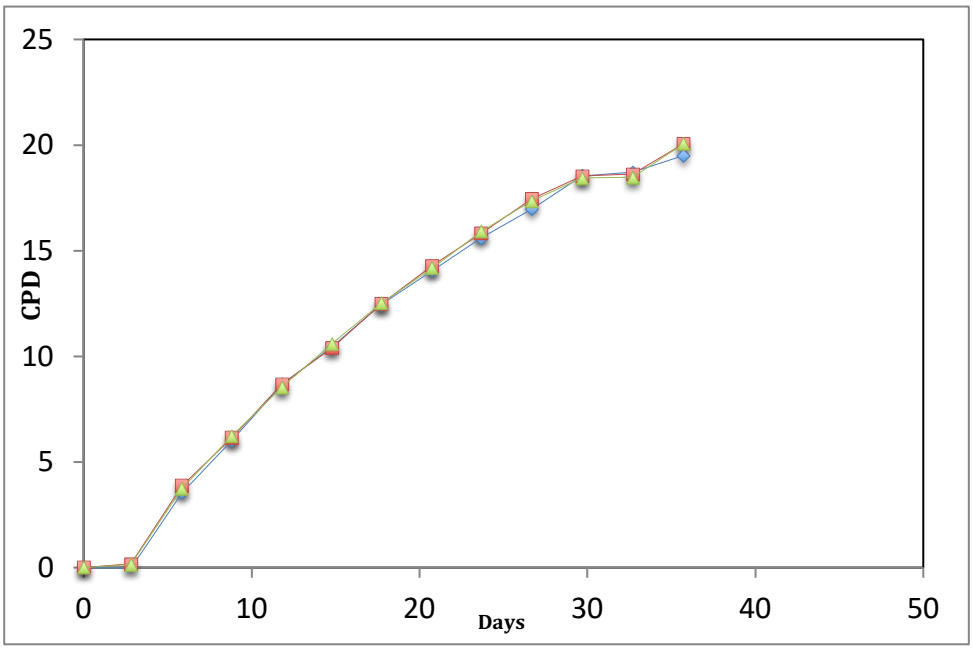

Figure 1. CPD data for three replicate serial cultures of human CD34$^+$-selected cord blood cells.

II. TORTOISE Test™ Simulation Quality

By simulating the replicate CPD data, the TORTOISE Test™ is able to discover unknown cell kinetics factors responsible for the growth of the serial cell cultures. The quality of the computer simulations is determined by the Simulation Quality Score (**SQS**). The SQS is a root mean square error determination of how well the TORTOISE Test™ software simulations describe the experimental CPD data. SQS scores ≤ 1.0 are acceptable for confident analyses. Scores ≤ 0.5 are ideal for the highest confidence analyses.

Mean SQS = 0.35 ± 0.03 (n = 10 determinations; $p < 0.0001$; 95% CI = 0.33-0.37)

An example of a simulation using the highest confidence cell kinetics factors discovered with the TORTOISE Test™ software is shown in **Fig. 2**.

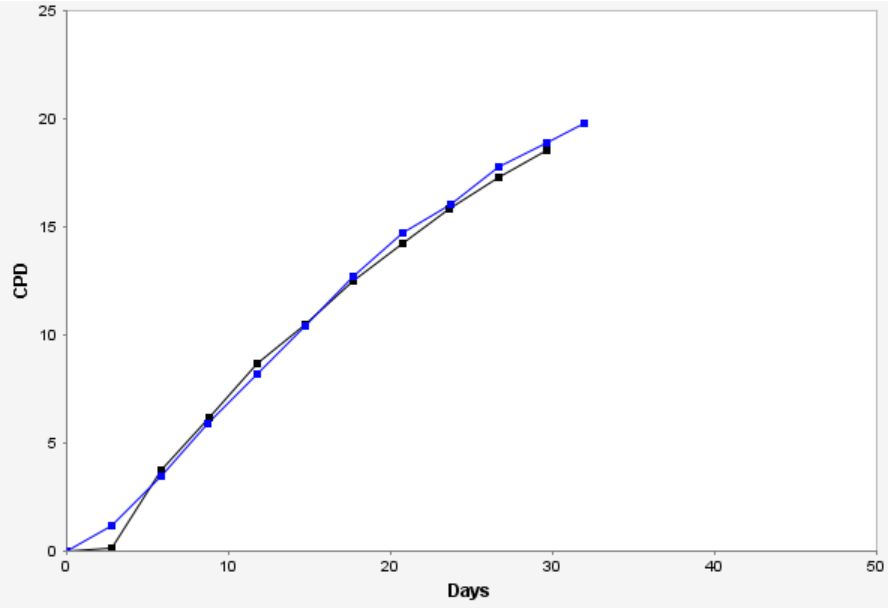

Figure 2. A CPD data simulation example. Cell kinetics factors determined from the simulation analysis of the CPD data shown in **Fig. 1** were used to simulate the mean CPD data. **Blue**, simulation. **Black**, mean CPD data.

III. The Initial Hematopoietic Stem Cell (HSC) Number

The most significant cell kinetics factor determined by the TORTOISE Test™ is the initial stem cell fraction of the starting cell preparation.

Initial HSC fraction = **1785 ± 553 HSC for every 10,000 mononuclear cells**
(n = 10; p = < 0.0001; 95% CI = 1389-2181)

IV. HSC Cell Kinetics Properties in Culture

The TORTOISE Test™ has the capability of discovering the cell kinetics properties of tissue stem cells in culture. **Fig. 3** shows that, in the evaluated cultures, HSCs are predicted to undergo periods of both symmetric self-duplication (**Fig. 3**, upward excursions) and periods of primarily asymmetric self-renewal, which keeps their number constant (**Fig. 3**, plateau segments). However, with continued serial passage, the stem cells exhibit the characteristic decline in number and fraction due to a characteristic low rate of symmetric self-renewal divisions (See Table 1 data).

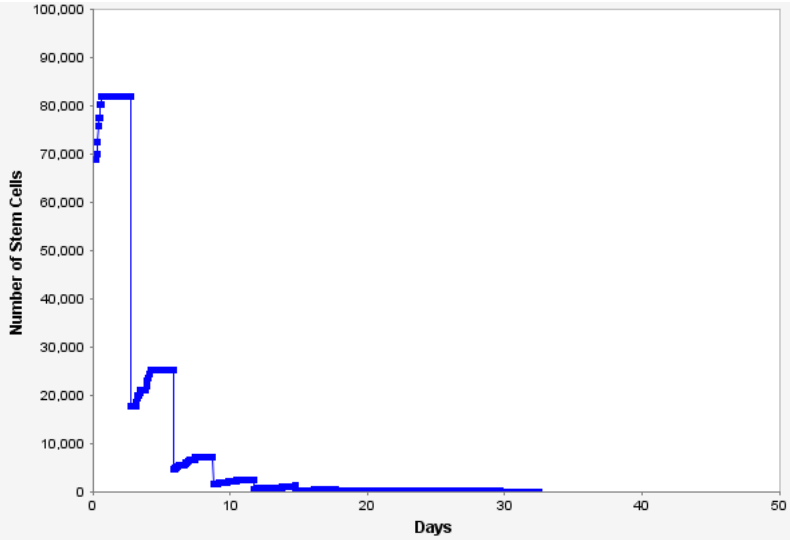

Figure 3. TORTOISE Test™ determined cell kinetics properties of cord blood HSCs during serial culture. Early upward excursions are indicative of periods of significant HSC symmetric self-renewal divisions, which increase the HSC fraction. **Plateau segments** are indicative of periods of HSC asymmetric self-renewal divisions, which maintain a constant number of HSCs. **Vertical declines** are due to 1:5 serial passages every 3 days.

V. General Cell Kinetics Factors

In addition to *stem cell* specific-cell kinetics factors, the TORTOISE Test™ provides, for the first time, estimates of cell kinetics properties of the two other major types of cells in complex human cell cultures: 1) actively dividing committed progenitor or transiently amplifying cells; and 2) terminally arrested differentiated cells. **Table 1** outlines the cell kinetics properties determined for each of the three cell types, including HSCs.

Table 1. Initial cell kinetics properties for different cord blood mononuclear cell types

	Mean ± SD	(p)[1]	95% CI
HSCs			
Initial fraction	**0.179 ± 0.055**	**< 0.0001**	**0.139 - 0.218**
Death rate	**0.03 ± 0.03**	**0.011**	**0.009 - 0.05**
Asymmetric cell cycle time	10h ± 3.8h	< 0.0001	7.4 - 13
Symmetric cell cycle time	8.5h ± 3.3h	< 0.0001	6.2 - 11
Symmetric self-renewal rate	**0.14 ± 0.09**	**0.0006**	**0.08 - 0.20**
HSC unit generation number[2]	8.0 ± 1.9	< 0.0001	6.8 - 10
Committed progenitor cells			
Death rate	0.06 ± 0.039	0.0008	0.03 - 0.09
Cell cycle time	9.8h ± 3.0h	< 0.0001	7.6 - 12
Arrested Differentiated cells			
Death rate[3]	0.31 ± 0.18	< 0.0001	0.21 - 0.41

[1] n = 10; [2] number of cell divisions by committed progenitor cells before terminal differentiation arrest occurs; [3] estimated directly from cell count viability data, n = 14. *Note: Bolded values are* ***potency factors****. 1-symmetric self-renewal rate =* ***Specific Replenishment Rate of Mature Functional Cells***

VI. Cell Type-Specific Kinetics Profile

In addition to a unique look at how the specific cell kinetics properties of stem cells evolve during serial culture, the TORTOISE Test™ software provides a direct comparison to the respective cell kinetics of committed progenitor cells and arrested differentiated cells (**Fig. 4**). These data are ideal of defining and relating specific biomarkers for each of the three types of cells. This analysis capability can also be used to follow how supplemented culture factors affect the relative cell kinetics of these three interdependent cell types.

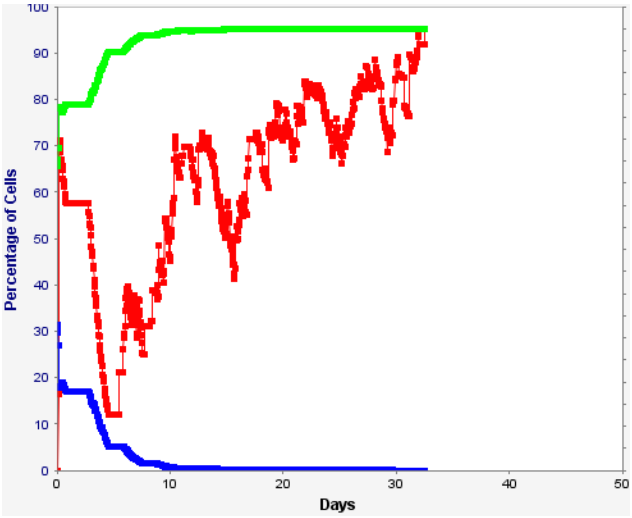

Figure 4. Respective changes in the three interdependent cell types present in CD34^{+}-selected cord blood cell cultures during serial passage. **Blue**, percent HSCs; **Red**, percent arrested differentiated cells; **Green**, percent total cycling committed progenitor cells and arrested differentiated cells. The difference between the green and red plots can be derived to inspect the cell kinetics of cycling committed progenitor cells separately.

CONTACT

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