Predicting Hematotoxicity in Drug Development with HemaTox™ Assays

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PURPOSE

In vitro colony-forming unit (CFU) assays allow for the assessment of hematopoietic progenitor cell growth and may be used to assess hematotoxicity in vitro. CFU assays have been validated for their ability to predict in vivo hematotoxicity, such as maximum tolerated dose for some hematopoietic progenitor cell lineages.[1-2] However, while CFU assays are the gold standard for hematotoxicity evaluation, these semi-solid medium-based assays are low throughput and require expertise in colony identification.

STEMCELL Technologies developed HemaTox™ assays to assess the toxicity of drugs on the growth and lineage-specific differentiation of human CD34+ hematopoietic stem and progenitor cells (HSPCs) into one of three specific progenitor cell lineages (erythroid, myeloid, or megakaryocyte). These liquid medium-based assays, which show similar drug toxicity trends to those identified in CFU assays, can be performed in a 96-well format. Furthermore, HemaTox™ assays allow for flexible treatment regimens and improve the ability to evaluate effects of anti-proliferative drugs in vitro.

METHODS

FIGURE 1. HemaTox™ Assay Workflow

On Day 0, pre-qualified cryovials of cord blood (CB) CD34+ cells are thawed and their viability assessed. CD34+ cells are plated in a 96-well culture plate at 500 - 3000 cells/well (depending on lineage of interest) in the presence of test or control article. Three replicates are initiated for each test condition, as well as a negative control initiated with an equivalent amount of solvent (solvent control). The culture plates are placed at 37°C, 5% CO_2 for 7 - 10 days.

After the appropriate incubation period, a portion of the cells from each well are stained to evaluate live erythroid cells (CD71 and CD235A/Glycophorin A), live myeloid cells (CD15 and CD13), or live megakaryocytes (CD41 and CD45) by flow cytometric analysis. The total number of live erythroid cells ($CD71^+CD235A^-$, $CD71^-CD235A^+$, and $CD71^+CD235A^+$), live myeloid cells ($CD13^+CD15^-$, $CD13^-CD15^+$ and $CD13^+CD15^+$), or live megakaryocytes ($CD41^+CD45^+$) in the test/control article-treated cultures are compared to the solvent control cultures to determine the percent of control growth.

RESULTS

Correlation with the CFU-GM Assay for Toxicity Levels of a Wide Range of Myelosuppressive Compounds

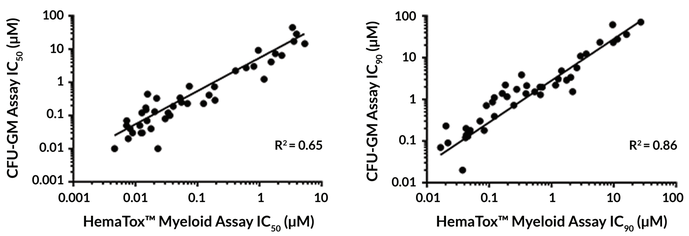
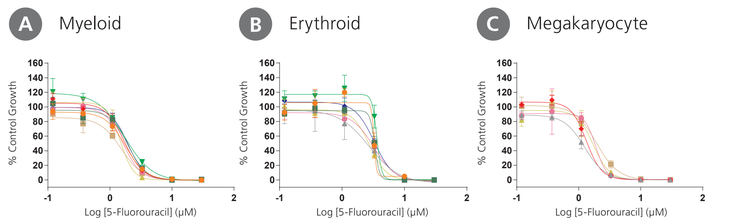


FIGURE 2. Correlation Between IC_{50} and IC_{90} Values for 43 Test Compounds Using the CFU-GM Assay and the Liquid-Based HemaTox™ Myeloid Assay

RESULTS

High Reproducibility with Pre-Qualified Primary Stem Cells and Flexible Treatment Regimens



IC_{50} Average	Standard Deviation
1.77 µM	0.22 (n = 7)

IC_{50} Average	Standard Deviation
3.13 µM	0.42 (n = 7)

IC_{50} Average	Standard Deviation
1.57 µM	0.29 (n = 5)

FIGURE 3. Comparison of HemaTox™ Assays Between Experiments with Multiple Donor Cell Lots

Dose-response curves were generated from titrations of 5-fluorouracil added to human CD34+ cells from five to seven donor lots (represented in different colors) in HemaTox™ (A) Myeloid, (B) Erythroid and (C) Megakaryocyte assays. In each assay, similar IC_{50} values were obtained with cells from different donors and in different experiments with cells from the same donor. Shown are values (% of control growth) normalized to the number of cells in the solvent control cultures.

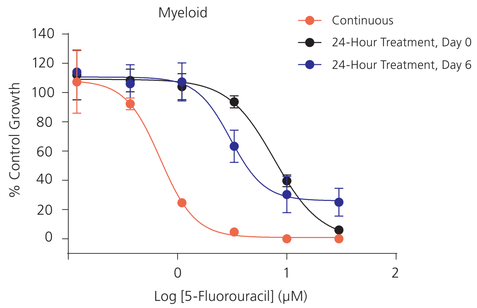


FIGURE 4. Short- and Long-Term Treatment Regimens Possible with HemaTox™ Assays

CD34+ cells were exposed to 5-fluorouracil continuously for the entire duration of the culture (Red, Continuous), transiently for 24 hours on day 0 followed by washout of the drug (Black, 24-Hour Treatment, Day 0), and transiently for 24 hours on day 6 (Blue, 24-Hour Treatment, Day 6), when committed myeloid progenitors were already present. Error bars represent triplicate culture wells from a single representative donor.

Summary

The HemaTox™ assay:

- Demonstrates high experimental reproducibility and low variability between pre-qualified cell lots
- Is capable of flexible treatment regimens, including short-term, long-term, and transient drug exposure
- Produces correlative toxicity trends with the gold standard CFU-GM assay
- Has an improved ability to detect toxicity in anti-proliferative drugs compared to the CFU assay

Improved Ability to Evaluate Anti-Proliferative Drug Effects

Traditional CFU assays primarily quantitate the effects of a drug on colony numbers and not effects on colony size that may result from inhibited cell proliferation. Azidothymidine (AZT) is well known to perturb hematopoiesis in patients, but does not exhibit high toxicity in CFU assays. HemaTox™ assays can detect changes in both cell differentiation (by assessing the expression of cell surface markers used to distinguish between specific cell populations) and cell proliferation (by absolute cell counts).

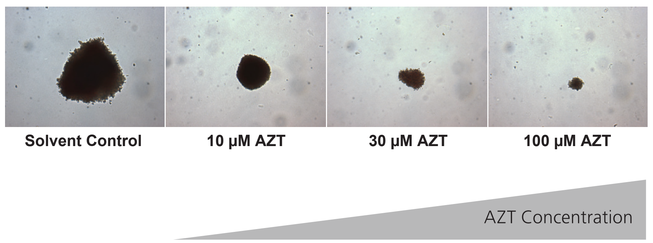
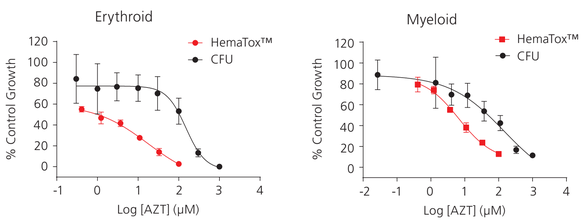


FIGURE 5. Strong Anti-Proliferative Effects of AZT can be Qualitatively Observed in the CFU Assay by Changes in Colony Size

Shown are erythroid (BFU-E) colonies at 10X magnification in a CFU assay after 14 days of culture in the absence and increasing presence of AZT.



	IC_{50} Average	Standard Deviation
HemaTox™ Erythroid	0.62 µM	0.58 (n = 4)
CFU Erythroid	84.13 µM	45.52 (n = 5)
HemaTox™ Myeloid	6.61 µM	2.73 (n = 5)
CFU Myeloid	35.57 µM	15.57 (n = 7)

FIGURE 6. AZT Demonstrates Greater Toxicity in HemaTox™ Assays when Compared with CFU Assays

Representative dose-response curves for AZT based on colony numbers (Black, CFU) and lineage-specific cell numbers (Red, HemaTox™). The table shows the average IC_{50} values from 3 - 5 multiple donor lots and 4 - 7 independent experiments.

References

1. Pessina A et al. (2003) Toxicological Sciences 75: 355–67.
2. Pessina A et al. (2009) Toxicology In Vitro 23: 194–200.