# Services for Predicting Hematotoxicity in Drug Development with HemaTox™ Assays

## Predicting Hematotoxicity

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

HemaTox™ assays have been developed for assessing 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 as identified in CFU assays, can be performed in a 96-well format. Furthermore, HemaTox™ assays allow for flexible treatment regimens and improve ability to evaluate the effects of anti-proliferative drugs in vitro.

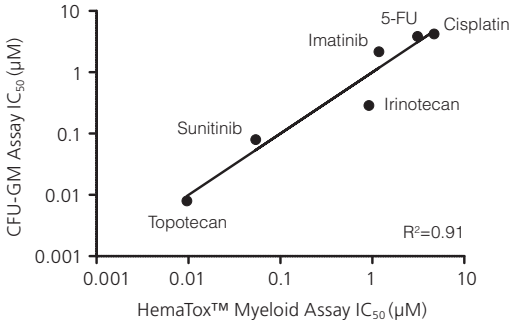



**Figure 1.** Correlation Between $IC_{50}$ Values for Six Drugs Measured Using the CFU-GM Assay and the 96-Well Plate HemaTox™ Myeloid Assay

Graph shows correlation between $IC_{50}$ values for 6 myelosuppressive drugs measured using the CFU-GM assay and the HemaTox™ Myeloid assay.

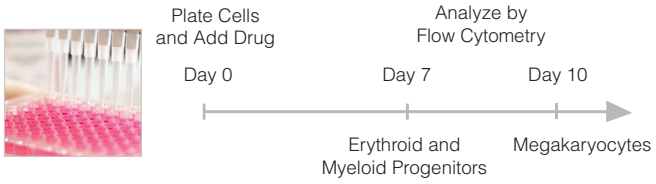



**Figure 2.** HemaTox™ Assay Workflow

HemaTox™ assays allow for high-throughput testing of compounds in 96-well format. Erythroid and myeloid progenitor cells can be analyzed by flow cytometry after only 7 days of culture and megakaryocytes can be analyzed after 10 days.

## Flexible Treatment Regimen

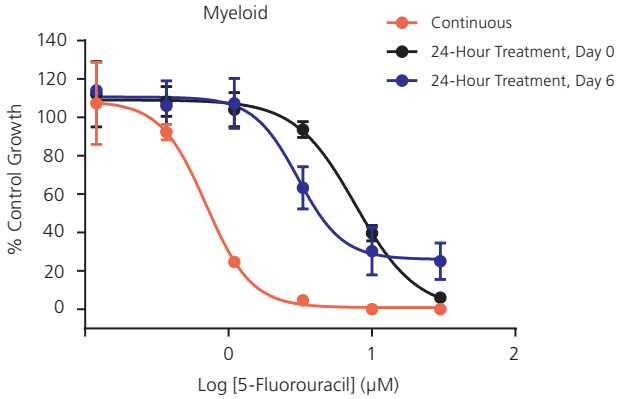
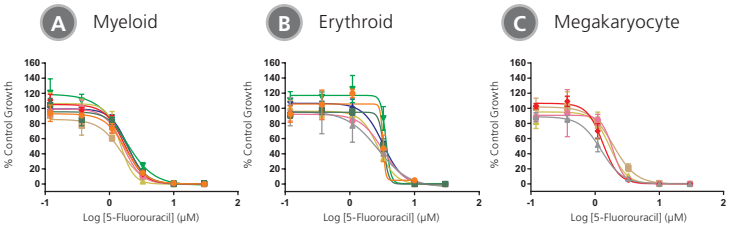



**Figure 3.** Short- and Long-Term Treatment Regimens Possible with HemaTox™ Assays

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.

## High Experimental Reproducibility and Low Variability Between Pre-Qualified Cell Lots




| $IC_{50}$ 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 4.** 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 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.

# Services for Predicting Hematotoxicity in Drug Development with HemaTox™ Assays

## Improved Ability to Evaluate Anti-Proliferative Drug Effects in HemaTox™ Assays: a Case Study of AZT

Azidothymidine (AZT) is an antiviral nucleoside analog that targets viral polymerases but can also inhibit cellular polymerases, leading to decreased cell proliferation and ultimately the suppression of hematopoiesis, resulting in anemia and neutropenia. 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. The inability to quantify changes in colony size may explain why AZT, well known to perturb hematopoiesis in patients, does not exhibit high toxicity in CFU assays. In contrast, 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.

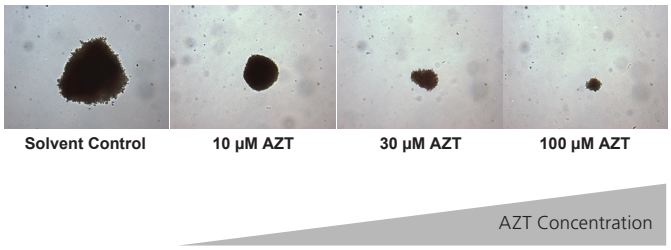
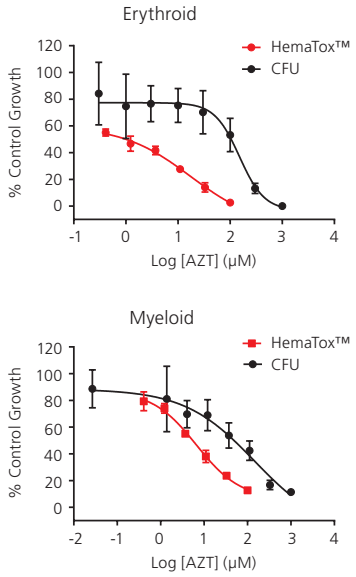



**Figure 5.** Strong Anti-Proliferative Effects of AZT can be Qualitatively Observed in the CFU Assay by Changes in Colony Size

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 three to five multiple donor lots and four to seven independent experiments.

## Why Choose Contract Assay Services (CAS) for HemaTox™ In Vitro Assays:

- CAS customizes each study to your particular needs, answering any questions and leveraging the expertise of STEMCELL scientists that developed these products
- $CD34^+$ cells are prequalified to ensure robust and reliable performance of the assay
- Assays are complemented by a suite of accessory in vitro and in vivo assays and over 18 years of experience in assessing hematotoxicity for global clients

If you are interested in learning more about Contract Assay Services at STEMCELL Technologies, visit **www.contractassay.com** or contact us at **contractassay@stemcell.com**.

## References

1. Pessina A, et al. (2003) Toxicological Sciences 75; 355–367
2. Pessina A, et al. (2009) Toxicology In Vitro 23; 194-200