Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol13Issue2abst008P.pdf

Evaluation of an Improved Throughput in-vitro Bone Marrow Toxicity Assay and its Application in Enabling Early Stage Discovery Safety.

The colony forming unit (CFU) assay is considered the gold standard in the in-vitro assessment of potential toxicity of drug candidates on bone marrow (BM) hematopoietic stem cells (HSCs) (ESAC 2006). In the CFU assay, compound-treated rat or human HSCs are cultured in a methylcellulose differentiation medium enabling cell proliferation and differentiation over a 10-14 day period, at which point CFUs are scored manually; thus the CFU assay is both time consuming and labour intensive and does not readily lend itself to a high-throughput system or application during the earlier stages of drug discovery. The aim of the present work was to evaluate, in comparison to the gold standard CFU assay, the application of a higher throughput BM toxicity assay that utilises a liquid differentiation medium and a proliferation endpoint (as measured by adenosine triphosphate (ATP) quantification). We also demonstrate the application of this higher throughput assay in enabling early stage drug discovery safety assessment.

Twenty-three in-house compounds, plus a positive control (5-Fluorouracil), were evaluated in the both the BM CFU assay and the BM ATP endpoint assay. For the CFU assay, CD34+ BM HSCs were cultured in compound-dosed MethoCult™ medium for 10 (rat) or 14 (human) days, followed by CFU enumeration and calculation of the IC_{50}. For the BM ATP assay, CD34+ BM HSCs were cultured in compound-dosed HemoGenix™ erythroid or myeloid linage differentiation medium, for 5 days (rat and human), followed by ATP quantification of cell number (CellTiter-Glo®, Promega™) and calculation of the IC_{50}. As a demonstration of the application of the BM ATP assay in enabling early stage discovery safety, the assay was used to evaluate whether the human BM toxicity of an in-house lead compound AZ-X (and its clinical competitors CC1 and CC2) was due to on- or off-target activity and also to assess the relative toxicity of the compounds.

Using Pearson correlation, a plot comparing the CFU-derived IC_{50} and the BM ATP-derived IC_{50} data from all 24 compounds revealed a significant correlation between the data from the two assay formats (r^2 = 0.85). Comparison of potency at the AZ-X, CC1 and CC2 primary target and IC50s from the BM ATP assay suggested that observed BM toxicity was a result of activity at the primary target. Additionally, the BM ATP assay demonstrated that the in-house compound AZX was no more toxic than the competitors.

In summary, we have demonstrated that the BM ATP assay is an excellent candidate as an improved throughput, quicker turnaround, less labour intensive and less costly alternative to the CFU assay. Critically, we have also shown that the BM ATP assay readily enables early stage discovery safety, using an example determining the on- or off-target pharmacological nature of compound-induced toxicity and how in-house compounds compare to competitors.