1. Abstract

A system consisting of human iCell® Cardiomyocytes (Cellular Dynamics International) and ApoTox-Glo™ assay reagents was assessed for the ability to distinguish potency and safety of anti-cancer compounds when compared to K562 cells. Acute cytotoxicity in in vitro culture models is linked to intrinsic cell type susceptibility, compound dosage, and exposure period. Conventional, single-parameter, membrane integrity or cell-health assays may reveal changes in viability or overall cell number, but rarely provide valuable insight into the cytotoxic mechanism by which cells are eliminated. We have developed a sequential addition, homogeneous, same well assay method that measures cellular viability, cytotoxicity and caspase activity as biomarker surrogates for defining cytotoxic mechanism. The resulting signals are fully compatible because the reporting molecules are spectrally distinct (fluorescence and luminescence), and are sufficiently sensitive to be miniaturized into high density plate formats. Herein we describe how the multiplex can be employed to generate human, cell-specific potency and safety profiles of anti-cancer compounds by comparing the iPSC-derived cardiomyocytes with the K562 leukemia cell line.

2. Triplex Principles

3. Multiplexed Assay Chemistry

4. Multiplexing General Viability Assays

5. Validation of ApoTox-Glo for Use with iPSC-derived Cardiomyocytes

6. Histone Deacetylase Inhibitor

7. Proteasome Inhibitor

8. Tyrosine Kinase Inhibitor

9. Summary and Conclusions

- A system consisting of human iCell® Cardiomyocytes and ApoTox-Glo™ assay reagents is an ideal mammalian cell model system to assess cytotoxic mechanisms.
- The assay can be readily miniaturized into high density formats.
- Cytotoxic mechanisms can be partially defined using the multiplexed viability, cytotoxicity and caspase activation chemistry (ApoTox-Glo™).
- Potency and selectivity can be evaluated within the context of disparate cell models.
- Data on safety (off-target effects) and potency (on-target effects) can be compiled using differentiated and cancer cell types.

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