Abstract 1664-216

GENOTOXICITY ASSESSMENT USING A NEW, HIGH SPECIFICITY AND HIGH SENSITIVITY HUMAN CELL ASSAY

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Abstract

Recent analyses of published genotoxicity and carcinogenicity assessments make rather uncomfortable reading for genetic toxicologists: a positive result from an in vitro *mammalian genotoxicity test is not a very reliable indication of potential carcinogenicity. High sensitivity is compromised by poor specificity. Over-prediction of carcinogenicity by the current* in vitro *tests causes many unnecessary* in vivo *tests (waste of animals, time, and money). We recently described a new* in vitro *reporter assay for the human GADD45a gene which provides high sensitivity along with high specificity. Assays from over 150 compounds, comprising further carcinogens and non-carcinogens, deliberately including many examples where other* in vitro *mammalian cell genotoxicity tests have given misleading indication of carcinogenicity. Example data collected by flow cytometry will be presented along with a summary of trials of robustness and reproducibility, and assays performed in the presence of S9.*

Introduction

❖ **Genotoxicity causes/contributes to significant failure in pharmaceutical development at the preclinical stage**

25-30% of drugs are positive in genotoxicity tests at the candidate selection stage .

About 15% of compounds are positive in the *in vivo* assays, post-candidate selection

❖ **Deployment of an HTS genotoxicity assay earlier in discovery could potentially enable compound prioritisation as early as hit-to-lead (H2L) to reduce late-stage failure**

Current *in vitro* mammalian cell genotoxicity liability screening assays are cut-down versions of the GLP regulatory assays - low throughput, time consuming, and compound hungry i.e. not screening solutions.

The current *in vitro* mammalian assays are inaccurate (compounds with positive results are tested in animals!)

❖ **Late-stage preclinical failure is very costly**

Rejection of a compound due to genuine genotoxicity can cost **$1M to $20M in wasted development**

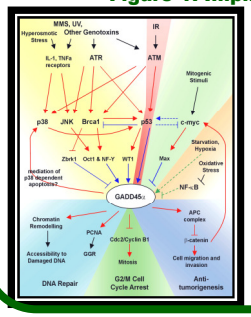
Delaying the release to market of a new blockbuster drug because of late mechanistic studies to resolve conflicting genotoxicity data costs about **$1M per day in lost revenue**

❖ **The GADD45a-GFP "GreenScreen HC" assay developed by Gentronix is a highly accurate, high-throughput 96-well microplate screening assay**

The assay uses a GFP reporter to exploit the genotoxin dependent transcription of the mammalian gene GADD45a (Figure 1) to give an indication of a compound's real genotoxic liability **[1]**.

GreenScreen HC is more accurate than the current *in vitro* genotoxicity assays

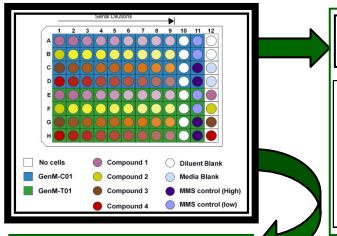
Figure 1. Implied GADD45a Regulation



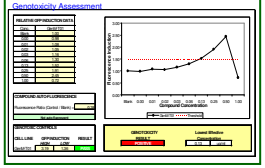
The GADD45a gene is a transcriptional target of many key tumour suppressors and oncogenes. Following genotoxic stress the protein interacts with a number of other key proteins to mediate processes such as DNA repair, G2/M checkpoint arrest, and apoptosis **[2]**.

Red connectors indicate an effect on protein activity.
Blue connectors indicate an effect on transcriptional activity.
Green connectors indicate an effect on mRNA stability.
Dashed connectors indicate that the mechanism of regulation is unclear.

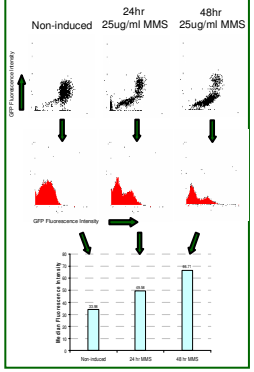
Figure 2. Measurement Choice from Microplates



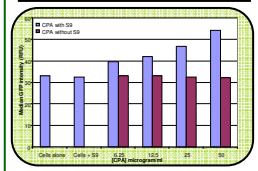
a). HTS plate reader – bulk cell measurement



b). Flow cytometer – lower throughput



c). Flow cytometer – additional utility



S9 extracts confound bulk cell optical measurements (colour & particulates). Here, cyclophosphamide (CPA) is shown giving correct positive data (3h exposure, 20h recovery) using flow cytometric analysis.

Results from HTS format studies

Inter-laboratory trial

❖ 4 labs, 16 compounds, 4 times: robustness and reproducibility trial.
- 86% (221 / 256) of expected genotoxicity calls across entire trial.

In-house testing

❖ The GADD45a-GFP assay has been validated with over 150 compounds.
- 75 compounds: initial validation paper [1]
- 62 compounds: an extended validation with marketed pharmaceuticals.
- 40 proprietary non-pharmaceutical compounds

External laboratory testing

❖ >500 compound tests in an Early Adopter Programme

Resolving data conflicts

❖ We have now tested 43 compounds uniquely positive in *in vitro* mammalian cell tests (negative Ames)

- 28 of 43 were negative in the *in vivo* MNT: 27 of these were correctly negative with GreenScreen HC (exception: ciclopirox olamine, a possible *in vitro* clastogen)
- 15 of 43 were positive in the *in vivo* MNT: 11 were correctly positive with GreenScreen HC. (exceptions: thiabendazole, *in vivo* unreproducible; stavudine, *in vivo* positive only at high concentrations; 2,4-dichlorophenol, *in vivo* mechanism not clear; pyrazinamide, weak response *in vivo*)

Conclusions from all studies

These validation studies have shown that GreenScreen HC has:

High sensitivity - assay responds to wide range of genotoxic mechanisms of action: direct acting mutagens, aneugens, topoisomerase inhibitors, DNA/nucleotide synthesis inhibitors, and ROS generators.

High specificity - assay does not produce positive results for non-genotoxins.

High utility - data from GreenScreen HC assay could be useful in early (H2L) identification of compounds with genotoxicity liability

❖ A compound positive in GreenScreen HC: likely positive *in vivo*, and *in vitro* mammalian cell assay
❖ A compound negative in GreenScreen HC: likely negative *in vivo*, negative or "false positive" *in vitro*

Summary

The GADD45a-GFP GreenScreen HC assay is highly predictive of biologically relevant genotoxicity.

As a 96-well microplate assay, GreenScreen HC has the qualities of high-throughput and low compound requirement that would facilitate its application in early screening for genotoxic liability.

References: [1] Hastwell *et al.* (2006), Mutat. Res. **607**, 160-175; **[2]** Zhan *et al.* (2005), Mutat. Res. **569**, 133-143