ToxTracker ACE and TubulinTracker provide mechanistic insight into the mode of action of aneugenic substances

I. Brandsma, M. Hoogenboom-Geijer, R. Derr, N. Moelijker, T. Osterlund and G. Hendriks

Toxys B.V., Robert Boyleweg 4, 2333 CG, Leiden, the Netherlands.

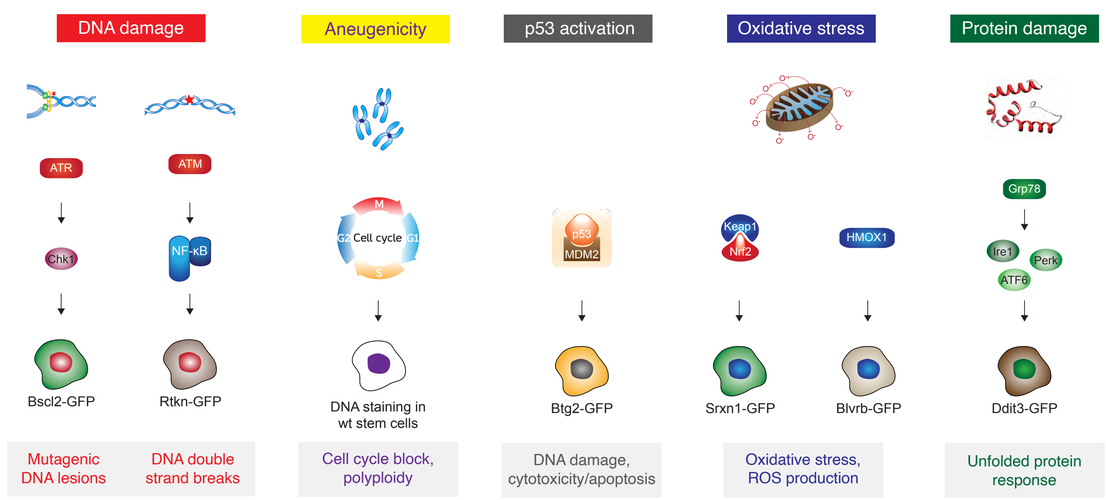
Introduction

Understanding the mode of action (MOA) and the classification of clastogens and aneugens is important for regulatory considerations. ToxTracker ACE is a mammalian stem cell-based reporter assay that detects the activation of specific cellular signalling pathways upon exposure to compounds to provide insight into the MOA. Selective activation of the different DNA damage reporters together with a DNA stain enables the rapid and quantitative evaluation of the clastogenic and/or aneugenic potential of compounds.

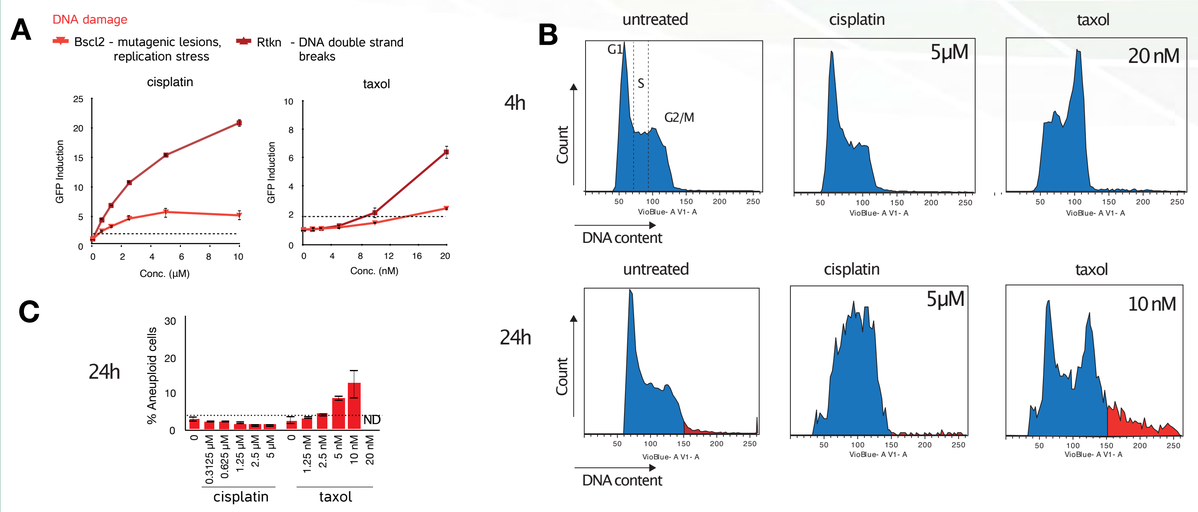
Aneuploidy, the presence of an abnormal number of chromosomes, can be caused by any process that interferes with chromosome segregation during mitosis, including microtubule disruption or inhibition of the Aurora A/B/C cell cycle kinases. Here, we established a reporter assay, TubulinTracker, to study the effect of substances on GFP-tubulin stability, to provide more insight into the cause of aneuploidy. The mechanistic insight into the MOA provided by this assay is important for hazard identification and part of weight of evidence approaches.

ToxTracker ACE

- The ToxTracker assay is a stem cell-based reporter assay consisting of 6 GFP-reporter cell lines.
- GFP-tagged biomarkers are activated upon specific cellular responses to DNA damage or other stress.
- Activation of the biomarkers is analyzed using flow cytometry.
- ToxTracker ACE includes cell cycle analysis and polyploidy detection for aneugen vs clastogen evaluation.

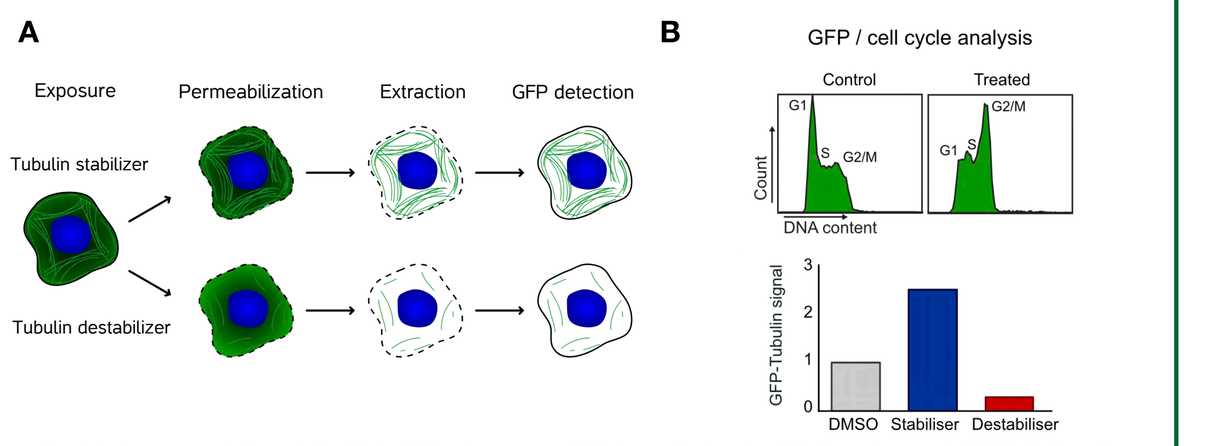


Aneugens and clastogens in ToxTracker ACE



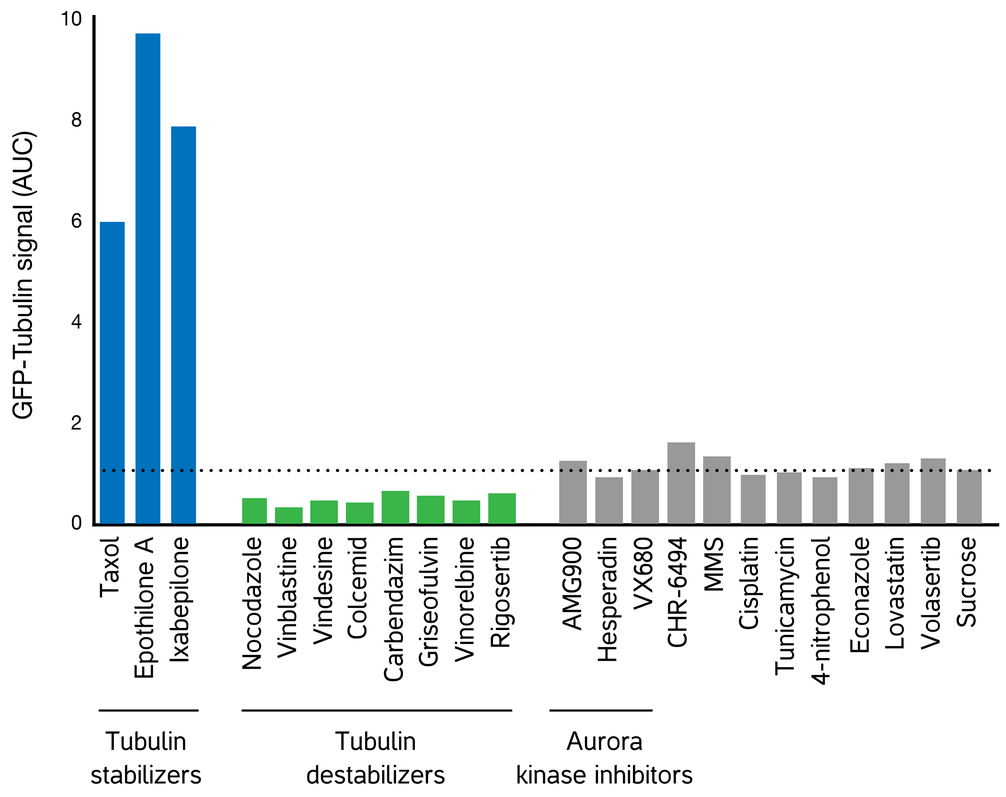
ToxTracker ACE analysis of clastogenic and aneugenic compounds. A) Graphs showing activation of DNA damage reporters in the ToxTracker. Assay was performed after 24 hours of exposure and GFP intensity was measured in intact cells. The dashed line indicates the threshold for a positive test (2-fold induction). B) Examples of cell cycle profiles after 4 and 24 h of treatment. Aneuploid cells (>4n) are shown in red. C) Quantification of the percentage of aneuploid cells after 24 hours of treatment. A threshold for aneuploidy (4%, dashed line) was calculated based on vehicle control data (average + 2xSD). ND: not determined due to cytotoxicity.

TubulinTracker



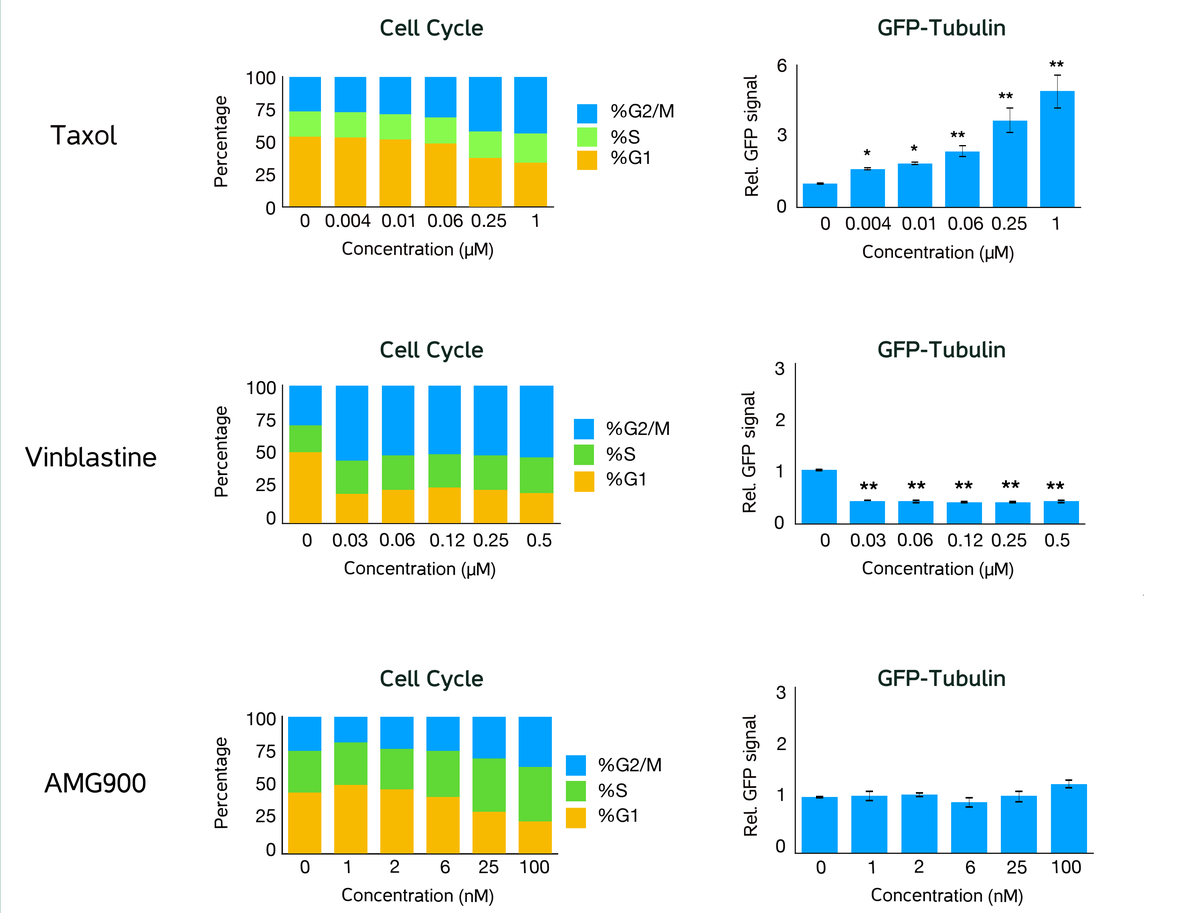
Graphical representation of the TubulinTracker assay. A) Schematic showing tubulin polymers in GFP-tubulin cells exposed to a microtubule destabilizer or stabilizer. After treatment, DNA is stained and cells are permeabilized to extract free tubulin. After fixation, the remaining polymerized GFP-tubulin in the cells and DNA stain intensity is quantified using flow cytometry. B) Schematic showing the effect of tubulin stabilizing and destabilizing compounds on the cell cycle and the GFP-tubulin signal. The GFP-tubulin signal is normalized to vehicle control.

Summary TubulinTracker



Overview of Tubulin stabilising and destabilising compounds. The mES TubulinTracker assay was validated using 23 compounds. GFP-tubulin cells were treated for 4h, using 10 concentrations of the test substances. To classify substances, the area under the curve (AUC) was calculated for the exposures, normalizing both the exposure concentration and the fold-change in GFP-signal. Compounds stabilizing microtubuli showed a large increase in AUC, while tubulin destabilizing compounds showed a decrease in AUC. For compounds not affecting tubulin stability, the AUC was around 1 (dotted line).

Tubulin poisons disrupt microtubule dynamics



Effect of compound exposure on the cell cycle and GFP-tubulin signal. Left panels, show the percentage of cells at each cell cycle phase, after 4h of treatment with the indicated compounds. Right panels, the analysis of GFP-tubulin signal in G2/M cells only. Exposure to all substances lead to an increase in G2/M phase cells. Tubulin stabilizers significantly increased, while tubulin destabilizers significantly decreased the GFP-tubulin signal. Bars represent the mean for 3 biological replicates, error bars show SEM. *p<0.05 **p<0.005 treated vs DMSO control (t-test).

Conclusions

- ToxTracker ACE distinguishes aneugenic from clastogenic substances based on DNA damage reporter activation and cell cycle analysis.
- Further insight into the mode of action of aneugenic substances is obtained using TubulinTracker.
- Using a stable GFP-tubulin reporter cell line, microtubule poisons can be reliably identified.
- These assays provide insight into the MOA of genotoxic agents.