

# The ToxTracker reporter assay detects indirect genotoxicity by high levels of oxidative stress.

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### Introduction

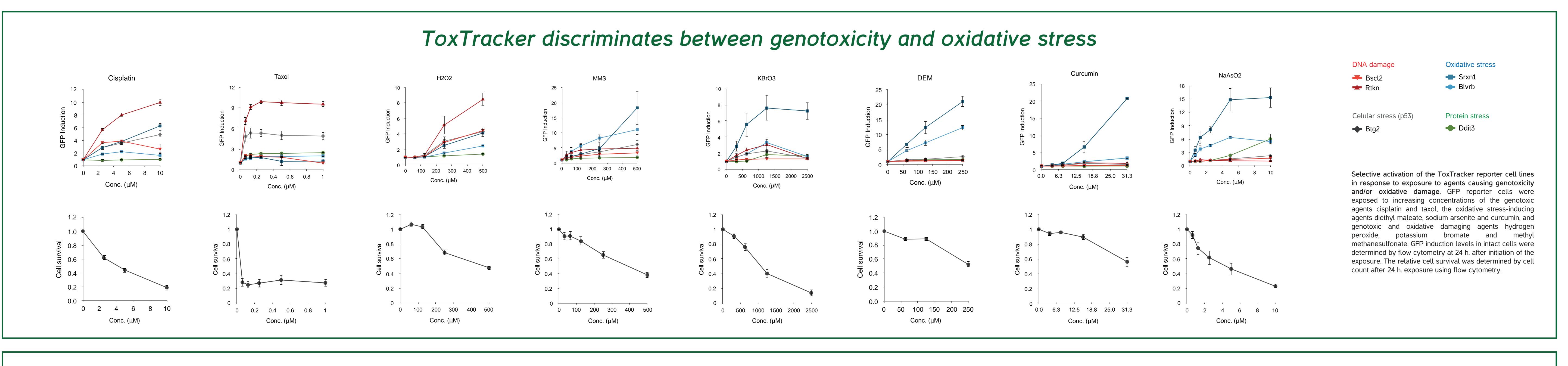
ToxTracker is a mammalian stem cell-based reporter assay that detects activation of specific cellular signaling pathways upon exposure to compounds. During the validation of this assay, we found that various compounds that give misleading positive results in the conventional in vitro genotoxicity assays did not activate the DNA damage reporters but did induce high levels of oxidative stress or protein damage in ToxTracker.

To investigate potential indirect genotoxic effects of compounds caused by high levels of oxidative stress, a selection of 8 different genotoxic and oxidative compounds were tested in ToxTracker in the presence of the ROS scavenger NAC (N-acetyl cysteine).

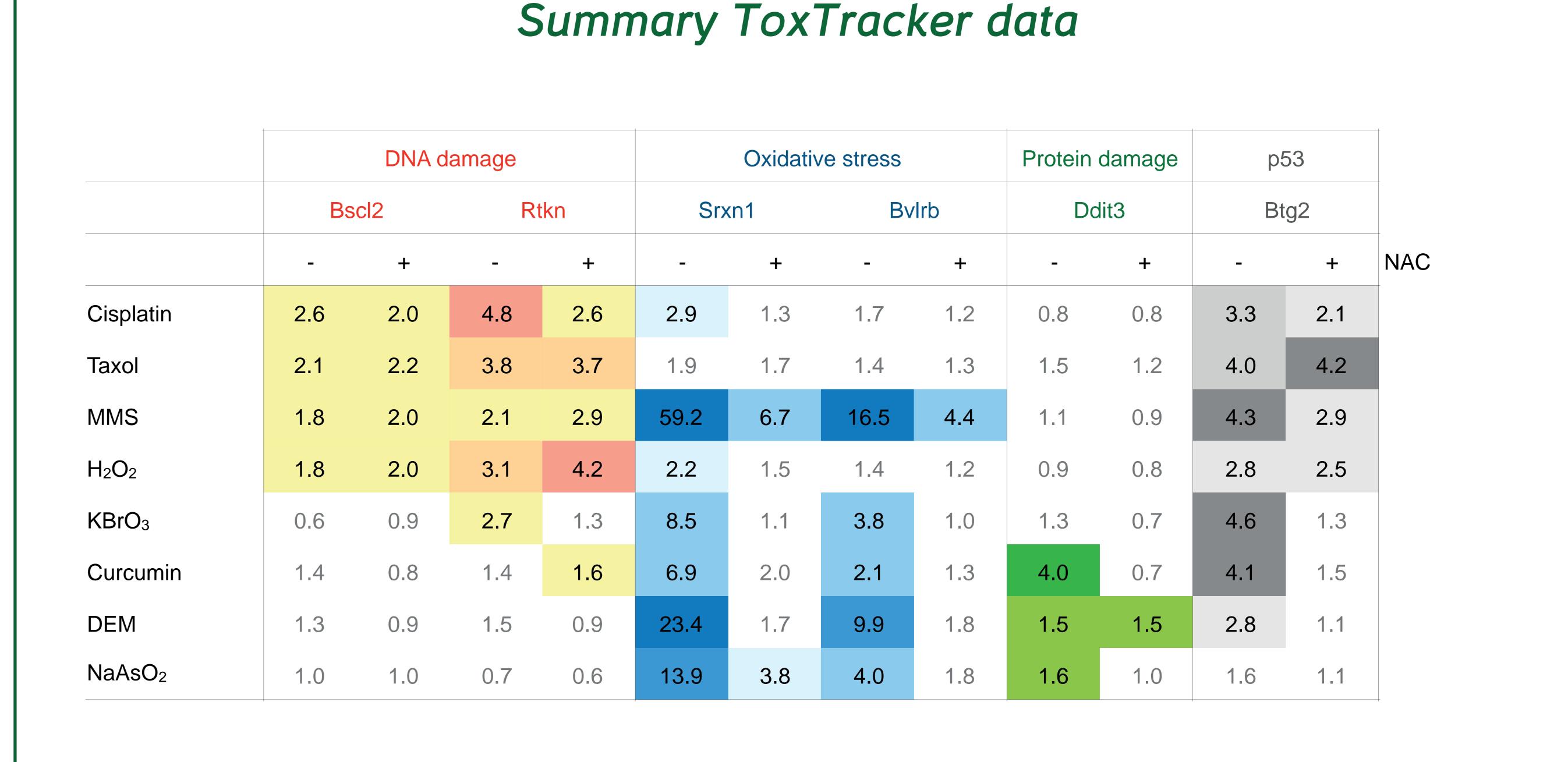
### The ToxTracker reporter assay Stem cell-based reporter DNA damage p53 activation Oxidative stress Protein damage assay consisting of 6 GFP reporter cell lines Exposure of reporter cell lines in 96-wells plates, in absence or presence of a bioactivation system Developed for in vitro (S9 liver extract) carcinogenicty hazard Ire1 Perk screening Cytotoxicity and GFP reporter detection by flow cytometry Provides insight into Tp Quantitiavite data analysis mechanisms of using Toxplot software genotoxicity DNA double- Cytotoxicity ROS Production ROS Production Unfolded

protein response

#### Compounds in this study Mode of action Agent N-acetyl cysteine Antioxidant DNA crosslinking agent Cisplatin Paclitaxel Microtubule stabilizer Hydrogen peroxide Oxidant Genotoxic DNA alkylating agent Methyl methanesulfonate Oxidant Potassium Bromate Diethyl maleate Oxidant Oxidant Curcumin Oxidant Curcumin NaAsO Sodium Arsenite Oxidant



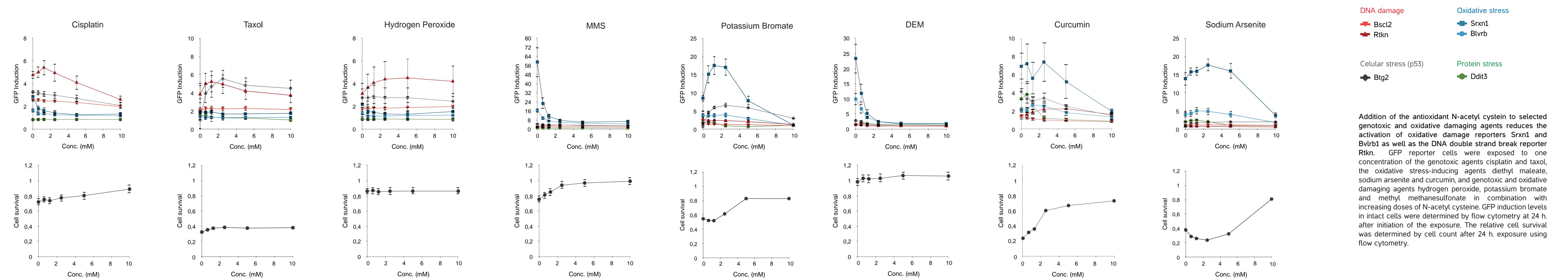
DNA lesions strand breaks



# average GFP induction for all six reporters in the presence and absence of N-acetyl cysteine.

Addition of the ROS scavenger N-acetyl cysteine reduces the activation of oxidative damage reporters Srxn1 and Bvlrb1 and for some of the compounds also the DNA double strand break reporter Rtkn. Heatmap shows the

# Activation of oxidative stress and genotoxicity reporters is reduced by ROS scavenger NAC



## Conclusions

- ROS scavenger NAC reduced the activation of the Srxn1-GFP and Bvlrb-GFP oxidative stress reporters for all compounds that induce oxidative damage.
- The ToxTracker assay in combination with a ROS scavenger can discriminate between direct DNA reactivity and indirect genotoxicity caused by oxidative stress.
- Cytotoxicity of the oxidative compounds was reduced by the addition of ROS scavengers, suggesting that induction of oxidative stress contributes to cytotoxicity.