Stem cells are the most sensitive screening tool to identify toxicity of GATA4-targeted small-molecule compounds

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Background
Cardiac transcription factors, such as GATA4, NKX2-5, and TBX5, are key regulators of cardiac development. They also play an important role in the development of pathological conditions such as cardiomyopathy and left ventricular hypertrophy and are therefore considered attractive drug targets. We have recently described a new family of GATA4-targeted compounds that inhibit cardiac hypertrophy. The aims of this study were to compare different cardiac and stem cell types in toxicity screening and to investigate the in vitro toxicity and structure-toxicity relationships of novel GATA4-targeted compounds.

Methods
Cell viability was studied in eight cell types: the H9c2 myoblast cell line, primary neonatal rat ventricular cardiomyocytes and fibroblasts, mouse embryonic stem cells (mESCs), mouse embryonic fibroblasts, mESC-derivatives from day 5 embryoid bodies, human induced pluripotent stem cells (hiPSCs) and hiPSC-derived cardiomyocytes (hiPSC-CMs). The cells were exposed to test compounds for 24 hours and the lactate dehydrogenase (LDH) and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were carried out to investigate necrosis and mitochondrial redox metabolism, respectively. Based on these results two compounds were chosen for high-content analysis of cell viability and proliferation using hiPSCs and hiPSC-CMs.

Results
None of the compounds induced necrosis even at the highest concentration studied (30 mikroM). The MTT assay however revealed significant structure-dependent and cell type-specific toxicity profiles for the structurally related compounds. The stem cells were the most sensitive cell types, whereas cardiomyocytes and fibroblasts were more resistant. The lead compound 3i-1000 induced a significant reduction in cell viability in hiPSCs (93-94% at 3-30 mikroM, P < 0.001) but had no effect on cell viability in hiPSC-CMs (Fig. 1). It also induced a 2.7-fold (P = 0.100) increase in cells positive for a fluorescent caspase reporter, indicating that stem cell death caused by some of the GATA4-targeted compounds is at least partially caspase-dependent.

Conclusions
The cell types used for toxicity screening have a major impact on the results and should thus be chosen carefully. Stem cells provide most sensitive model for toxicity screening. Identification of structural features responsible for stem cell toxicity in GATA4-targeted compounds allows further drug development towards non-toxic derivatives.