# Human Stem Cell-Derived Osteoblasts as an *In Vitro* Assay to Assess Bone Developmental Toxicity

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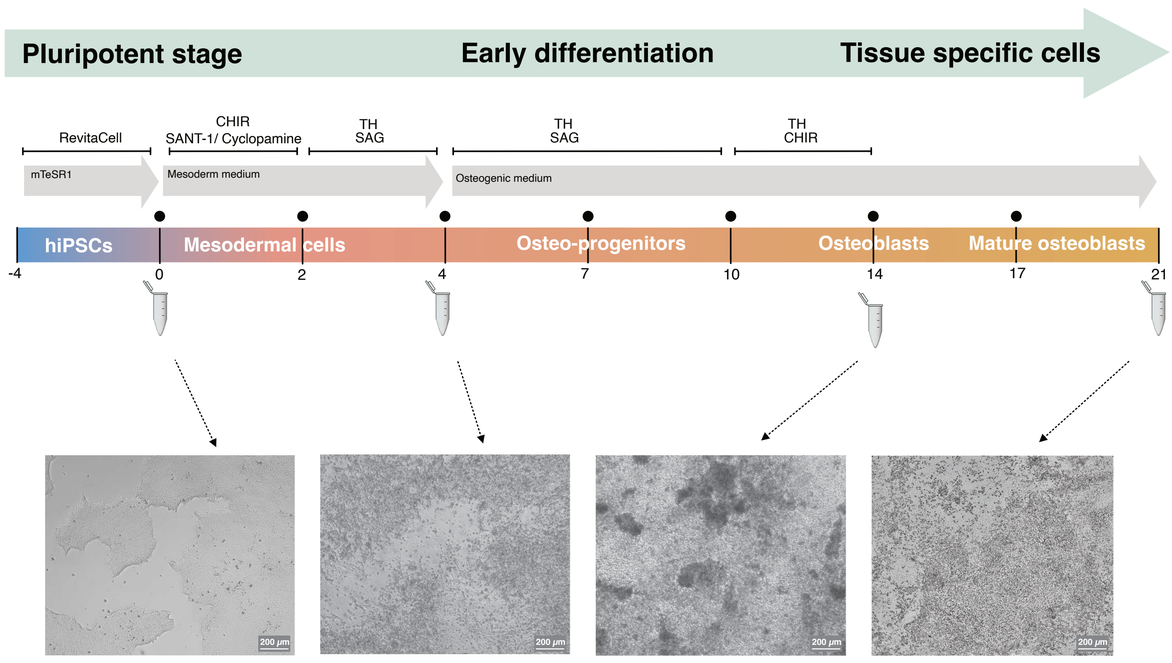
## Introduction

- Bone and skeletal abnormalities are among the most severe birth defects linked to medications. Several drugs, such as thalidomide and warfarin, have been reported to cause bone malformations, including shortening of bone length, radial dysplasia, and brachycephaly.
- We previously developed ReproTracker, a human induced pluripotent stem cell (hiPSCs)-based biomarker assay that predicts the teratogenicity of drugs and other chemicals. This assay utilizes three different lineage-specific cells: hepatocytes, cardiomyocytes, and neural rosettes.
- In this study, we introduced a new lineage-specific cell type to the ReproTracker platform, specifically aimed at capturing the direct effects of teratogenic agents on bone development.

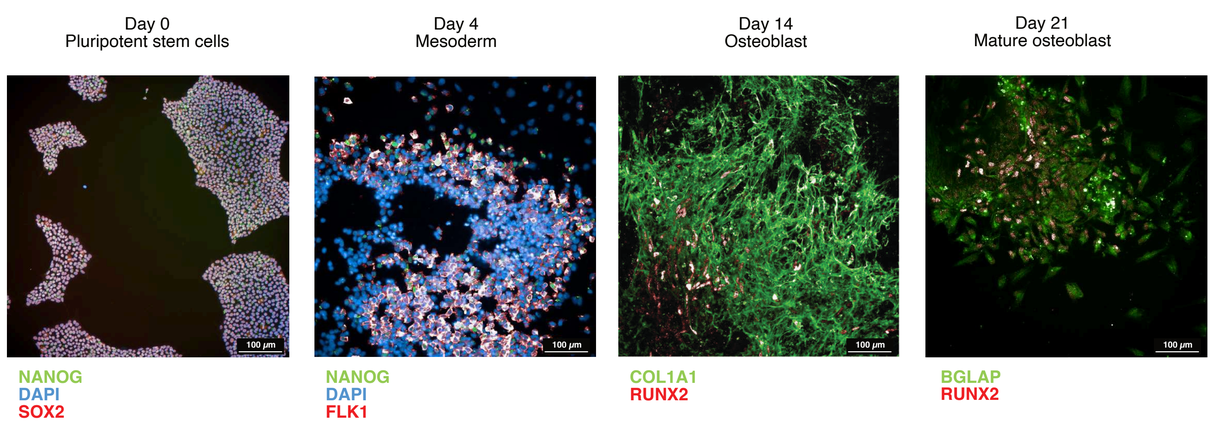
## Methods




Schematic representation of hiPSCs differentiation towards osteoblast-like cells with representative brightfield images at the different time points.

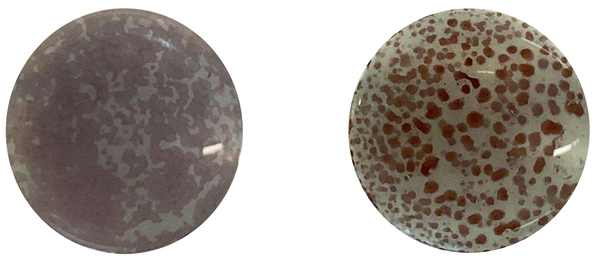
### Morphology assessment & biomarker expression analysis

#### Immunofluorescent staining



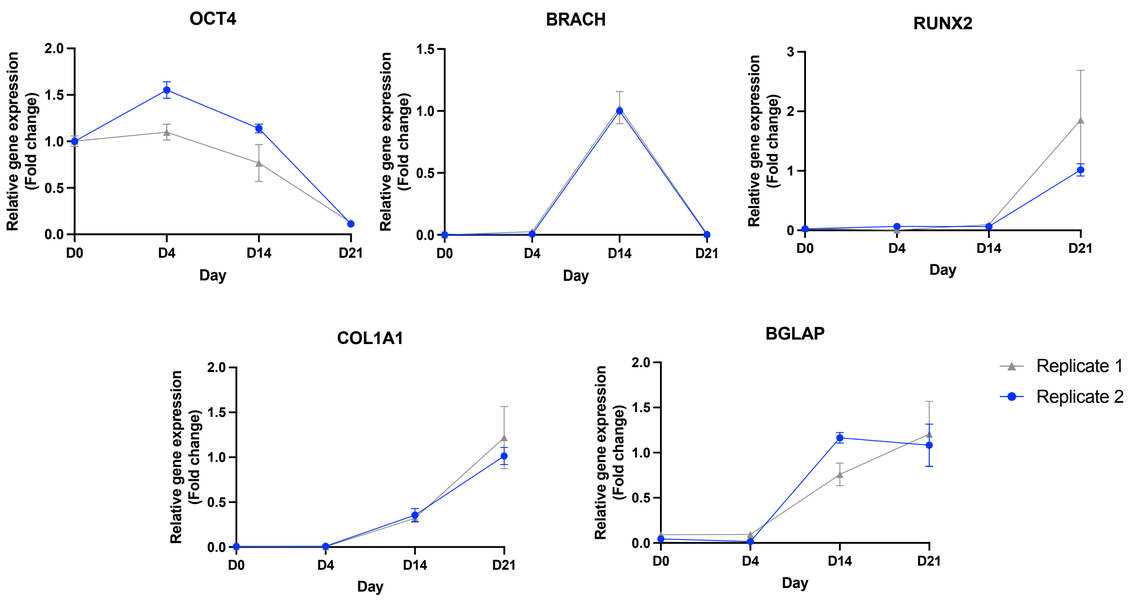

Immunostaining of the cultures at different developmental stages showing the expression of the relevant biomarkers.

#### Functional analysis: Alizarin red staining




Alizarin Red Staining indicates the presence of mineralization nodules in the osteoblasts at D21 compared to hiPSCs at D0.

#### Biomarker expression analysis

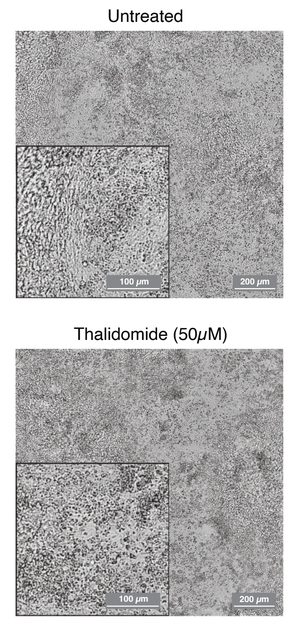



Gene expression of key bone developmental biomarkers at the different developmental stages. Biomarkers included represent pluripotency (*OCT4*), mesoderm (*BRACHYURY*) and osteoblasts (*RUNX2, COL1A1, BGLAP*). The effect of teratogenic compounds was determined by their ability to decrease gene expression of bone biomarkers at different stages of the differentiation.

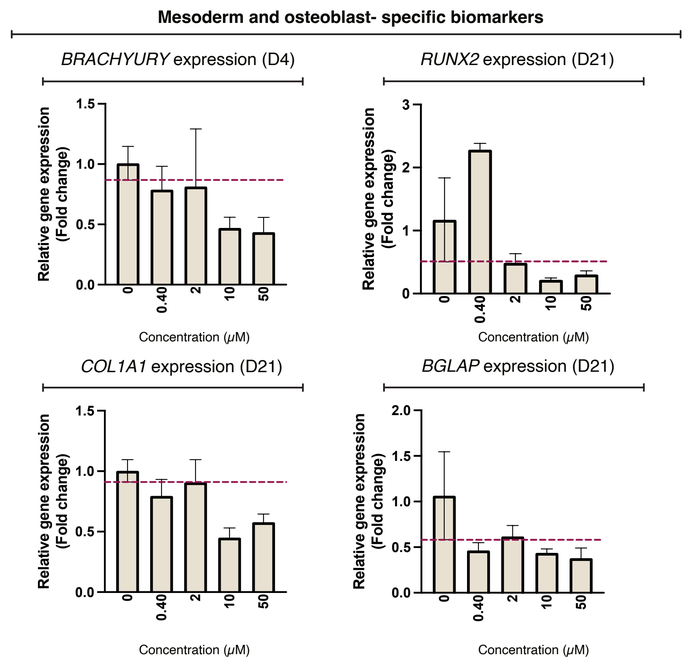
## Results

### Thalidomide

#### Morphology assessment



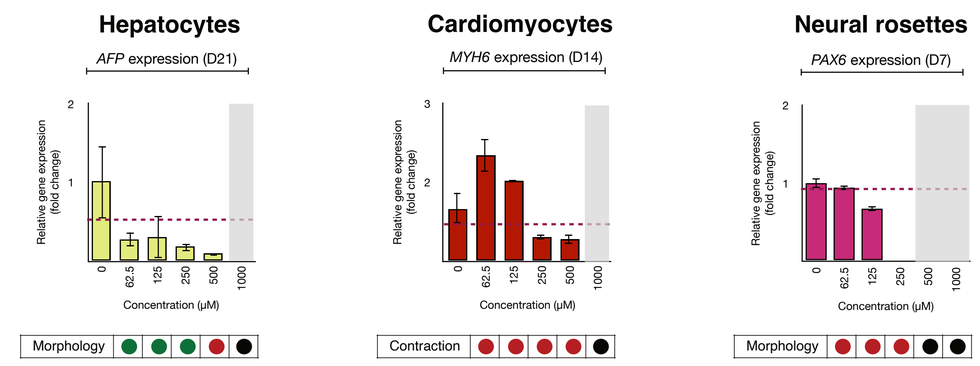

#### Biomarker expression analysis



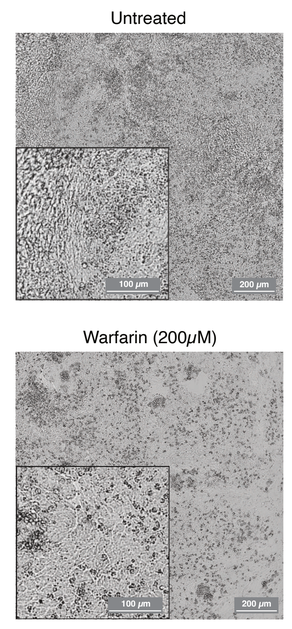

Exposure of differentiating cells to thalidomide selectively decreased expression of bone biomarkers and altered morphology at clinically relevant concentrations.

### Warfarin

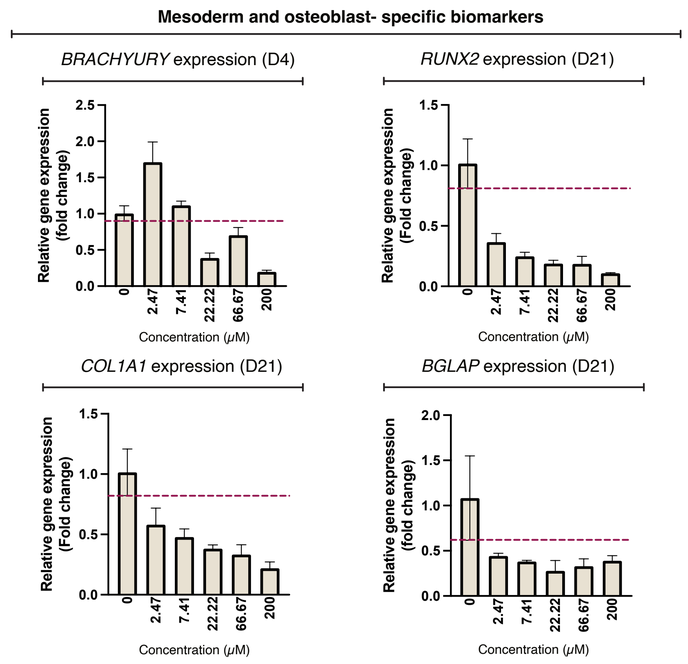
#### ReproTracker results




#### Morphology assessment




#### Biomarker expression analysis




#### Teratogen in human, ReproTracker and rat

| ReproTracker | Rat study | Human |
|---|---|---|
| LOAEL 2.47-7.41µM | LOAEL ~13 µM (based on 0.5 mg/kg) | Cmax ~18-20 µM (therapeutic dose) |

#### Non-teratogen in rabbit

| Rabbit study |
|---|
| LOAEL n.d. |

Inclusion of bone development into the ReproTracker portfolio increased sensitivity of the assay and detected teratogenicity of warfarin at the clinically relevant concentration.

## Conclusions

- Addition of the osteoblast lineage cells to ReproTracker allows identification of the teratogenic potential of chemicals in the development of bone.
- This assay has the potential to expand the spectrum of teratogenic agents detectable by ReproTracker during early in vitro teratogenicity screening.
- Inclusion of bone development into the ReproTracker portfolio allows to increase sensitivity of detection of Warfarin to concentrations below the clinically relevant concentration.

## Bibliography

Zujur et al. Stepwise strategy for generating osteoblasts from human pluripotent stem cells under fully defined xeno-free conditions with small-molecule inducers. https://doi.org/10.1016/j.reth.2019.12.010.