Improving clinical translation of neuroblastoma treatment effects by integration of preclinical experimental innovation and computational modeling

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Introduction

Development of quantitative systems pharmacology (QSP) models requires large datasets that can only be obtained by high-throughput preclinical experiments using e.g. zebrafish[1]. Subsequently translating pharmacological findings to higher vertebrates, including humans requires a drug exposure-effect relationship, for which experimental innovation needs to be integrated with computational modeling.

We present a new method to characterize an isotretinoin exposure-effect relationship in neuroblastoma (NB) zebrafish, and to quantify NB volumes more accurately, for improved translation to the clinic.

Methods

- The fluorescent NB zebrafish model[2] was used, which overexpresses MYCN and visualizes NB cells by Dβh-promotor controlled EGFP expression (figure 1).
- Fluorescent tumour area was quantified using stereofluorescence microscopy upon 1-7 days of treatment of 0 (control), 1, 1.5, and 2 uM isotretinoin (13-cis retinoic acid). An exploratory exposure-effect relationship was modelled in R using linear regression.
- A method was developed to use Optical Projection Tomography (OPT), a 3D-imaging technique, to accurately measure tumor volumes within the zebrafish upon isotretinoin exposure.

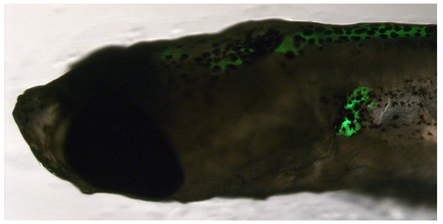
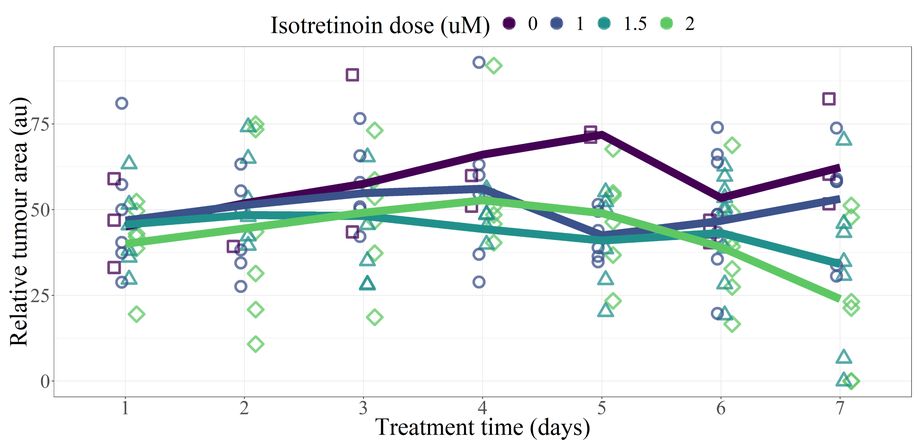


Figure 1. NB zebrafish disease model with fluorescent tumour and minor dorsal autofluorescence.

Results



↓ **Table 1.** Linear regression of isotretinoin treatment effect on relative tumour area (arbitrary units).

Dose group	Estimate (au)	p-value
Control	55.7	-
Low dose (1 uM)	-5.63	0.26
Middle dose (1.5 uM)	-11.6	0.021
High dose (2 uM)	-13.4	0.0080

← **Figure 2.** Relative tumour area (arbitrary units) over 7 days of isotretinoin treatment at 0 (control), 1, 1.5, or 2 uM. Symbols show tumour area, lines show loess smooth.

A isotretinoin dose dependent decrease in relative tumor area can be observed. This is especially clear after 4 days of treatment (figure 2, table 1).

To get more accurate measurements of the tumor volume, 3D images are taken. Following an optimized protocol, zebrafish were cleared to be sufficiently transparent for optical access of the tumors, while retaining the fluorescent signal from NB cells for quantification. Bespoke software was developed to reconstruct 3D NB volumes based on 2D fluorescence images taken from 400 angles (figure 3).

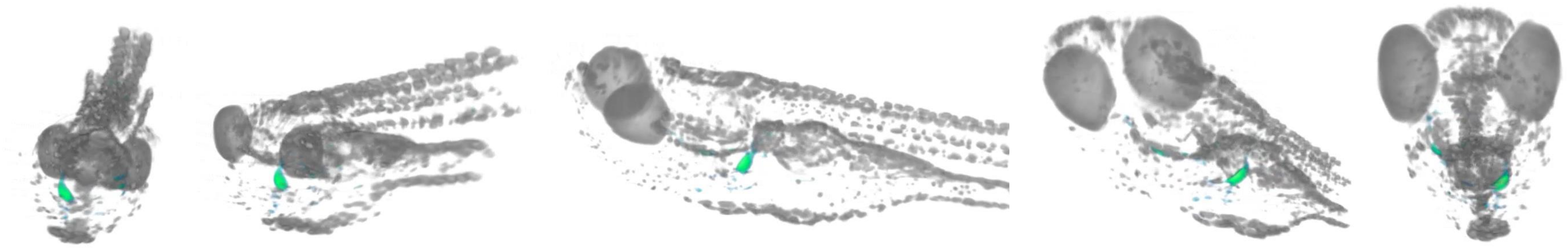


Figure 3. Optical Projection Tomography (OPT) based 3D reconstruction of GFP-fluorescent neuroblastoma tumour (in green) within a zebrafish

Conclusion

An exposure-effect relationship of isotretinoin treatment of NB zebrafish has been quantified based on external concentration and 2D measures. With the new method to measure 3D tumor volumes, combined with internal exposure measures, this quantitative relationship will be improved; essential for translation towards the clinic.

References

[1]Schulthess P and Van Wijk RC et al, CPT:PSP **7**, 285-287 (2018); [2] He, S et al. *Elife* **5,** e14713 (2016), [3]Tang, X et al, *IEEE Trans on NanoBiosci*, 16:5,367-374 (2017)

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