Zebrafish patient tumor-derived xenograft models as a screening system to find the right PDX-mouse model as well as to be used for individualized treatment of cancer patients

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Introduction

Due to the highly advanced nature of many modern cancer treatments, the pharmaceutical industry has been presented with a significant drug discovery challenge: to successfully select the most reliable, translational in-vivo models for the preclinical testing of new oncology therapies.

Also, another challenge the pharmaceutical industry faces is the fact that many drugs fail late-stage development. Here oncology holding one of the highest preclinical to clinical drug failure with 95% of preclinical drugs failing during clinical development. This typically relates to the diversity in genetic backgrounds seen across clinical trial patients, which correlates directly to the variety of drug responses observed during testing. This highlights the value and needs for models that can accurately predict individual patient responses to cancer drugs and understanding how to adopt a more individualized approach to clinical study design.

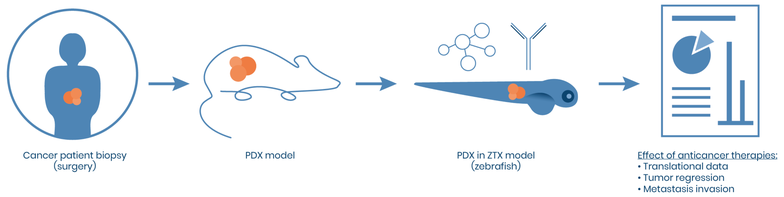
Aim

To evaluate a zebrafish tumor xenograft model (ZTX) as a tool for screening patient derived xenografts (PDX)- models to select the best models for rodent studies as well as a tool to evaluate metastases and finding responders and non-responders for specific treatments.

Methods

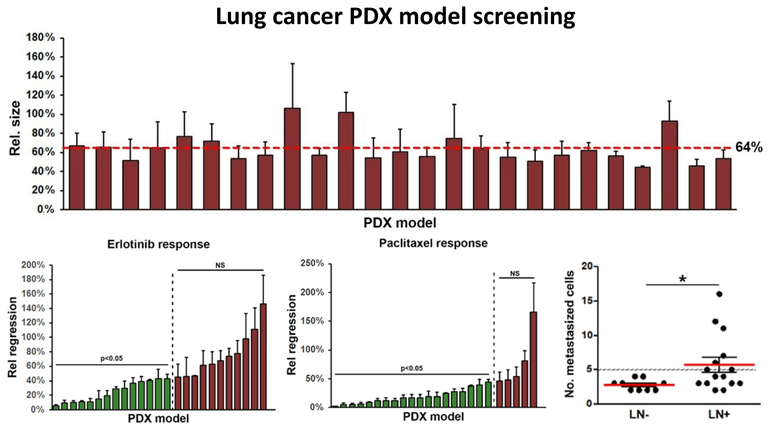
Here we generated zebrafish- and mouse- PDX models based on 20 breast cancer- and 30 lung cancer samples and compared the response efficacy of standard-of-care treatment on primary tumor growth/regression as well as metastatic dissemination in the zebrafish tumor xenograft model.

Lung- and Breast PDX models were chosen from Charles River´s PDX library representing a variety of sub-types, different degrees of differentiation, and different degrees of aggressiveness.

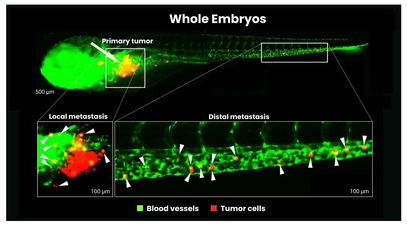


Tumor tissue was enzymatically and mechanically digested and dyed with a red fluorescent dye. Malignant and non-malignant cells from the tumor tissues were then implanted into the perivitelline space in zebrafish embryos (n=20 per group) aged 48 hours post fertilization (hpf). Implanted embryos were placed in separate wells in multi-well plates in PTU water containing vehicle (DMSO), paclitaxel, erlotinib, or docetaxel. Trastuzumab was administered intratumorally, via co-injection together with the tumor cells. The relative change in tumor size and metastasis three days after tumor implantation was evaluated by fluorescent imaging techniques. Drug efficacy was evaluated as the change in tumor size of drug-treated embryos relative to controls.

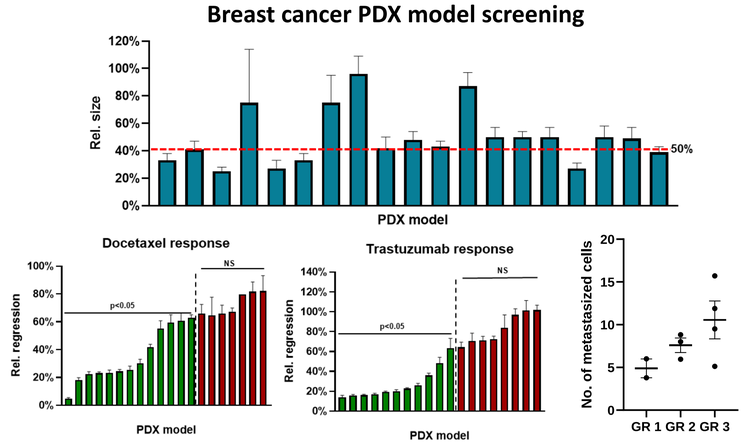
Results



	ZTX-Erl	PDX-Erl	ZTX-Pacli	PDX-Pacli
M1		NA		
M2		NA		
M3		NA		
M4		NA		
M5				
M6		NA		
M7		NA		
M8		NA		
M9		NA		
M10		NA		
M11		NA		
M12		NA		
M13		NA		
M14				
M15		NA		



- The ZTX model could predict patients with lymph node involvement with 100% accuracy and in situ cancer with 67% accuracy
- 25 of the 30 lung-cancer PDX models generated a sufficient amount of cells to be implanted. Of these 25, all models established tumors in the zebrafish larvae, with an average of 64% relative tumor size 3 days after implantation.
- Treatment with Erlotinib and Paclitaxel generated a diverse response among the tested models.
- 87% of the models (13 of 15) exhibited the same treatment response as was generated in mouse-PDX models.



	ZTX-Doce	PDX-Doce	ZTX-Trast	PDX-Trast
M1				
M2				
M3		NA		
M4		NA		
M5		NA		NA
M6				NA
M7		NA		NA
M8				
M9				NA
M10				
M11				NA
M12		NA		
M13		NA		
M14		NA		NA
M15		NA		NA
M16		NA		NA
M17		NA		
M18				NA
M19		NA		

- 19 of the 20 breast cancer PDX models implanted successfully in zebrafish larvae, with an average of 50% of relative tumor size 3 days after implantation.
- Increased dissemination of implanted tumor cells correlated with clinical data on advancing tumor stage from grade 1 to grade 3. The number of models/group was however too low (2-4 models/stage) for statistical calculations.
- Treatment response with Docetaxel and Trastuzumab was identical between the ZTX model and mouse PDX model in 73% of the tested models (14 of 19).
- The ZTX model could identify responders and non-responders to both docetaxel and trastuzumab.

Conclusion

- Here we present a powerful tool for evaluating drug efficacy in a highly translational manner.
- ZTX models are accurately predicting anti-tumor responses to commonly used drugs in lung cancer and breast cancer compared to mouse-PDX models and could find responders and non-responders to tested treatments.
- ZTX models provide a sensitive method for determining early dissemination and metastatic risk, providing a synergistic complement to mouse PDX-models with the ability to not only evaluate efficacy on tumor regression but also on metastasis dissemination.
- Due to these characteristics, the ZTX model is suitable not only for pre-clinical development, shortening time for drug development, but also for clinical development matching the right patient to the right treatment.