This abstract was submitted to the DTRF Research Workshop in September, 2018.

"Identifying targets for therapy in a novel genetic Xenopus model for desmoid tumor formation."

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In our group, we deployed CRISPR/Cas9 technology in *Xenopus tropicalis* - an aquatic vertebrate with external development and a genuine diploid genome highly syntenic to the human - to establish fast (< 3 months) and efficient genetic cancer models. We have recently established models for desmoid tumors (DT), retinoblastoma, choroid plexus cancer and pancreatic neuroendocrine cancer, amongst others.

Evidently, the translational value of these novel preclinical cancer models will rely on how they can be used to advance treatment options for patients. As such, we generated a methodology allowing semi-high throughput screening of new candidate dependency factors (therapeutic targets) via multiplexed CRISPR/Cas9-mediated genome editing. We have already successfully applied this approach, CRISPR/Cas9-mediated Negative-Selection Identification of Drug targets (CRISPR-NSID), to identify EZH2 as a new therapeutic target in DT. The EZH2 protein is a histone methyl transferase and is the functional enzymatic component of the Polycomb Repressive Complex 2 (PRC2). This protein, and epigenetic regulators in general, have recently emerged as possible druggable proteins for treating a range of human cancers. Further using the CRISPR/Cas9 methodology, we could show that the SET domain, which is the catalytic site of EZH2 was required for DT formation in *Xenopus.* Next, we performed experiments to address the clinical relevance of our findings. We found that the EZH2 protein is highly expressed in clinically resected human DT samples and we performed drug treatments with the EZH2 inhibitor GSK-126 in primary human desmoid tumor cultures and in our *Xenopus* DT model. While increased cell death was detected in the animal model, the response in the patient derived cell cultures was less consistent with only one out of four cells showing a clear response. Experiments with an alternative EZH2 inhibitor are currently on-going. We are also further exploring CREB3L1, a central mediator of collagen synthesis, as a possible dependency factor for DT.

Our results show that desmoid tumors are dependent on EZH2 expression, revealing epigenetic pharmacological inhibitors as novel candidates for a drug trial in DT. We believe that the CRISPR-NSID technology in *Xenopus tropicalis* will find broad applicability for in vivo identification of novel dependency factors in different types of cancer, thereby exposing new therapeutic strategies for cancer patients. This should further establish *Xenopus tropicalis* as a valuable pre-clinical cancer model.