RNA-sequencing of medulloblastoma PDOX models enables identification of both tumor and microenvironment specific biomarkers

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Facts & Figures

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Contributions	
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Project website:	www.itccp4.eu
Social media:	Linkedin - itccp4-p4-442191154

Challenge

Therapies for medulloblastoma (MB), a highly malignant pediatric brain tumor, often impose debilitating effects on the developing child, highlighting the need for molecularly targeted treatments with reduced toxicity. Therapies aimed at tumor-intrinsic mechanisms have translated to limited success thus far. More effective therapeutic strategies may also need to target elements of the tumor microenvironment. However, current understanding about the medulloblastoma microenvironment/ tumor stroma is limited, particularly its cellular composition.
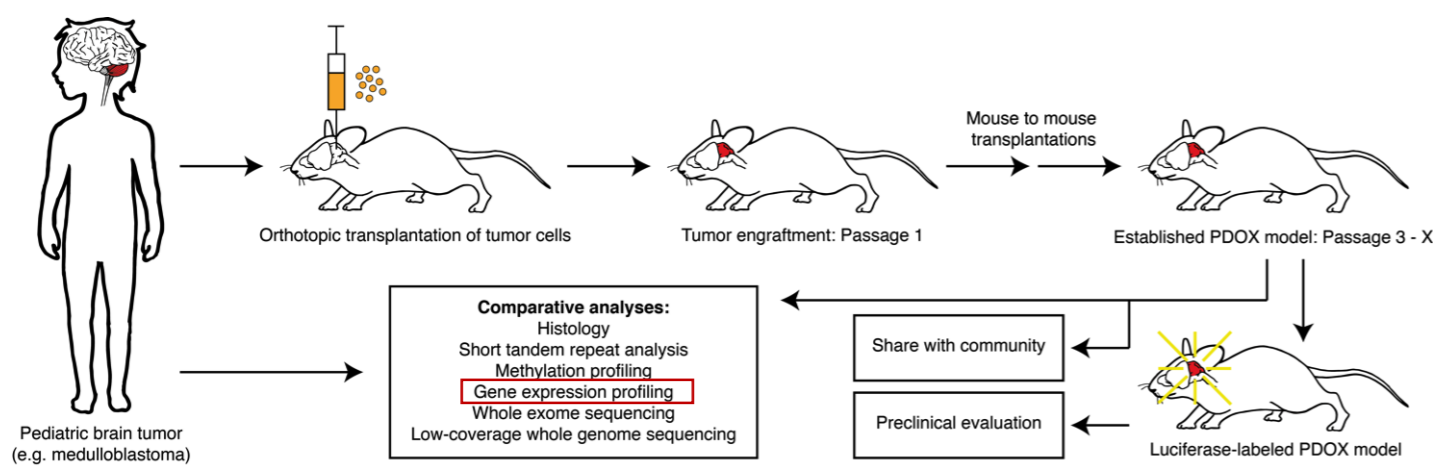
Approach & Methodology

Figure 1: Schematic illustration of the establishment and characterization pipeline of PDOX models of pediatric brain tumors (e.g. medulloblastoma). Tumors were injected in the brain region in the mouse corresponding to the location of the tumor in the patient.

As part of an IMI2-funded preclinical proof-of-concept platform for paediatric cancer (ITCC-P4), we performed whole-transcriptome sequencing and bioinformatics analyses of 16 patient-derived orthotopic medulloblastoma xenografts, in order to identify genes and biomarkers that compose the medulloblastoma microenvironment. Patient-derived orthotopic xenografts (PDOX) are an excellent model for biomarker and preclinical drug development. In PDOXs, mouse stroma supports human cancer cells, replacing the human stroma early after engraftment. Therefore, the transcriptome of PDOXs is composed of a mixture of human RNAs (deriving from tumor cells) and mouse RNAs (deriving from stroma) and a species-specific computational alignment approach allows for the simultaneous study of both tumor and microenvironment specific signals.
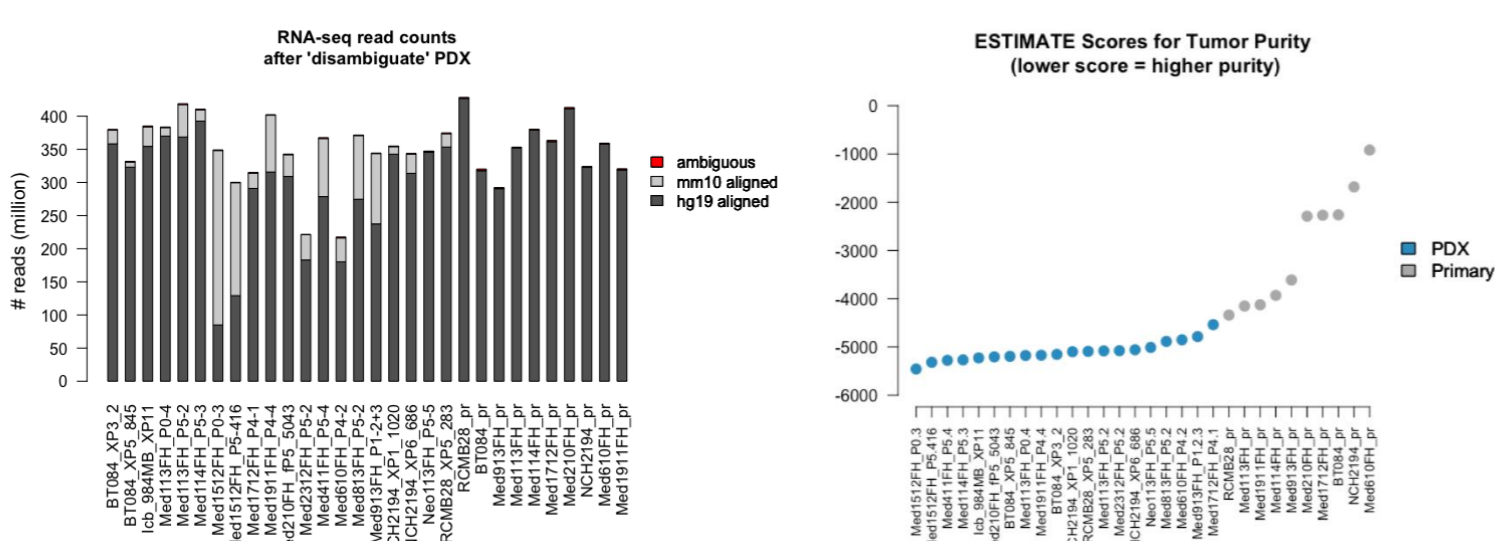
Results

Figure 2: The mouse fraction varied between 2% - 70% per PDOX model. The ambiguous fraction of reads was very low (<1%, left panel). Tumor purity estimates show the expected higher tumor purity for PDOX compared to primary tumors (right panel).
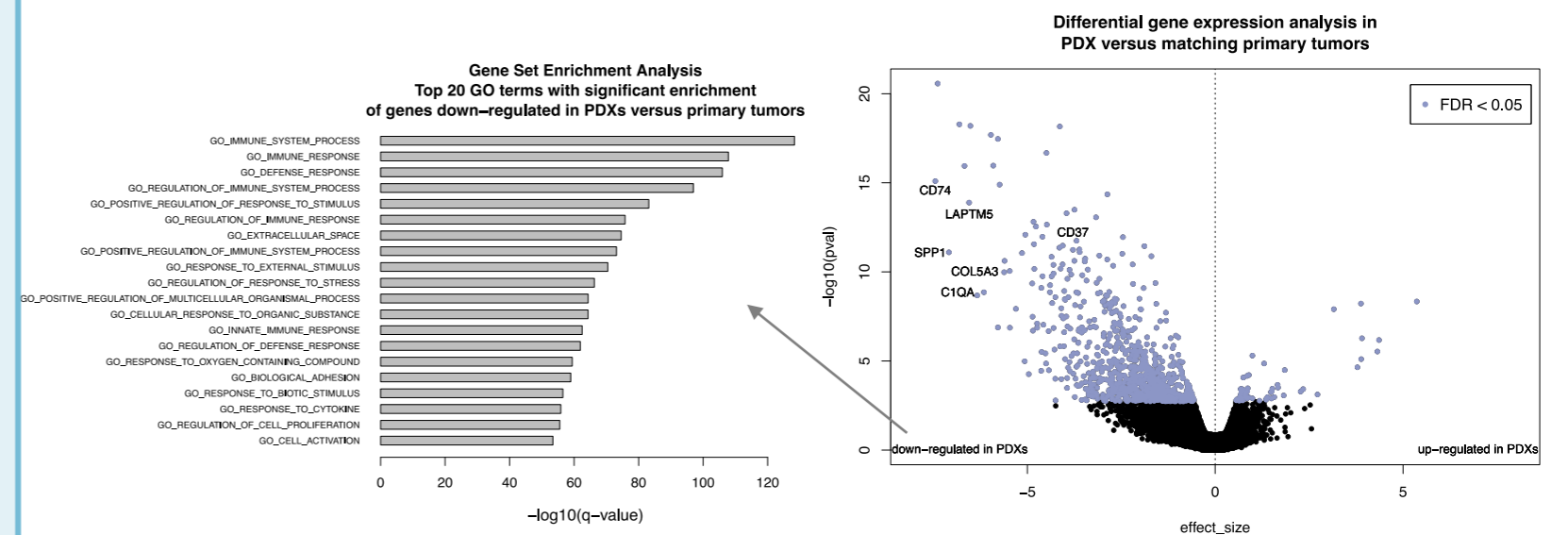

Figure 3: Differential gene expression analysis between the PDOX and primary tumors shows that a large fraction of genes (~800 genes) is significantly down-regulated, as shown in the volcano plot on the right panel. Further, these down-regulated genes are highly enriched for immune response-related genes and extracellular matrix components, supporting the hypothesis that the human tumor stroma is lost in the PDOXs. *LAPTM5*, for example, is highly expressed in the primary tumors, while expression is almost undetectable in the PDOX human fraction, and this gene is a marker for microglia (see Fig. 5, left panel).
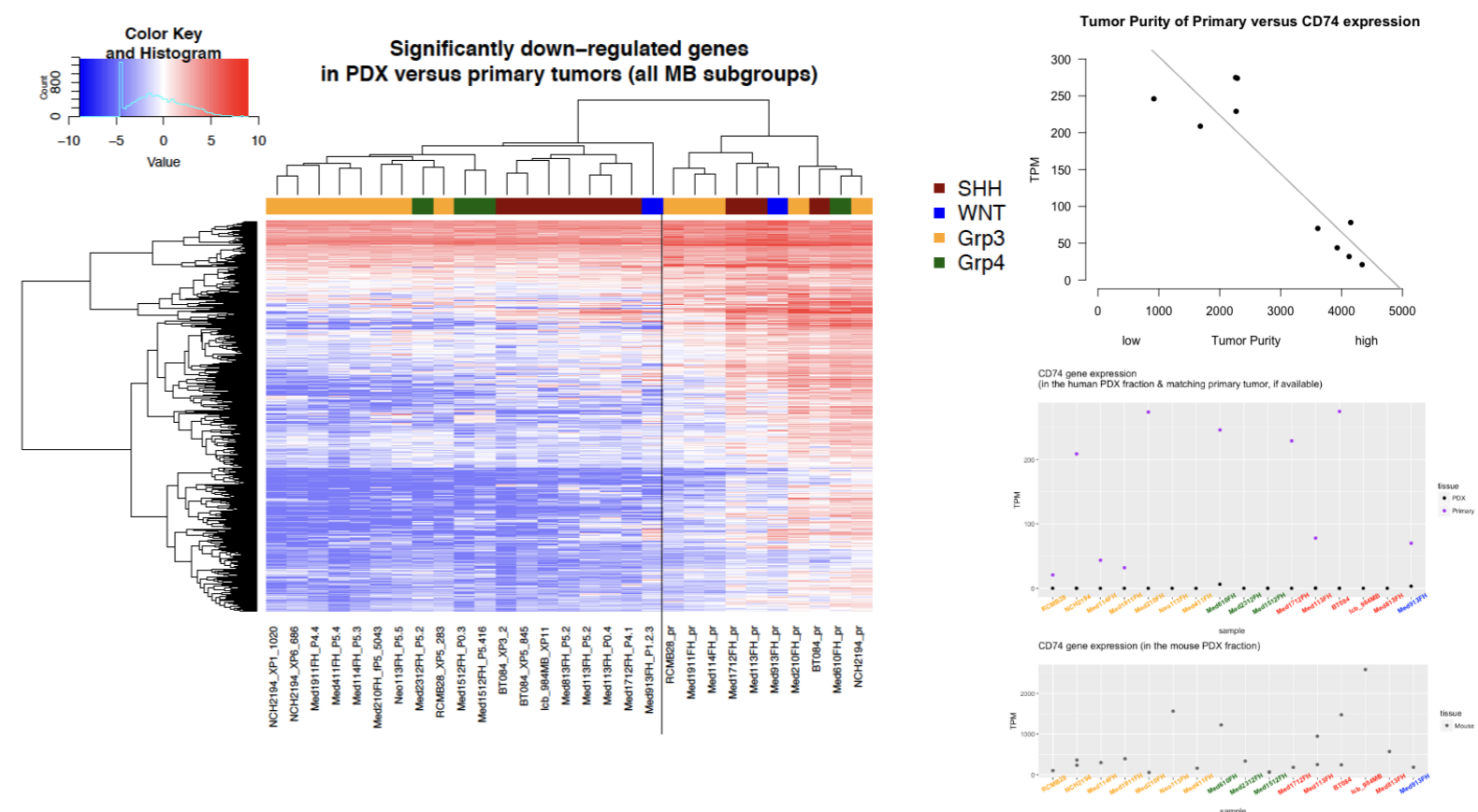

Figure 4: Stromal gene expression patterns: The heatmap on the left shows that the majority of genes significantly down-regulated have very low expression (TPM < 1, blue) left in the PDOX. *CD74* is an example of a gene that is of stromal origin across all medulloblastoma subgroups (right panel). Therefore, in the primary tumors, the expression of this stromal gene correlates well with tumor purity (plot in upper right corner). No systematic differences between early and late PDOX passages were observed, i.e. human stromal cells are lost by passage one, in line with a recent report in PDX of colorectal carcinoma (Chao et al., 2017).
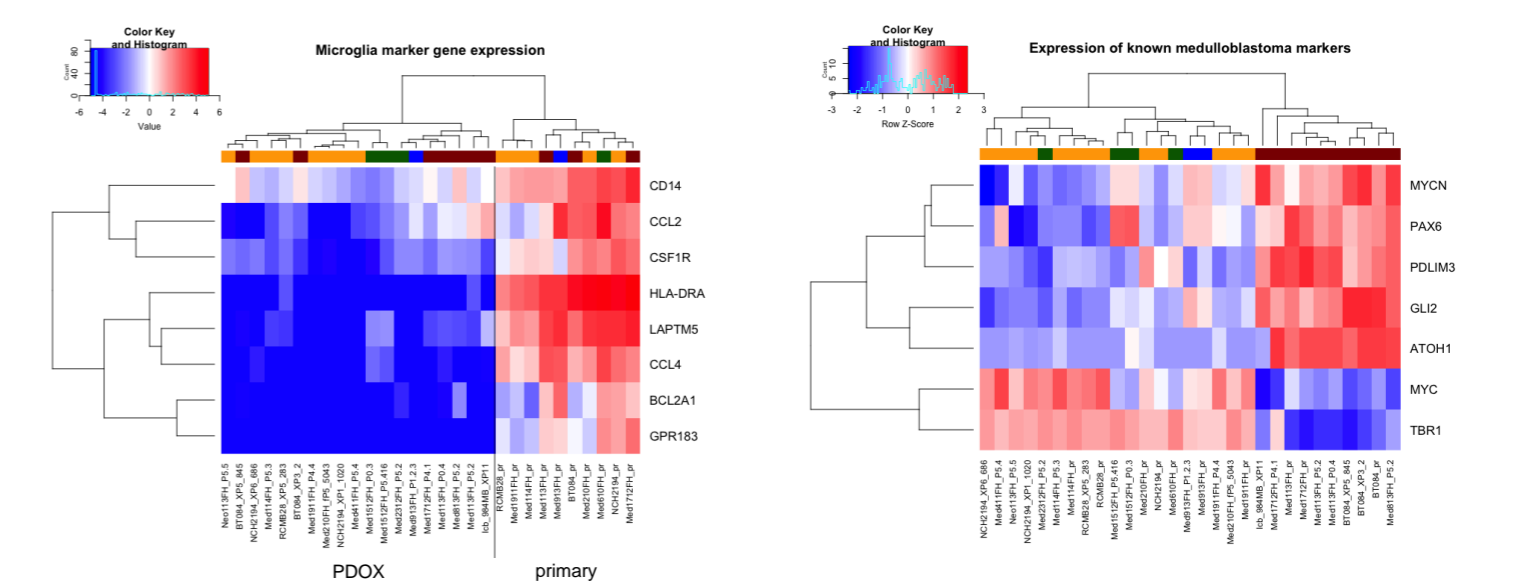

Figure 5: Identification of tumor and microenvironment specific gene markers. The heatmap on the left demonstrates that the expression of marker genes for microglia (as one example for cell types from the MB tumor microenvironment) is largely lost across all PDOX models compared to their primary counterparts. In contrast, genes known to be expressed in MB tumor cells or their respective cell of origin show no such down-regulation in expression (heatmap in right panel).

Value of IMI collaboration

- Systematic collection of rare preclinical pediatric tumor model systems for academia and pharmaceutical industry
- Funding for in-depth molecular classification (including RNA-seq) of model systems and matching patient material
- Access to novel drugs through the pharmaceutical industry
- Large-scale preclinical drug testing by CROs

Impact & take home message

RNA-sequencing analysis of patient-derived orthotopic xenografts provides a great opportunity to dissect the (medulloblastoma) tumor microenvironment for possible therapeutic targets. The results presented here, show that a sizeable number of genes known to be expressed in medulloblastoma tumor samples are not of tumor cell origin, but of stromal origin instead. Further, the majority of such stromal genes are replaced by mouse stroma, which may suggest that they play a role in tumor maintenance and could therefore possibly be exploited as therapeutic targets.