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An in vivo platform of pre-characterized renal cell carcinoma (RCC) patient-derived xenograft models allows the preclinical evaluation of patient-tailored intervention strategies

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Background and Aim

Renal cell carcinoma (RCC), is the most common kidney cancer of adults, originating in the lining of the proximal convoluted tubule. Prognosis is poor in patients with advanced or metastasized RCC. Drug resistance towards Standard of Care (SoC, incl. everolimus, sorafenib, or sunitinib) drugs develops frequently within months. Therefore, development of novel options to target acquired TKI resistance mechanisms in advanced and metastatic RCC is still an urgent medical need. Preclinical models with high translational relevance can promote the implementation of novel personalized therapies. To evaluate novel targeted therapies and their combinations in preclinical settings, patient-derived xenograft (PDX) models represent valuable tools.

Methods

Responsible local ethics committees approved usage of patient tissue and all animal procedures. In this study, RCC tissue from 167 patients was collected and xenotransplanted in mice. Partially, a multi-region approach, xenografting tissue from different regions of one tumor, was used. PDX models were characterized by immunohistochemistry (Ki-67, CD31, Pax2 and Pax8 antibodies), gene expression, copy number variations and mutational analyses. To evaluate in vivo drug response of RCC PDX models, mice transplanted with PDX tumors were treated with bevacizumab (i.p.), with everolimus, sorafenib, or sunitinib (p.o.). Adopted clinical response criteria for solid tumors (RECIST) were applied to classify the anti-tumor activity of the tested compounds in RCC PDX models. Next generation sequencing (NGS, panel) and transcriptome data were used to compare primary tumors and metastases.

Results

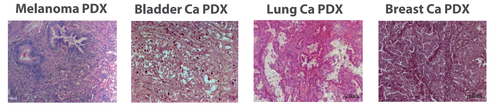
A comprehensive panel of subcutaneous RCC PDX models with well-conserved molecular and pathological features over multiple passages was established. The overall take for the RCC PDX in this study was 21%. Tumor growth characteristics were heterogeneous throughout the different models but were stable during in vivo passaging. Drug screening towards four SoC drugs, targeting the VEGF and PI3K/mTOR pathway, revealed individual and heterogeneous response profiles in the PDX, resembling the clinical situation. Intra-tumor heterogeneity can be assessed via PDX models from multi-tumor regions from one patient in our platform. Development of corresponding in vitro cell culture models from the PDX enables advanced high throughput drug screening in a personalized context. Analyzing novel targeted molecules is possible due to the pre-established molecular characterization of the PDX at the genomic and expression level. In conclusion, we established a new and molecularly characterized panel of RCC PDX models with high relevance for translational preclinical research.

Available PDX models at EPO

Evaluation of treatments with:

- Cell-based therapies
- Antibody-based therapies
- Oncolytic microorganisms
- Immune modulators

Melanoma PDX | **Bladder Ca PDX** | **Lung Ca PDX** | **Breast Ca PDX**



- Tumor growth data
- Treatment response data
- Expression data (RNAseq)

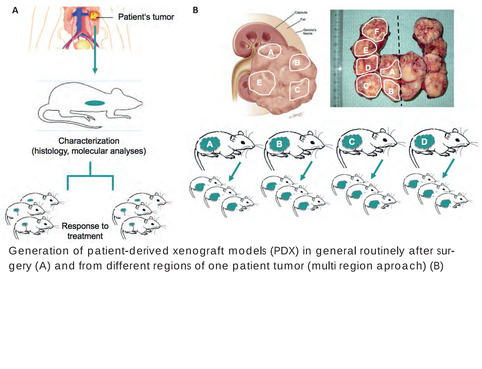
PDX	n
Breast	39
Gastrointestinal	
Cholangio	2
Colon	183
Gastric	17
Oesophagus	4
Pancreatic	52
Glioma*	29

PDX	n
Gynaecologic	
Endometrial	9
Cervical	4
Ovarian	29
Haematological	
ALL*	10
AML	13
Lymphoma (B & T cell)	25

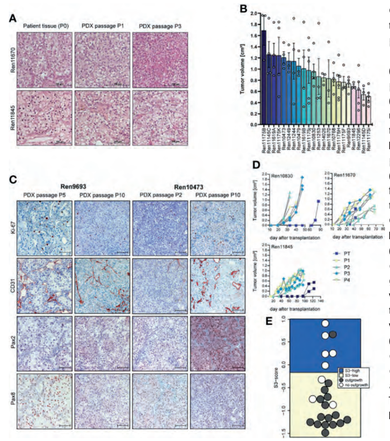
PDX	n
Head & Neck	85
Lung	58
NSCLC	47
SCLC	2
Melanoma	21
Mesothelioma	11
Neuroblastoma*	22
Neurendocrine	5

PDX	n
Sarcoma	31
Sarcoma paediatric*	42
Urological	
Bladder	2
Prostate	1
Renal	41

* PDX available through the IMI ITCC P4 platform

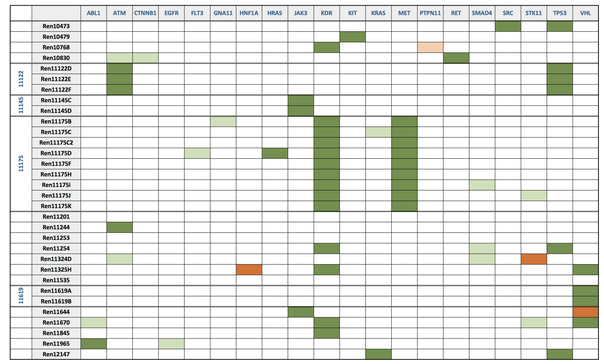


Generation of patient-derived xenograft models (PDX) in general routinely after surgery (A) and from different regions of one patient tumor (multi region aproach) (B)

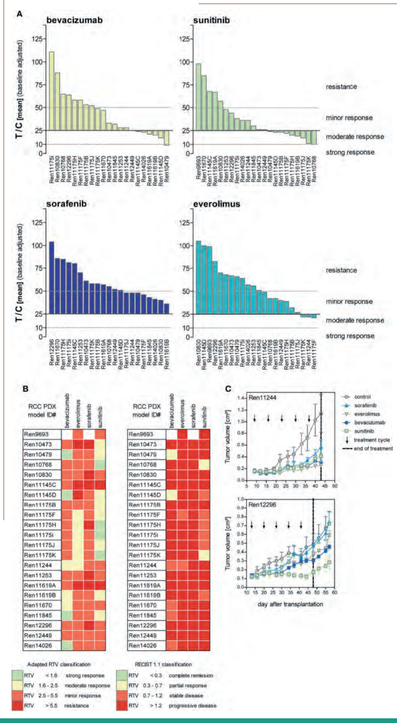


Characterization of representative PDX models from the RCC panel.

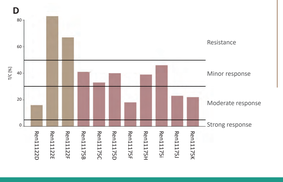
(A) Histological examination of patient and PDX tissue. (B) Tumor growth characteristic of untreated control mice reflecting the heterogeneous biology of RCC regardless of the molecular phenotype. (C) Representative IHC analyses from RCC in vivo passaged PDX tumors for Ki-67 (proliferation), CD31, PECAM1 (blood vessels), Pax2, Pax8 (renal markers). (D) Exemplary RCC PDX growth curves showing individual TV growth characteristic during in vivo passaging (PT = primary tumor passage, P1–P4 consecutive PDX tumor passages). (E) The gene expression-based ccRCC risk model (S3 score) was calculated for primary tumors and metastases from tissue of the Tübingen cohort collected for PDX generation.

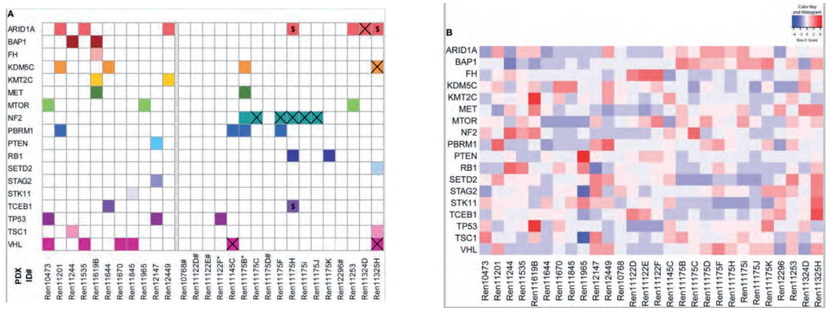


Genomic sequence variations of the RCC PDX models as detected by NGS using the Illumina TrueSeq Amplicon cancer panel (TSACP) performed on an Illumina MiSeq device. This panel covers 220 regions in 48 cancer-related genes. Single nucleotide variations (SNVs) in green, insertions/deletions in orange, low frequency data (5-20%) in light colors



Response characterization of renal cancer PDX models upon treatment with targeted therapies blocking angiogenic and proliferative pathways. (A) Mean tumor volume of treated mice compared to the mean tumor volume of control (T/C mean) from 22 PDX sensitivity studies (n = 3–5 individual animals per group) were illustrated in waterfall plots. Bevacizumab and sorafenib were not tested in Ren9693. Response was categorized as T/C: >50% = resistance/progressive disease (PD), <50% >30% = minor response/stable disease (SD), <30% >10% = moderate response/partial response (PR), and <10% = strong response/complete remission (CR) with zero line equal to T/C = 25%. In the 22 analyzed PDX models, treatment response was best for sunitinib, followed by bevacizumab and everolimus. Lowest response rate was observed for sorafenib. (B) Individual PDX models exhibit distinct response pattern against selected compounds. Adapted response criteria utilizing RTV (left) opposed to clinical RECIST response classification (right). Adapted RTV response classification mirrors T/C drug response well, whereas clinical RECIST classification yields dramatic lower responses. (C) Different response patterns were observed for individual RCC PDX models during drug testing, illustrating RCC heterogeneity and differences of intra-tumoral regions. (D) Differences in treatment response of PDX derived from different regions of two asvanced patient tumors. Examplary treated-to-control (T/C) data for everolimus is shown (> 50% T/C = resistance, 50-30% T/C = minor response, 30-5% T/C = moderate response, < 5% T/C = strong response).





Somatic mutation analysis in RCC PDX models and expression of mutated genes. (A) Using RNA sequencing data, 31 genes that are frequently mutated in RCC were analyzed for somatic mutations. The matrix includes those variants in 18 genes either not included in gnomAD 3.1 or had allele frequencies below 0.0001. The panels separate the models by the tissue source site of their primary tumors and metastases (left: Magdeburg, right: Tübingen). For the Tübingen cases, mutation data from NGS panel sequencing of the primary tumors were also available (except for those marked with *). Overlapping mutations detected in both the primary tumor and the PDX model are highlighted by a cross. $: Variants with low coverage in the primary tumors. #: No somatic mutation found. (B) Gene expression of the 18 mutated candidate genes described in (A).

Conclusions

The use of personalized PDX models can guide oncologists in selecting the best possible treatment option for the corresponding donor patient. Due to time constraints, personalized PDX generation is not always possible for all patients. Large panels of PDX models reflecting heterogeneity and molecular and pathologic features of the disease are of great value for identification and validation of predictive biomarkers and of novel treatment options.

A molecularly characterized RCC PDX panel as a drug testing platform was established:

- 46 PDX models established primarily from untreated patients
- take rate for RCC PDX was 21%, no PDX from tumor with neoadjuvant treatment
- heterogenous doubling times above 15 days
- mutational landscape reflects clinical situation
- molecular profiling of RCC PDX using RNAseq confirms clinically relevant heterogeneity
- treatment data show the unique predictive power of this platform for preclinical drug testing