Abstract Presentation
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Patient-derived xenograft and PDX-derived cell line models from glioblastoma for drug development and identification of molecular signatures

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Introduction

Glioblastoma is the most common malignant brain tumor in adults, with about 90% of tumors developing *de novo*. Their heterogeneity, aggressiveness and infiltrative growth limit success of current standard of care (SoC) Temozolomide (TMZ), and efficacy of various new therapeutic approaches. There is a need for *in vitro* and *in vivo* models reflecting the complex biology of these tumors to analyze the molecular mechanisms of tumor formation and resistance, as well as to identify new therapeutic targets.

Methods

In vivo PDX models: We established of 39 PDX glioma models on immunodeficient mice, 15 out of these by orthotopic (i.cer.) transplantation. 23 s.c. and 4 orthotopic PDX models were screened for sensitivity towards SoC TMZ and a set of drugs with different modes of action and have been analyzed for mutations and gene expression profiles using RNA sequencing.

PDX-derived cell lines: s.c. PDX tumor tissue was harvested and a single cell suspension prepared by mechanic and enzymatic breakup using the Miltenyi GentleMACS® system. PDX-derived cells were cultured following the PromoCell Primary Cancer Culture System® (C-28081) protocol for 4-8 weeks. To analyze chemosensitivity, established PDX-derived cell lines were incubated with selected compounds in the Incucyte® Live-Cell Analysis system for up to 72 h with confluency measurements every 2 h.

Results

Established s.c. PDX models show individual mutation and gene expression profiles, resembling proposed molecular GBM subtypes mesenchymal, proneural and classical, and therefore recapitulate the heterogeneity seen in patient tumors.
S.c. PDX show individual growth and chemosensitivity profiles. Matching orthotopic PDX models have a reduced sensitivity towards most tested drugs, possibly due to the blood-brain barrier and altered microenvironment

PDX-derived GBM cell cultures are free of murine cells, show individual morphology and are stable over > 15 passages. FACS analyses show a high expression of stem cell markers CD15 and CD133 compared to the glioma cell line U87MG. First PDX-derived cell lines show similar response patterns towards selected drugs in vitro as their matching s.c. PDX.
PDX-derived cell lines engraft and grow after s.c. inoculation with a reduced expression of individual stem cell markers.

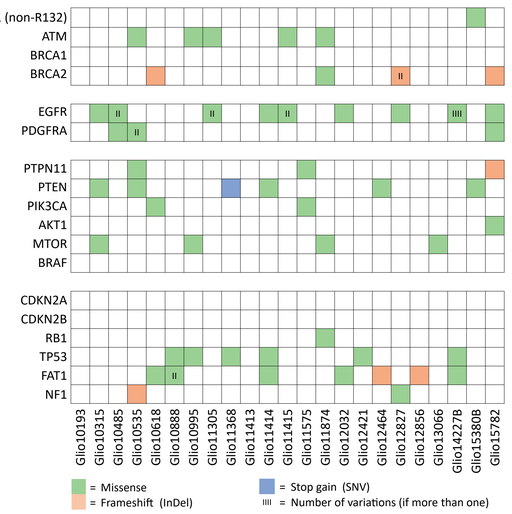
Conclusions

-> Our GBM PDX panel offers a valuable tool for efficacy screenings in s.c PDX and validation in matching orthotopic PDX
-> available RNA seq data allows for selection of models expressing desired targets and analyses of correlation between monitored phenotypes and gene expression signatures
-> Panel of PDX derived cell lines further streamline screenings and help reduce animal numbers

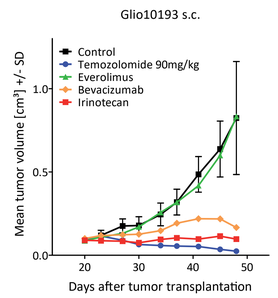
(A) Patient data of established PDX

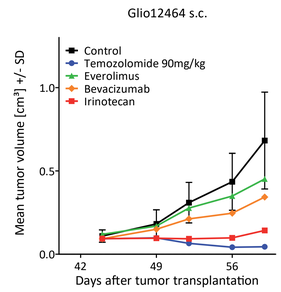
PDX	Patient		Tumor Characteristics	
	sex	age	Diagnosis	past treatments
Glio10193	male	n.a.	GBM Grade IV	no
Glio10315	male	n.a.	GBM Grade IV	no
Glio10485	female	n.a.	GBM	n.a.
Glio10535	male	n.a.	GBM Grade IV	no
Glio10618	female	66	GBM Grade IV	no
Glio10888	male	n.a.	GBM Grade IV	no
Glio10995	female	n.a.	GBM	n.a.
Glio11305	male	71	GBM Grade IV	no
Glio11368	male	49	GBM Grade IV	no
Glio11413	female	n.a.	GBM	n.a.
Glio11414	female	60	GBM Grade IV	recurrence, yes
Glio11415	male	55	GBM Grade IV	recurrence, n.a.
Glio11575	male	66	GBM Grade IV	recurrence, n.a.
Glio11874	male	63	GBM Grade IV	no
Glio12032	male	69	GBM Grade IV	no
Glio12421	male	61	GBM Grade IV	no
Glio12464	female	62	GBM Grade IV	no
Glio12827	male	60	GBM Grade IV	recurrence, n.a.
Glio12856	female	77	GBM Grade IV	no
Glio13066	male	68	GBM Grade IV	no
Glio14227B	male	61	GBM Grade IV	no
Glio15194	female	67	GBM Grade IV	no
Glio15379	male	5	Ependymom	no
Glio15380B	female	49	GBM Grade IV	no
Glio15782	female	79	GBM	no
Glio15807	male	42	GBM Grade IV	no

(B) Selected mutations in PXD

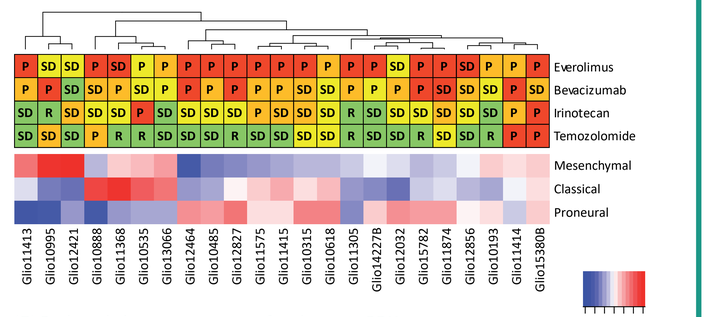


(C) Sensitivity tests s.c.

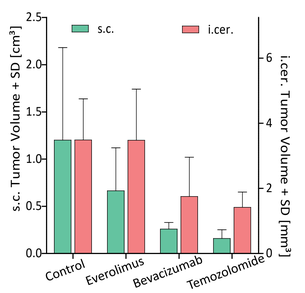




(D) Sensitivity of s.c. PDX and molecular subtypes



(E) Reduced chemosensitivity of orthotopic PDX



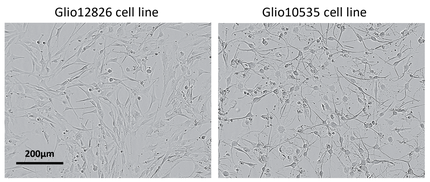
PDX		Glio10535	Glio10618	Glio12032	Glio13066
Everolimus	s.c.				
	i.cer.				
Bevacizumab	s.c.				
	i.cer.				
Irinotecan	s.c.				
	i.cer.				
Temozolomide	s.c.				
	i.cer.				

Relative Tumor Volume (RTV)
P: progression, RTV > 1.7
SD: stable disease, RTV 0.34 – 1.7
R: response, RTV < 0.34

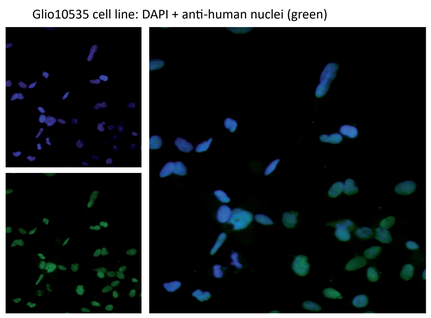
T/C optimal
> 50 %, no response
50 - 25 %, minor response
25-10 % , moderate response
< 10 %, strong response

(A) Available clinical data from patients that provided tumor tissue for PDX generation. (B) Mutation analysis of 23 s.c. PDX models revealed individual profiles with mutations common in GBM: EGFR, in the PI3k/Akt/mTOR signaling pathway, TP53 and FAT1. All models were IDH1-wt regarding codon R132. (C) Examples of drug testings of two s.c. glioma PDX models with model-specific growth characteristics and treatment responses, n=3-5. (D) Clustering of PDX models regarding their expression of gene sets characteristic for proposed molecular subtypes mesenchymal, proneural and classical (Wang et al., 2017) and corresponding sensitivity profiles. (E) Chemosensitivity testing of selected orthotopic PDX revealed reduced sensitivity towards most drugs than their matching s.c. PDX models. n=3-5.
(Reference: Alcaniz et al., 2023)

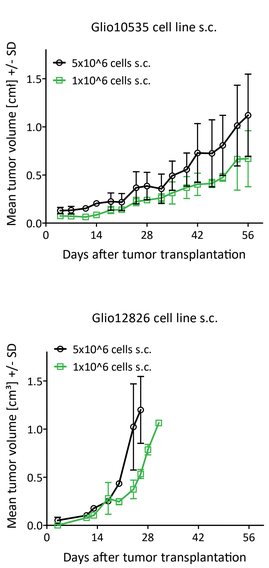
(A) Morphology of PDX-derived cells



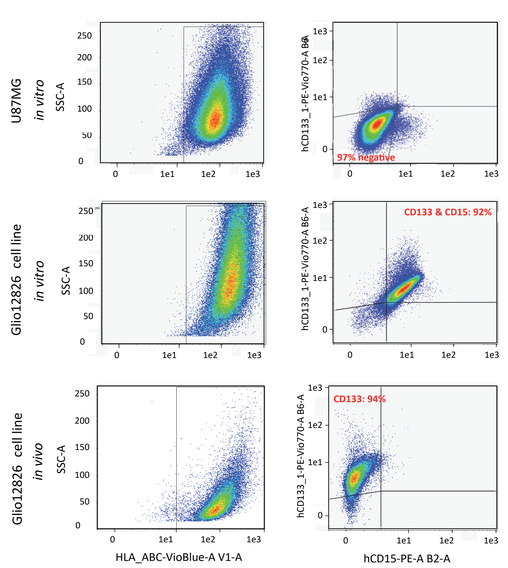
(B) Anti-human nuclei staining



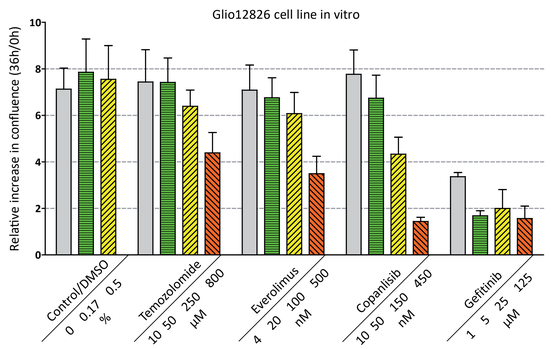
(C) *In vivo* growth of PDX-derived cells



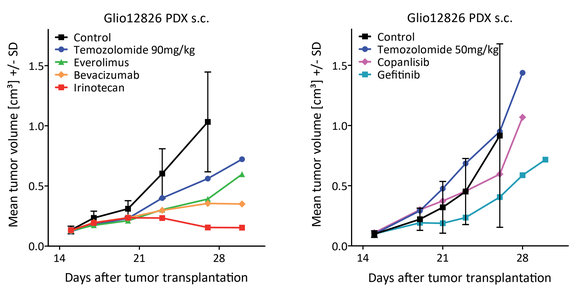
(D) HLA, CD15 and CD133 expression



(E) Growth and chemosensitivity of PDX-derived cells



(F) *In vivo* chemosensitivity of matching s.c. PDX



(A) Morphology of PDX-derived cells growing *in vitro*. (B) Anti-human nuclei staining (green) and DAPI counterstaining (blue) of Glio10535 cell line. (C) Growth of Glio10535 cell line and Glio12826 cell line after inoculation of two different cell number s.c. n=2 (D) FACS staining of Glio12826 cell line growing *in vitro* and *in vivo* s.c. confirm human origin of cells. Comparisons with U7MG show an increased expression of selected stem cell markes *in vitro*. (E) Change of confluence of PDX derived Glio12826 cell line after incubation with SoC temozolomide, EGFR inhibitor gefitinib, PI3K inhibitor copanlisib and mTOR inhibitor everolimus after 36h. n=3. (F) Sensitivity of Glio12826 s.c. PDX towards different drugs, including standard of care temozolomide and different targeted therapies. n=3-5 .

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