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Integrating PDX GBM In Vivo Models with Patient History and Whole Exome Sequencing: Advancing Relevance and Precision in Preclinical Studies

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ABSTRACT

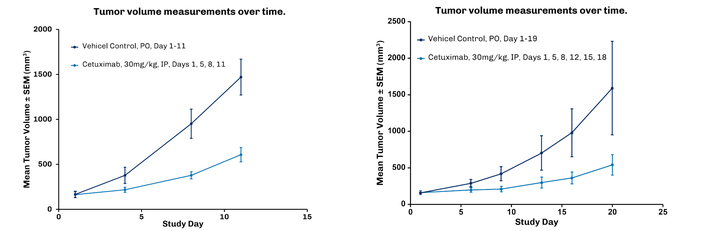
Glioblastoma poses a significant challenge in oncology, necessitating improved translational models that faithfully replicate the intricate tumor microenvironment. Patient-derived xenograft (PDX) models are key in glioblastoma multiforme (GBM) preclinical research. PDX models (subcutaneous and orthotopic) provide a more clinically relevant representation compared to traditional CDX xenografts. Analyzing PDX tumors in their native environment allows for a nuanced understanding of GBM biology, tumor-stroma interactions, and treatment responses. This abstract explores the rationale and advantages of utilizing PDX GBM in vivo models coupled with patient diagnosis and treatment history, empowered by tumor whole exome sequencing (WES), to enhance the relevance of preclinical studies in evaluating GBM-specific treatments. TD2 has access to the over 100 GBM PDX models with fully annotated patient details and treatment history. Moreover, 68 of these PDXs also have WES data, that allows most appropriate model selection to ensure alignment with the therapeutic development strategy. Below is a detailed analysis of two GBM PDX models, GBM6 and GBM46, using patient history and WES data to define criteria for success in assessing novel preclinical therapeutic agents. GBM6, was obtained prior to treatment from a 65-year-old man with glioblastoma multiforme, that contains 642 mutations, including EGFR VIII amplification. GBM46, was obtained from a 55-year-old man with prior treatment, contained 646 mutations, including RAS pathway alterations and EGFR (MET and FLT3) amplification. When grown subcutaneously the anti-tumor activity of cetuximab against GBM6 and GBM46 resulted in 41% and 48% growth inhibition, respectively. Traditional treatments have also been evaluated in the orthotopic setting, with temozolomide (TMZ) showing a nominal improvement in lifespan (11%) as expected for the unmethylated MGMT found in the GBM6 model. By contrast, GBM46 from a patient with prior treatment with TMZ, OSI-774, radiation, and BCNU, exhibited a 58% increase in overall lifespan when treated with TMZ alone. Orthotopic PDX GBM models preserve many molecular characteristics, enabling the study of intrinsic GBM mutations for developing novel therapeutic strategies. While orthotopic models better capture GBM's dynamic nature, traditional xenograft systems remain valuable as rapid screening tools. Models with defined clinical treatment history and WES data allows for testing new agents in models that more closely resemble the clinical development path for a given new agent and aids in deciphering factors influencing therapeutic resistance. These models have the potential to reshape preclinical research paradigms, accelerating the translation of promising therapeutic agents increasing the likelihood of clinical benefit for patients with glioblastoma multiforme.

MATERIAL AND METHODS

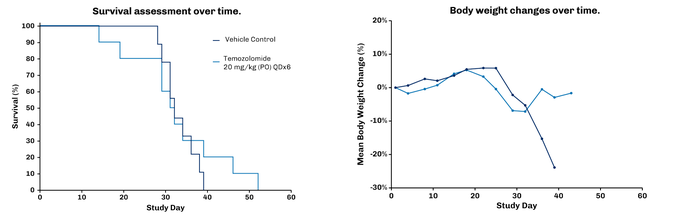
- PDX GBM6 and GBM46 were received from Mayo Clinic (Rochester, MN) as cryopreserved coarse tumor suspensions.
- Subcutaneous tumors were serially passed once prior to being processed into a cell suspension (50% RPMI media/ 50% Matrigel™) and implanted into study mice. Female athymic nude mice (Hsd:Athymic Nude-Foxn1^{nu}) at 6 week6s old were obtained from Envigo (Indianapolis, IN) were implanted subcutaneously with the cell suspension into the right flank at 0.1ml/mouse.
- Short-term explants were established to create single tumor cell suspension for intracranial implantations. Once the subcutaneous tumors reached >1000mm^3, the explants were placed in Matrigel coated tissue culture flasks containing DMEM (Lonza; Walkersville, MD) supplemented with 2.5% fetal bovine serum (FBS; Omega Scientific; Tarzana, CA) to promote the growth of tumor cells over fibroblast. Under inhaled isoflurane anesthesia female athymic nude mice (Hsd:Athymic Nude-Foxn1^{nu}) at 6 week6s old were obtained from Envigo (Indianapolis, IN) were inoculated intracranially with a 3 µl suspension of GBM6 or GBM46 tumor cells (approximately 3 x 10^5 cells/mouse) in sterile saline. Implant coordinates were 1mm anterior and 2mm lateral to the bregma, at a depth of 3mm. A 0.05 mg/kg injection of buprenorphine (Reckitt Benckiser Healthcare; UK) was provided approximately 30 minutes prior to cell injection.
- Temozolomide (Selleckchem, Houston, TX) and cetuximab (ImClone Systems Incorporated, Branchburg, NJ) were prepared for treatment as described below:
  - Cetuximab (Erbitux) – FDA approved EGFR antagonist that works by blocking the growth of cancer cells. Dissolved in 0.9% saline (Braun Medical; Bethlehem, PA), administered at 3mg/ml (10ml/kg), intraperitoneally.
  - Temozolomide (TMZ) – FDA approved alkylating agent that damages DNA and can kill cancer cells. Dissolved in 1% DMSO (Sigma Aldrich; St. Louis, MO), 0.5% Tween 80 (Sigma Aldrich; St. Louis, MO), and 98.5% Saline (Braun Medical; Bethlehem, PA), administered at 2mg/mL or 5mg/ml (10 mL/kg), orally.
- Animal care and use was conducted in alignment with animal welfare regulatory requirements in an AAALAC-accredited facility.

Model Selection based on WES & RNA Seq

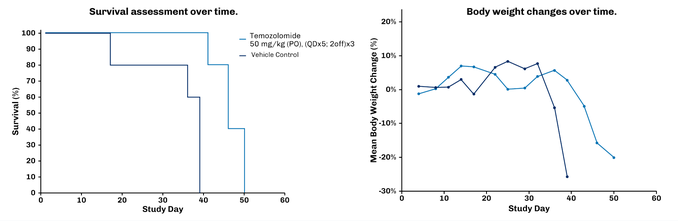
Subcutaneous GBM6 and GBM46



Orthotopic GBM6



Orthotopic GBM46



Published Clinical Trial Data for Cetuximab

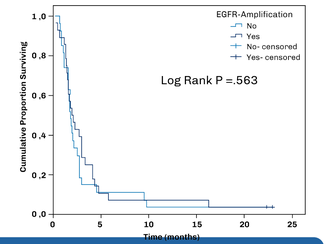
Neyns, B., Sadones, J., Joosens, E., Bouttens, F., Verbeke, L., Baurain, J.-F., D'Hondt, L., Strauven, T., Chaskis, C., In't Veld, P., Michotte, A., De Greve, J. (2009). "Stratified phase II trial of cetuximab in patients with recurrent high-grade glioma." Annals of Oncology, 20, 1596–1603. https://doi.org/10.1093/annonc/mdp032

Efficacy of cetuximab in patients with and without EGFR gene amplification

	EGFR amplified (n = 28), no. of patients (%)	EGFR nonamplified (n = 27), no. of patients (%)	All patients, no. (%) (n = 55)
PR	2 (7.1)	1 (3.7)	3 (5.5)
SD	10 (35.7)	7 (25.9)	17 (30.9)
DCR	12 (42.9)[a]	8 (29.6)[a]	20 (36.4)
PD	16 (57.1)	19 (70.4)	35 (63.6)

[a]Fisher's exact test (two sided) $P = 0.4$.
EGFR, epidermal growth factor receptor; PR, partial response; SD, stable disease; DCR, disease control rate (=CR + PR + SD); PD, progressive disease; CR, complete response.

Kaplan–Meier curves of progression-free survival according to epidermal growth factor receptor amplification status.



Clinical Trial Data for Temozolomide

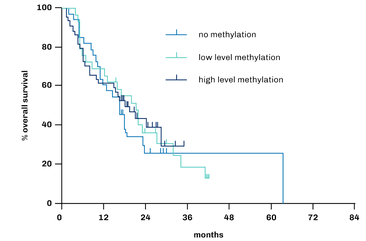
Vaubel, R. A., Tian, S., Remonde, D. A., Schroeder, M. A., Mladek, A. C., Kitange, G. J., Caron, A., Kollmeyer, T. M., Grove, R., Peng, S., Carlson, B. L., Ma, D. J., Sarkar, G., Evers, L., Decker, P. A., Yan, H., Dhruv, H., Berens, M. E., Wang, Q., Marin, B. M., Klee, E., Califano, A., Lachance, D., Eckel-Passow, J. E., Verhaak, R., Sulman, E. P., Burns, T. C., Meyer, F. B., O'Neill, B. P., Tran, N. L., Giannini, C., Jenkins, R. B., Parney, I. F., & Sarkaria, J. N. (2020). Genomic and phenotypic characterization of a broad panel of patient-derived xenografts reflects the diversity of glioblastoma. Clinical Cancer Research, 26(5), 1094–1104. https://doi.org/10.1158/1078-0432.CCR-19-0909.

Univariate analysis of clinical and molecular factors in GBMs.

variable	# (%)	median survival (months)	P
age ≤ 45 years	58 (10.9)	19.4	< 0.0001
age > 45 years	474 (89.1)	7.6	
male	311 (58.5)	9.1	0.78
female	221 (41.5)	8.1	
biopsy only	153 (30.9)	3.8	< 0.0001
resection	342 (69.1)	12.0	
no adjuvant therapy	81 (17.4)	2.0	< 0.0001*
adjuvant therapy without TMZ	72 (15.5)	6.7	
adjuvant therapy with TMZ	312 (67.1)	12.7	
10q intact	70 (18.0)	8.6	0.81
10q LOH	318 (82.0)	9.1	
EGFR not amplified	301 (56.6)	7.9	0.33
EGFR amplified	231 (43.4)	9.8	
negative EGFR expression	10 (2.0)	8.2	0.59
weak EGFR expression	63 (12.6)	8.0	
moderate EGFR expression	147 (29.4)	7.6	
strong EGFR expression	280 (56.0)	9.4	

Overall survival in 532 GBMs was stratified according to key clinical and molecular factors. Of note, not all variables were retrievable in all 532 cases. *P < 0.0001 for all three intergroup comparisons. GBM = glioblastoma; TMZ = temozolomide; LOH = loss of heterozygosity; EGFR = epidermal growth factor receptor

MGMT promoter methylation and response to temozolomide in GBMs.

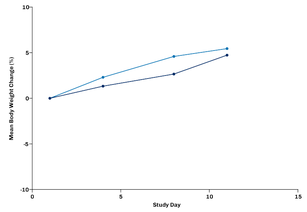


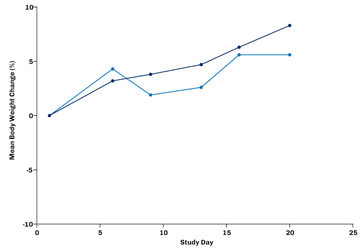
In the entire cohort, 106 GBMs had known MGMT promoter methylation status and were treated with temozolomide (TMZ) as part of the postsurgical adjuvant regimen. 33 (31.1%) were negative for methylation, 29 (27.4%) had low-level methylation, and 44 (41.5%) had high level methylation. No significant survival differences were seen (P = 0.89). Likewise, MGMT promoter methylation was not an independent prognostic factor on multivariate Cox regression analysis using the same variables as in the upper portion of Table 3 (hazard ratio = 0.92; 95% CI = 0.61—1.4; P = 0.71; N = 140).

CONCLUSIONS

- Based on the WES and RNASeq data PDX GBM6 and GBM46 both had >600 mutation and harbored an EGFR amplification.
- Both PDXs produced reliable orthotopic disease progression coupled with corresponding body weight loss. Subcutaneous tumor growth was also reproducible and did not result in any body weight loss.
- Treatment with cetuximab (30mg/kg, IP) against subcutaneous GBM6 or GBM46 was well tolerated and produced a mild and consistent anti-tumor response (TGI =~40%).
- In the referenced cetuximab phase II clinical trial, these subcutaneous preclinical results were predicative to the clinical outcome, with 35.7% stable disease (SD) and 7% partial response (PR) in EGFR amplified patients.
- Treatment with temozolomide (20mg/kg, PO or 50mg/kg, PO) against orthotopic GBM6 or GBM46 was well tolerated and produced consistent results in both GBM PDXs. GBM46 was mildly sensitive to treatment, resulting in a nominal improvement in survival. However, GBM6 was insensitive to TMZ treatment.
- In the referenced temozolomide clinical trial data, temozolomide treatment alone was not effective, producing 12.7 month in medial survival. This was an improvement over the 6.7 month survival when treatment did not include TMZ.

Subcutaneous GBM46





PDX GBM Model Characterization

Genomic database contains WES on a total of 83 models and 66 models have RNASeq available

106 characterized PDX models derived from patients at the Mayo Clinic

MATERIAL AND METHODS

- Viable PDX material (GBM6 and GBM46) is implanted either orthotopically (intracranially) or subcutaneously (right upper flank) in female athymic nude mice (Harlan). Mice were treated with cetuximab (Erbitux), EGFR target antibody. Subcutaneous tumor growth changes were tracked by caliper measurements. Orthotopic disease progression was monitored via body weight changes and clinical observations.
- Orthotopic
- Animal care and use was conducted in alignment with animal welfare regulatory requirements in an AAALAC-accredited facility.