CERTIS™ ONCOLOGY INTELLIGENCE

SNO Society for NeuroOncology

A Multi-Targeted Approach to Evaluate Therapeutic Selection and Efficacy in Preclinical GBM Models

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ABSTRACT (NUMBER EXTH-61)

Glioblastoma (GBM) is a devastating primary brain cancer with approximately 10,000 new US diagnoses annually. The current standard of care (SOC) for GBM includes surgical resection followed by radiation therapy (RT) and temozolomide (TMZ), however, there is near universal recurrence and development of resistance after treatment. Relapse in disease is tightly linked with dynamic changes in gene expression during tumor evolution, highlighting the need for stronger preclinical GBM models. Here, we report developing a pair of patient-derived xenograft (PDX) models from surgical resections obtained from initial GBM incidence in a patient (BarneyOI™ Cancer Model CRT00433) and subsequent recurrence following treatment (BarneyOI Cancer Model CRT00435) to study disease progression and novel treatment strategies. Recent studies have suggested the utilization of combination therapy approaches for GBM patients to address TMZ resistance. To this end, Certis has developed a personalized, AI-based approach to predict and test combination therapies in preclinical cancer models. Accuracy of the predicted sensitivities to treatment was evaluated *in vivo* using both subcutaneous (SC) and orthotopic (OT) mouse models. Luciferase-tagged CRT00433 and CRT00435 spheroid cell lines were transduced with firefly luciferase and implanted intracranially by stereotactic surgery for OT monitoring. Optical bioluminescence imaging (BLI) and murine-scale MRI from Aspect Imaging were used to assess therapeutic response of AI-predicted combination therapies and SOC treatment.

METHODS

For *in vitro* assays, PDX cell lines were plated in 384-well plates (1,000 cells/well) and compounds were added as single agents or in combination. Plates were read at D4 or D7 with CellTiter-Glo® (CTG). Synergy analysis was determined using SynergyFinder2[1]. For subcutaneous PDX studies, $3x10^5$ cells were implanted into the right rear flank of female NOG mice. For OT PDX studies, CRT00433 and CRT00435 cell lines were transduced with firefly luciferase and $3.0x10^5$ cells were implanted intracranially (X:1.5, Y:0.5, Z:-3.0mm from Bregma) into female NOG mice. To monitor tumor growth, SC tumors were measured via caliper twice weekly, and OT tumors were imaged using bioluminescence weekly with the Vilber NEWTON 7.0 FT500. Therapeutics were formulated and administered per manufacturer's instructions or based on peer-reviewed, published literature. CertisAI™ pre-trained cancer models are a set of proprietary machine learning models trained on experimental high-throughput monotherapy and combination experiments[2-4], encompassing over 4,500 investigational and FDA-approved drugs tested against 10 major indications. Normalized transcripts per million (TPM) values from a set of 9 predictive biomarker signatures for CRT00435 were used as input into a pre-trained CertisAI (brain) machine learning model to obtain monotherapy and combination therapy predictions. Drugs were additionally screened for predicted blood-brain barrier (BBB) permeability using a custom BBB prediction model trained on a curated diverse molecular database of over 7800 small molecules[5].

CITATIONS

Authors: Certis Oncology Solutions, San Diego, CA. **References:** **1** Ianevski A, et al. SynergyFinder 2.0: Visual Analytics of Multi-Drug Combination Synergies. *Nucleic Acids Res.* Apr. 2020. **2** Yang W et al. Genomics of Drug Sensitivity in Cancer (GDSC): A Resource for Therapeutic Biomarker Discovery in Cancer Cells. *Nucleic Acids Res.* Jan. 2013. **3** Barretina J et al. The Cancer Cell Line Encyclopedia Enables Predictive Modelling of Anticancer Drug Sensitivity. *Nature*. March 28, 2012. **4** Zagidullin B et al. DrugComb: An Integrative Cancer Drug Combination Data Portal. *Nucleic Acids Res.* Jul. 2, 2019. **5** Meng F et al. A Curated Diverse Molecular Database of Blood-Brain Barrier Permeability with Chemical Descriptors. *Sci Data*. Oct. 29, 2021. **6** Rajan, R et al. In Vitro and In Vivo Drug-Response Profiling Using Patient-Derived High-Grade Glioma. *Cancers* (Basel). June 22, 2023.

RESULTS

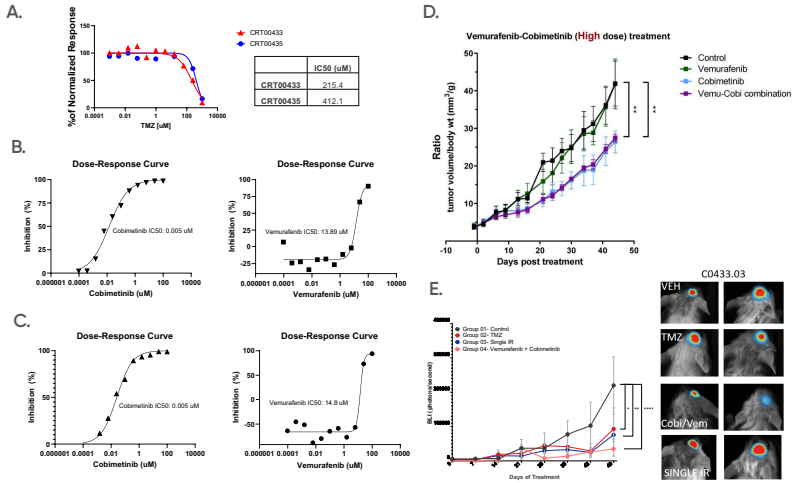


Figure 1. ***In vitro* and *in vivo* assessment of SOC and synergistic compounds in a matched pair of GBM models (CRT00433 and CRT00435). A.** *In vitro* IC50 response to TMZ (CTG). **B-C.** *In vitro* dose response of Cobimetinib and Vemurafenib in CRT00433 (**B**) and CRT00435 (**C**). **D.** Single and combination pharmacological results of Vemurafenib and/or Cobimetinib in a SC implantation of CRT00433 (Published Data[6]) **E.** Intracranial implantation of luciferase-tagged PDX model CRT0433 treated with SOC or synergy-predicted combination therapy.

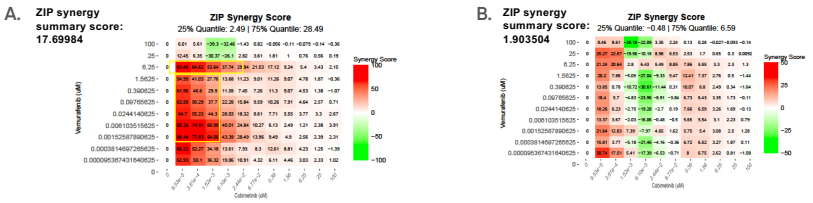
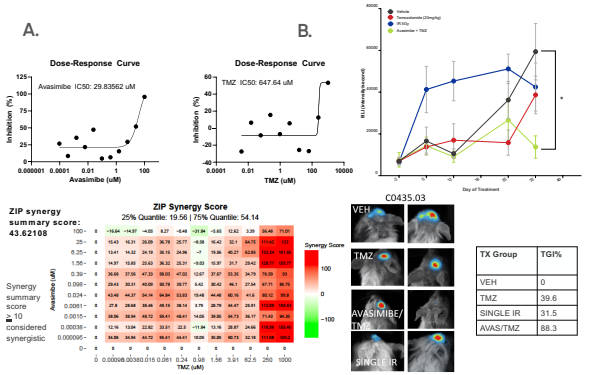


Figure 2. ***In vitro* assessment of synergistic compounds in a patient-matched, naïve or SOC-treated, recurrent model of GBM. A.** ZIP synergy analysis of Cobimetinib and Vemurafenib in CRT0433 (treatment-naïve resection) demonstrating robust synergy in this model. **B.** Synergy analysis with combinatorial Vemurafenib and Cobimetinib in the treated/recurrent model (CRT00435) fails to demonstrate the synergistic effects observed in the naïve patient-derived model shown in Figure 2A.

Figure 3. CertisAI Therapy Predictions for GBM CRT00435. Gene expression predictive biomarkers from CRT00435 were used as input into the CertisAI Predictive Oncology Intelligence platform. A ranked list of FDA-approved monotherapy and combination drugs were selected and further screened for BBB permeability using the CertisAI BBB permeability machine learning classifier.



TX Group	TGI%
VEH	0
TMZ	39.6
SINGLE IR	31.5
AVAS/TMZ	88.3

Figure 4. CertisAI therapy predictions were used to assess *in vitro* synergy and orthotopic efficacy in CRT00435. A. *In vitro* CTG assessment using one of the CertisAI predicted synergistic combinations (Avasimibe and TMZ). B. OT efficacy assessment using SOC and synergistic combinations predicted by CertisAI.

CONCLUSIONS

These results demonstrate a novel, combinatorial platform with which to more efficiently identify therapeutic candidates and assess efficacy in GBM. Combining expression profiling, predictive biomarker analysis, machine learning, and functional *in vitro* and *in vivo* testing, represents a promising new approach in overcoming this deadly disease's overall ability to adapt to an ever-changing molecular and genetic landscape that leads to therapy resistance.

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