

UC Irvine

UC Irvine Previously Published Works

Title

EXTH-19. EVALUATING THE ANTI-TUMOR EFFECT OF A NOVEL THERAPEUTIC AGENT, MAGMAS INHIBITOR, IN MALIGNANT GLIOMA

Permalink

<https://escholarship.org/uc/item/3pr2b1tq>

Journal

Neuro-oncology, 22(Suppl 2)

ISSN

1522-8517

Authors

Di, Kaijun
Du, Senjie
Lepe, Javier
et al.

Publication Date

2020-11-01

Peer reviewed

Kira Downey¹, and Hideho Okada¹; ¹University of California, San Francisco, San Francisco, CA, USA, ²Agios Pharmaceuticals, Cambridge, MA, USA

Recent studies have demonstrated the impacts of 2-HG on the anti-tumor immune response in isocitrate dehydrogenase (IDH)-mutant gliomas. Our laboratory has reported that mutant IDH1 (IDH1^{R132H}) suppresses STAT1, the master regulator of IFN-responses and IFN-inducible chemokines, thereby contributing to the “cold” tumor environment. Our data also indicated that inhibition of 2-HG production, through the use of a small molecular inhibitor of mutant IDH, can restore anti-tumor immunity. As the IDH1^{R132H} mutation has been shown to elicit a CD4⁺ T-cell response in human leukocyte antigen (HLA)-DR1 hosts, to establish a clinically relevant mouse model of IDH1^{R132H} glioma, we generated a novel IDH1^{R132H} glioma cell line syngeneic to the *HLA-A2/HLA-DR1*-transgenic mice. The cell line expresses the IDH1^{R132H} protein, produces ~65 µg/ml 2-HG *in vitro*, and forms 2-HG producing orthotopic glioma *in vivo*. Furthermore, *ex vivo* sorted tumor-associated myeloid cells (TAMCs) demonstrate high levels of intracellular 2-HG (60–80ng/1M cells), suggesting 2-HG may be taken up by these TAMCs *in vivo*. Treatment of tumor-bearing mice with vorasidenib, a pan-mutant IDH inhibitor, resulted in a 10-fold reduction of 2-HG levels in the tumor cells, correlate reduction in intracellular 2-HG levels in TAMCs, and 2-fold smaller tumors at time of sacrifice. Analysis of tumor tissues using the NanoString platform revealed enhanced pro-inflammatory IFN-related responses as a result of IDH1^{R132H} inhibition with vorasidenib. Furthermore, vorasidenib treatment increased CD4⁺ tumor-infiltrating T-cells coupled with upregulation of HLA-DR on tumor-infiltrating myeloid cells, suggesting the opportunity for enhanced antigen presentation. Currently ongoing studies are evaluating the benefit of combining this enhanced immune response with additional immunomodulatory treatments, such as vaccine therapy and immune checkpoint inhibition. These data represent highly translational findings as our major histocompatibility complex (MHC)-humanized model addresses current challenges in the study of IDH1^{R132H}-specific immunity and utilizes a clinically relevant inhibitor in phase 3 clinical development.

EXTH-16. TREATMENT OF THREE DIFFERENT BRAF-V600E POSITIVE BRAIN TUMORS WITH VEMURAFENIB AND DABRAFENIB/TRAMETINIB: A CASE SERIES

Nikita Dhir, Sheila Chandras, Chibuzo O'Suoji, and Mohamad Al-Rahawan; Texas Tech University Health Sciences Center, Lubbock, TX, USA

BACKGROUND: The BRAF-V600E gene is a protein kinase involved in regulation of the mitogen activated protein kinase pathway (MAPK/MEK) and downstream extracellular receptor kinase (ERK). The BRAF-V600E mutation has a significant role in the progression of pediatric brain tumors. 85% of pediatric CNS tumors express the BRAF mutation. Thus, BRAF targeted therapy in pediatric CNS malignancies has potential to become the standard of care for tumors expressing this mutation. **OBJECTIVE:** Current pediatric CNS brain tumor treatment focuses on chemotherapy and radiation, causing significant toxic side effects for patients. The significance of this case series lies in relaying our experience using targeted therapy in BRAF-V600E positive CNS pediatric brain tumors. **METHODS:** We followed the disease course, progression, and treatment of three pediatric patients with three different CNS tumors. Each of these individuals was treated with surgical resection, chemotherapy, and/or radiation as per standard protocol. When that modality failed to reduce tumor progression, we found that each of their different tumors was BRAF-V600E positive and they were all started on targeted therapy. **DISCUSSION:** Vemurafenib, Dabrafenib, and Trametinib are BRAF-V600E/MEK inhibitors that were initially used to treat melanomas. However, more research has shown that various pediatric CNS tumors are BRAF-V600 positive. Therapy with these BRAF inhibitors has been shown to slow tumor progression, but toxicity can be severe. This case series shows one patient with successful tumor regression, one patient with prolonged disease stabilization, and one patient with initial response but subsequent progression and ultimate death. It has been shown that using BRAF inhibitors in lower grade CNS tumors are more effective than higher grade CNS tumors. **CONCLUSION:** The success of Vemurafenib and Dabrafenib/Trametinib in causing pediatric CNS tumor regression is promising, but further studies are needed to solidify their role in pediatric CNS cancers.

EXTH-17. LOCAL DELIVERY OF CYTOKINES AND SYNTHETIC IMMUNOMODULATORS INCREASES T CELL INFILTRATION AND SIGNIFICANTLY IMPROVES SURVIVAL IN A POORLY IMMUNOGENIC MODEL OF GLIOBLASTOMA

Alexander Haddad, Jordan Spatz, Sara Collins, Matheus Pereira, Sabraj Gill, Megan Montoya, Polly Chuntova, Jacob Young, Nikhil Mummaneni, Noriyuki Kasahara, and Manish Aghi; University of California, San Francisco, San Francisco, CA, USA

BACKGROUND: Severe local and systemic immune suppression in glioblastoma (GBM) contributes to the failure of single-agent immunotherapies

in clinical trials. In this study, we evaluated the efficacy of locally delivered combination immunotherapy in a poorly immunogenic murine GBM model. **METHODS:** Immunomodulators used in these studies included: IL-15 and IL-7 (T cell activation), LIGHT (T cell tumor infiltration), FLT3L (dendritic cell maturation/proliferation), a surface T cell engager (T cell killing of tumor cells), and a bispecific PD-L1/T cell engager (T cell killing targeted to PD-L1-expressing cells). We first assessed T cell-mediated cytotoxicity *in vitro* against SB28, a poorly immunogenic murine GBM cell line, after expressing these immunomodulators in combination. Next, tumor growth inhibition *in vivo* was evaluated in syngeneic C57BL/6 mice, initially by establishment of intracranial tumors with pre-transduced SB28 cells, and subsequently by delivering these immunomodulators to pre-established naive SB28 tumors using neural stem cells (NSCs) and retroviral replicating vectors (RRV). **RESULTS:** SB28 cells transduced with immunomodulators activated dose-dependent T cell-mediated cytotoxicity *in vitro*. Mice with pre-transduced intracranial SB28 gliomas showed significantly longer survival (minimum survival: 60 days, long-term survival in 57% of mice) vs. control mice (median survival: 20 days) ($p < 0.001$), and significantly increased tumor infiltration of CD4⁺ and CD8⁺ T cells. NSC- and RRV-mediated immunomodulator delivery to pre-established SB28 gliomas also resulted in significantly increased survival of treated mice vs. controls (median survival: 31 days vs. 22 days, $p < 0.001$). Immunomodulator-treated tumors again showed significantly increased infiltration of CD4⁺ and CD8⁺ T cells, along with decreased CD11b⁺ cell infiltration. **CONCLUSIONS:** A novel combination therapy for GBM immunotherapy activated T cell killing of SB28 GBM cells *in vitro* and achieved a significant survival benefit *in vivo*, associated with anti-tumor alterations to the GBM tumor micro-environment. Further studies to optimize the efficiency of combinatorial immunomodulator delivery are currently underway.

EXTH-18. ENGINEERED EXOSOMES AS GENE DELIVERY TOOLS FOR THE TREATMENT OF GLIOBLASTOMA

Malcolm McDonald¹, Anwar Hossain², Eric Momin³, Irtiza Hasan⁴, Sanjay Singh², Satoshi Adachi², Joy Gumin², Daniel Ledbetter², Marc Daou², Sricharan Gopakumar¹, Lynette Phillips², Brittany Parker Kerrigan², and Frederick Lang²; ¹Baylor College of Medicine, Houston, TX, USA, ²MD Anderson Cancer Center, Houston, TX, USA, ³University of Wisconsin, Madison, WI, USA, ⁴University of Florida Jacksonville, Jacksonville, FL, USA, ⁵Juntendo University, Tokyo, Japan

Although we previously showed that exosomes are capable of delivering anti-glioma microRNAs (miRs) to brain tumors (Lang *et al.* 2018), our studies revealed significant opportunity to 1) improve packaging and delivery efficiency of exosomes and 2) expand the repertoire of anti-glioma miRs. We hypothesized that incorporation of viral proteins into exosomes would enhance miR packaging and cell entry. To test this hypothesis, we engineered exosomes that express retroviral Gag and VSVg proteins (eExos). Specifically, HEK293T cells were transfected with Gag, VSVg, and with Cre-recombinase containing plasmid (pCre) to generate eExos-pCre. After 48hrs eExos-pCre were isolated by differential ultracentrifugation. Western analyses verified Gag and VSVg in eExos-pCre, and PCR documented pCre in these exosomes. Next, U87 cells harboring a dsRed-eGFP-loxP reporter-gene were treated with eExos-pCre or control exosomes. Flow cytometry demonstrated that eExos-pCre resulted in 82% conversion of red cells to green, compared with controls (2% conversion), verifying the effectiveness of eExos to deliver plasmids containing anti-glioma agents. To identify effective anti-glioma miRs, we conducted a high-throughput screen of 539 miRs against 7 glioma stem cell lines (GSCs) and identified miR-124-2, miR-135-a-2, and Let7i as the most potent anti-glioma miRs. We then studied the ability of eExos to package and deliver plasmids of these miRs either singly (eExos-miR-124, eExos-miR-135, eExos-miRLet7i) or as a tri-cistronic plasmid (eExos-miR-124-135-Let7i). Although eExos-miR-124, eExos-miR-135, and eExos-miRLet7i significantly decreased *in vitro* proliferation in all three GSCs ($p < 0.01$), eExos-miR-124-135-Let7i were most effective ($p < 0.001$). In *in vivo* studies, mice harboring GSC231 gliomas were injected with each of the eExos-miRs. Most significant improvement in survival was seen with eExos-miR-124-135-Let7i (median 75 versus 32.5 days for controls, $p < 0.001$). We conclude that eExos are a novel delivery strategy for human gliomas and that a tri-cistronic plasmid of miR-124-135-Let7i is highly effective against GBM and worthy of clinical translation.

EXTH-19. EVALUATING THE ANTI-TUMOR EFFECT OF A NOVEL THERAPEUTIC AGENT, MAGMAS INHIBITOR, IN MALIGNANT GLIOMA

Kaijun Di¹, Senjie Du¹, Javier Lepe¹, Nitesh Nandwana², Bhaskar Das², and Daniela Bota¹; ¹Department of Neurology, UC Irvine, Irvine, CA, USA, ²Department of Medicine and Pharmacological Sciences, The Icahn School of Medicine at Mount Sinai, New York, NY, USA

BACKGROUNDS: Glioblastoma (GBM) is an aggressive infiltrative brain tumor, and has an extremely poor prognosis despite the use of multiple treatment modalities, including surgery, radiation, and chemotherapy.

Meanwhile, mitochondrial changes represent a significant part of cancer cell biology since cancer cells must survive and adapt to challenging micro-environments, specifically in conditions where tumor growth makes oxygen and glucose scarce. As GBM is characterized by extensive hypoxia-induced phenotypic changes such as abnormal vascular proliferation and necrosis, regulation of mitochondrial function could be a novel approach for treating GBM that currently lacks effective therapies. Magmas (mitochondria-associated protein involved in granulocyte-macrophage colony-stimulating factor signal transduction) is a nuclear gene that encodes for the mitochondrial import inner membrane translocase subunit Tim16. We previously demonstrated that a novel Magmas inhibitor, BT#9, significantly exerted anti-tumor effect in glioma *in vitro*, and may cross the blood brain barrier *in vivo*, indicating that Magmas inhibitor may be a new chemotherapeutic agent for the treatment of GBM. **METHODS:** In this study, the antitumor effect of Magmas inhibitor BT#9 was tested in an orthotopic xenograft model of human GBM. The molecular mechanism of BT#9 was investigated using glioma cell lines. **RESULTS:** The mice were tolerated to BT#9, and there was no statistical difference in the weight of animals between the control and MTD (Maximum-tolerated Dose, 50mg/kg) groups. The immunocompromised mice, intracranially implanted with human D-54 GBM xenografts, survived significantly longer than the controls ($P < 0.5$) when treated with BT#9 at MTD. *In vitro* study showed that the MAP kinase pathways are involved in BT#9-induced tumor suppression. **DISCUSSION:** This is the first study on the role of Magmas in glioma *in vivo*. Our findings suggested that Magmas plays a key role in glioma survival and targeting Magmas by Magmas inhibitor has the potential to become a therapeutic strategy in glioma patients.

EXTH-20. SYNGENEIC B7-H3-SPECIFIC CAR T-CELLS HAVE POTENT ANTI-BRAIN TUMOR ACTIVITY VIA LOCAL OR SYSTEMIC DELIVERY

Dalia Haydar¹, Zhongzhen Yi², Haley Houke², Martine F. Roussel², Chris DeRenzo², Stephen Gottschalk², and Giedre Krenciute²; ¹Dr., Memphis, TN, USA, ²St. Jude Children's Research Hospital, Memphis, TN, USA

BACKGROUND: We and others have identified B7-H3 (CD276) as a promising target for CAR T-cell-based immunotherapies for pediatric brain tumors. So far, B7-H3-CAR T cells have only been studied in xenograft models for brain tumors, which do not recapitulate the immunosuppressive tumor microenvironment (TME). To overcome this obstacle, we decided to adapt the immune competent GL261 murine glioma model which mimics human disease and host immune barriers. **METHODS:** To evaluate their safety and efficacy, murine B7-H3-CAR T-cells were generated using retroviral particles encoding a 2nd generation B7-H3-CAR with a CD28.z signaling domain. Expansion, persistence, and anti-tumor activity were evaluated *in vitro* and *in vivo*. Components of the brain TME were then evaluated using flow cytometry and immunostaining. **RESULTS:** B7-H3-CAR T cells only killed B7-H3+ tumor cells, secreted significant levels of IFN γ and IL-2 in an antigen-dependent manner and expanded an average of 85-fold in repeat stimulation assay with B7-H3+ tumor cells in contrast to control CAR T-cells. *In vivo*, intratumoral (2×10^6) or systemic (3×10^6) injection of syngeneic B7-H3-CAR T-cells into mice with orthotopic GL261 glioma induced complete regression in 60% of treated mice resulting in a significant survival advantage. Mice showed no evidence of acute or long-term toxicities related to CAR T-cell infusions. We confirmed this encouraging safety profile by systemic administration of a high dose (1×10^7) B7-H3-CAR T-cells and performing histological analyses of all major organs on day 14 post T-cell injection, which showed no notable signs of injury or on-target/off-tumor toxicities. **CONCLUSIONS:** We successfully generated syngeneic B7-H3-CAR T-cells and have demonstrated that these cells have potent anti-tumor activity in the immune competent GL261 glioma model via local or systemic delivery without apparent toxicities. Our study paves the way for future testing of B7-H3-CAR T-cells in early phase clinical studies.

EXTH-21. INTRINFUSION CATHETER MOVEMENT FACILITATES INCREASED INFUSATE VOLUME DISPERSED IN AGAROSE GEL

Jason Mehta, Gabrielle McRoberts, and Christopher Rylander; The University of Texas at Austin, Austin, TX, USA

Glioblastoma has a 5 year survival of only 5.5% and a median patient survival of 12 to 15 months even with gold standard treatment. One potential method of improving treatment of glioblastoma is the use of convection-enhanced delivery (CED) which utilizes local delivery of therapeutics to the brain. However, clinical trials have shown an inability of standard catheters to deliver therapeutics to the entire target area. In this study, we explore the potential of controlled catheter movement to increase the volume dispersed (V_d) of indigo carmine dye in agarose gel brain tissue phantoms. We use four catheter control protocols: stationary, continuous retraction, continuous in-

sertion, and intermittent insertion using a single port stepped catheter. The continuous retraction group resulted in consistent catheter clogging caused by the continued insertion of the catheter and therefore was removed from further analysis. V_d and backflow distance was quantified for all other catheter movement protocols using optical images captured throughout the infusion. Catheter retraction resulted in an increase in V_d of 51% while intermittent insertion resulted in a V_d increase of 24% compared to the stationary catheter. Additionally, a 37% reduction in backflow distance was seen with the retracting catheter when compared to the stationary catheter. These results are further supported by a simplified computational model that we have created. The computational model simulates the infusion of indigo carmine dye through an agarose gel brain tissue phantom and shows an increase in V_d of over 100% with catheter retraction. The increased V_d and decreased backflow distance afforded by the retracting catheter, suggests that the use of catheter movement may be a useful technique in increasing drug dispersal in tumorous tissue. Additional work in live and excised tissue should be conducted to confirm these results and an exploration of optimal needle movement protocols is necessary.

EXTH-22. THE CNS PENETRATING TAXANE TPI 287 AND THE AURKA INHIBITOR ALISERTIB IMPROVE SURVIVAL IN VIVO

Brian Williams, Cory Zumar, Muge Sak, and Norman Lehman; University of Louisville, Louisville, KY, USA

Glioblastoma is the most common primary malignant brain tumor in adults and has a poor prognosis with current standard of care. The AURKA inhibitor alisertib exhibits antiproliferative activity against glioblastoma *in vitro* including our previous work. Unlike current clinically used taxane drugs, the novel taxane TPI 287 penetrates the CNS. In this study we stereotactically implanted human GB9 xenografts into nude mice brains and tested the activity of alisertib alone, TPI 287 alone and both together compared to control. Five days after implantation treatment was started using twice daily 20 mg/kg alisertib orally administered 5 days per week and/or 18 mg/kg TPI-287 administered intravenously every 4 days for a total of 3 treatments. Survival was assessed as well tumor volume using MR imaging at 2 weeks and 4 weeks after tumor implantation. Monotherapy with alisertib improved survival, which was further improved with the addition of TPI 287 ($p=0.0058$). TPI 287 alone did not significantly improve survival. Tumor volume was significantly decreased in all treatment groups at 2 weeks compared with control. At 4 weeks the alisertib and TPI 287 groups showed a trend toward decreased tumor volume with a significant decrease in the combination therapy group. This data supports the potential use of this combination therapy in human trials.

EXTH-23. MULTIVALENT TARGETED CYTOLYTIC AGENTS FOR GLIOBLASTOMA TREATMENT

Puja Sharma¹, Denise Herpai¹, Callie Roberts¹, Izabela Fokt², Waldemar Priebe², Stephen Tatter¹, John Rossmeis³, and Waldemar Debinski¹; ¹Wake Forest University, Winston-Salem, NC, USA, ²MD Anderson, Houston, TX, USA, ³Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA

Glioblastoma (GBM) complexity and heterogeneity requires treatment that addresses those pathobiological features. We have been developing selective cytotoxic agents able to target at the same time several GBM-associated factors. The chosen targets are responsible for the disease progression and/or recurrence as well as for resistance to the existing therapies. As a proof of concept, a Phase I clinical trial of a cytotoxic cocktail targeting IL-13RA2 and EphA2 receptors demonstrated dramatic anti-tumor responses in dogs with spontaneous gliomas that represent the closest translational model to human disease. To this end, we have developed multivalent agents that target four receptors specific to GBM: IL-13RA2, EphA2, EphA3, and EphB2, the combined expression of which covers virtually the whole tumor micro-environment. We have designed a multivalent protein termed QUAD 3.0 that contains an IgG1 scaffold, ephrinA5, which is a ligand for the EphA2, EphA3, and EphB2 receptors, and IL-13.E13K, a mutated version of interleukin 13 (IL-13), which binds preferentially to IL-13RA2. In QUAD 3.0, there is a cysteine at the C-terminal end of the protein to allow site-specific conjugation to cytotoxic cargo. QUAD 3.0 bound effectively to the four receptors *in vitro* and *in vivo*. QUAD 3.0 was conjugated to a modified form of *Pseudomonas* Exotoxin A (PE38QQR) and highly potent DNA binding agents based on modified doxorubicin (WP936, WP1737 and WP1244). All conjugates were highly cytotoxic to established and primary GBM cells with IC_{50} 's < 50 nM. We also treated the first dogs with QUAD 3.0-PE38QQR and QUAD 3.0-WP936 at a dose of 1.6 mg/ml using real-time monitored convection-enhanced delivery and observed up to 60% of tumor shrinkage and long-term survival. Thus, multivalent targeted agents demonstrate highly promising anti-tumor activity as single pharmaceutical, off-the-shelf agents. We also expect that our targeted drug candidates produce immune responses against tumors amplifying their cytolytic anti-tumor effect