Targeting Metabolic Abnormalities in Glioblastoma Cells

Landon J. Hansen¹, Rui Yang¹, Simranjit Singh¹, Yiping He¹

¹Duke University School of Medicine, Preston Robert Tisch Brain Tumor Center
Department of Pathology, Durham, NC 27710
Disclosures

• L.H, R.Y, and Y.H have filed an internal disclosure form for combination therapies described in this document
Introduction

- *MTAP* deletion occurs in ~50% of Glioblastomas
- MTAP is a metabolic enzyme in the methionine and adenine salvage pathway
- *MTAP* deletion can theoretically sensitize cells to purine deprivation or methionine depletion, but this has not previously been demonstrated in GBM
- Tumor microenvironment may provide purines to *MTAP*-null tumor cells, complicating the *in vivo* efficacy of purine deprivation treatment and necessitating the use of combination therapies

Methods

• Patient-derived GBM cells were cultured in human neural stem cell media (STEMCELL, cat# 05751) supplemented with EGF, FGF, and Heparin. CCK8 was used to quantify cell viability following in vitro drug treatment. Absorbance was measured using a Tecan Infinite M200 Pro plate reader.

• Patient-derived GBM cells with retrovirally-introduced luciferase expression were implanted in the right caudate nucleus of athymic nu/nu (nude) mice using a stereotaxic frame to generate orthotopic xenografts. Treatment began 4 weeks after tumor implantation. Mice received daily I.P. injections of L-Alanosine 225mg/kg for the specified duration. Tumor response to treatment was monitored by bioluminescence on an IVIS Lumina XR imager and analyzed using Living Image software. Subcutaneous tumors were measured by handheld calipers.

• Gene expression microarray (Affymetrix) and RNAseq were processed by the Duke Sequencing and Genomic Technologies core resource.
Figure 1. MTAP deletion sensitizes GBM cells to purine deprivation. A) Copy number analysis by qPCR on genomic DNA confirms MTAP deletion in a panel of patient derived GBM cells. B) Schematic illustrating a potential therapeutic strategy to target MTAP null GBM. C) MTAP null cells show increased sensitivity to L-Alanosine +/- 5’dAdo. D) Sensitivity of patient derived cell line panel to L-Alanosine + 5’dAdo. E) MTAP restoration in patient-derived cell lines Mut#1 and Mut#3 (left, middle) confers resistance to L-Alanosine + 5’dAdo, while MTAP knockout by CRISPR/Cas9 in U251MG cells (right) sensitizes cells to L-Alanosine + 5’dAdo.
Figure 2. Purines in the microenvironment can rescue cells from L-Alanosine treatment

A) Media supplementation with purines (adenine, adenosine, or ATP) rescues MTAP null cells from L-Alanosine-induced toxicity, simulating what potentially happens in vivo.

B) ENT1/2 inhibitor dipyridamole is able to block the rescue of L-alanosine treated cells by Adenosine.

C) Schematic showing hypothesis for how purine supplementation rescues cells from L-Alanosine treatment and combination strategy for overcoming this mechanism.
Figure 3. L-Alanosine demonstrates potent anti-tumor effects in vivo. (A, B) L-Alanosine causes delayed tumor growth (A) and prolonged survival (B) in a subcutaneous patient-derived xenograft model, n=6 animals per arm. (C) Bioluminescent monitoring shows response of intracranial xenografts to L-Alanosine treatment after 21 days. (D) Average tumor growth over 42 days of L-Alanosine treatment by bioluminescence, n=8 animals/arm. (E) Kaplan-Meier survival of nude mice with intracranial xenografts depicted in (D). (F) Bioluminescence during and after treatment from a second patient-derived intracranial xenograft model, n=8.
Glioblastoma cells with *MTAP* deletion are sensitized to *de novo* purine synthesis inhibition. These results are potentiated by the addition of the MTAP substrate, 5’dAdo. *MTAP* WT cells are less sensitive to L-Alanosine and are protected by 5’dAdo, which is converted into additional adenine stores in MTAP-expressing cells only.

Exogenous supplementation of cell culture media with adenine, adenosine, or ATP results in the rescue of L-alanosine-treated cells by uptake of purines across the cell membrane, revealing potential for tumor rescue by exogenous purines in the tumor microenvironment. Nucleoside transport inhibitors such as Dipyridamole or Dilazep demonstrate the potential to enhance purine deprivation treatment of *MTAP*-null tumors by blocking purine uptake from the microenvironment.

In order to better understand the cellular response to purine deprivation therapy, we performed proteomic and gene expression microarray analysis on L-Alanosine-treated tumor cells *in vitro*, and RNAseq on L-Alanosine treated tumor xenografts in vivo. Pathway analysis of upregulated genes revealed activation of AMPK and FoxO signaling as a response to nutrient stress.

Additionally, enzymes involved in the *de novo* purine synthesis pathway were upregulated along with several of the interacting pathways, such as glycolysis, which provide the metabolites utilized in purine production.

HSP90 provides a scaffold for purine production in a complex called the purinosome. We tested whether combination of L-Alanosine with HSP90 inhibitors could improve anti-tumor efficacy by countering the tumor’s efforts to increase purine synthesis. HSP90 inhibition (17-AAG) in combination with L-Alanosine heightened the selectivity against *MTAP*-null cells *in vitro* and augmented the *in vivo* tumor response. This highlights the potential to improve the efficacy of purine deprivation therapy through combinatorial approaches. The efficacy of additional combinations, including standard of care drug Temozolomide, remain to be demonstrated.
Summary Points

- *MTAP* deletion is a common event in glioblastoma and represents an opportunity for targeted metabolic therapy due to compromised purine production in *MTAP*-null tumor cells.

- An inhibitor of *de novo* purine synthesis, L-Alanosine, demonstrates potent anti-tumor effects *in vitro* and *in vivo*, is transported across the blood brain barrier, and is well tolerated, suggesting potential for clinical use.

- The efficacy of L-Alanosine can be improved with additional purine-targeting agents, such as purine transport inhibitors which can block uptake of purines from the tumor microenvironment.

- Tumor cells demonstrate nutrient deprivation and stress-response signaling after L-alanosine treatment and attempt to upregulate purine production through a variety of mechanisms.

- Combination therapies utilizing L-Alanosine have the potential to selectively target *MTAP*-null GBM, which represents a large portion of GBM patients.