The addition of CB-839 to TMZ significantly reduces glioma aspartate and glutamate in an IDH mutated patient derived glioma xenograft model

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Abstract

Background: IDH mutated gliomas are critically dependent on glutaminase for glutamate biosynthesis. CB-839 is a novel glutaminase-1 inhibitor which has demonstrated efficacy in both genetically engineered and patient derived IDH mutated glioma cells, especially in combination with radiation. These preclinical studies evaluate the pharmacodynamic and pharmacokinetic impact of combining CB-839 with TMZ in patient derived xenograft (PDX) glioma models.

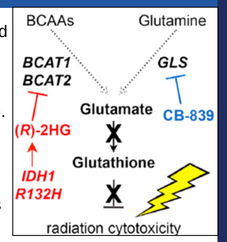
Methods: GBM164 is a PDX model derived from an IDH1 mutant, 1p/19q non-codeleted glioma. D- and L-2HG levels in untreated GBM164 tumors were compared to GBM6 tumors (IDH wildtype glioma PDX) by GC/MS. Athymic nude mice bearing GBM164 flank tumors were treated with CB-839 (200 mg/kg PO BID x 9 doses) and/or temozolomide (50 mg/kg PO daily x 5 doses) and sacrificed four hours after the last dose to harvest plasma, normal brain and flank tumor. Tumor metabolomics were assessed by GC/MS. DNA damage signaling in tumors was assessed by Western blotting for KAP1 / Chk1 / Chk2 phosphorylation and H2AX / Rad51. Tumor, plasma and brain pharmacokinetics were assessed by LC/MS/MS.

Results: Total 2HG levels in GBM164 were 18-fold higher than GBM6. CB-839 monotherapy reduced tumor glutamate (18% reduction, p = 0.15) and aspartate (30% reduction, p = 0.06) when compared to vehicle, however these changes did not reach statistical significance. The combination of CB-839 and TMZ more significantly reduced both tumor glutamate (30% reduction, p = 0.03) and aspartate (34% reduction, p < 0.001) when compared to TMZ monotherapy. CB-839 did not significantly increase DNA damage compared to vehicle, and the combination of CB-839 and TMZ did not significantly increase DNA damage compared to TMZ monotherapy. The mean tumor: plasma ratios of CB-839 concentrations were 0.68 and 0.58 in mice treated with CB-839 monotherapy and CB-839/TMZ, respectively. The mean brain: plasma ratios of CB-839 concentrations were 0.07 and 0.12 in mice treated with CB-839 monotherapy and CB-839/TMZ, respectively.

Conclusions: The addition of CB-839 to TMZ significantly reduces glioma aspartate and glutamate in an IDH1 mutant PDX glioma model, without any impact on DNA damage. Survival studies are in progress to assess the efficacy of CB-839 when used in combination with RT and/or TMZ.

Background[1]

Branched chain amino acid transaminase (BCAT) is necessary to synthesize glutamate from branched chain amino acids (BCAA).



Excess (R)-2HG (2-hydroxyglutarate) in gliomas with IDH mutation (e.g. IDH1 R132H) inhibits BCAT expression.

This defect causes IDH mutant gliomas to become reliant on glutaminase (GLS) for glutamate and glutathione biosynthesis from glutamine.

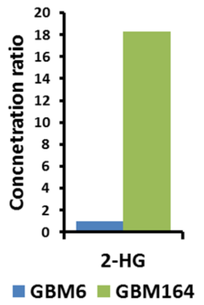
CB-839 is a novel, potent, selective and reversible inhibitor of GLS activity.

CB-839 induces further depletion of glutamate in IDH mutated glioma cells, and is associated with enhanced radiation cytotoxicity.

These preclinical studies evaluate the pharmacodynamic and pharmacokinetic impact of combining CB-839 with TMZ in patient derived xenograft (PDX) glioma models.

1. McBrayer SK, Mayers JR, DiNatale GJ, et al. Transaminase Inhibition by 2-Hydroxyglutarate Impairs Glutamate Biosynthesis and Redox Homeostasis in Glioma. Cell. 2018 Sep 20;175(1):101-116.e25.

GBM164 – IDH mutant glioma PDX model expresses 2-HG



GBM164 is an IDH mutant patient-derived xenograft glioma model.

2-HG concentrations in GBM164 and GBM6 (an IDH wildtype PDX glioma model) were assessed using GC/MS.

Total 2HG levels in GBM164 were 18-fold higher than GBM6.

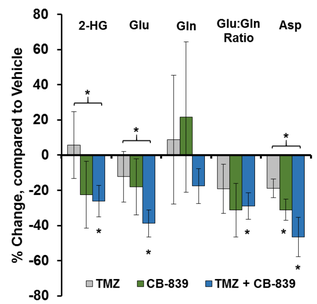
Treatment

Athymic nude mice harboring GBM164 flank tumors were randomized between four treatment arms (n = 4-7 per arm)

Arm	Drug	Dose	Day 1	Day 2	Day 3	Day 4	Day 5
Placebo	Vehicle	N/A	X X	X X	X X	X X	X
TMZ	TMZ	50 mg/kg	X	X	X	X	X
CB-839	CB-839	200 mg/kg	X X	X X	X X	X X	X
TMZ + CB-839	TMZ	50 mg/kg	X	X	X	X	X
	CB-839	200 mg/kg	X X	X X	X X	X X	X

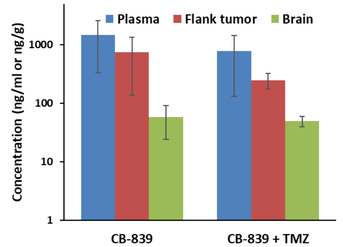
Mice sacrificed 4 hours after dose on Day 5
Plasma/brain/tumor harvested for:
Pharmacokinetics (all) – LC/MS/MS
Metabolomics (tumor) – GC/MS
DNA damage signaling (tumor) – Western Blots

Tumor metabolomics



- CB-839 reduces tumor 2-HG, glutamate and aspartate by 22%, 18% and 30%, respectively, compared to vehicle, and increases glutamine by 22%.
- TMZ + CB-839 reduces tumor 2-HG, glutamate, glutamine and aspartate by 26%, 39%, 18% and 46%, respectively, compared to vehicle. TMZ + CB-839 reduces tumor 2-HG, glutamate, glutamine and aspartate by 30%, 30%, 24% and 34%, respectively, compared to TMZ monotherapy.

Pharmacokinetics

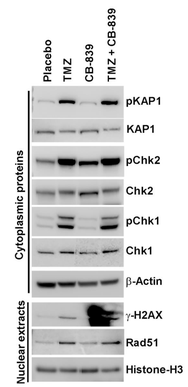


- Tumor: plasma concentration ratio = CB-839 = 0.68; CB-839 + TMZ = 0.58
- Brain: plasma concentration ratio = CB-839 = 0.07; CB-839 + TMZ = 0.12

DNA damage signaling

CB-839 did not increase DNA damage compared to vehicle.

The combination of CB-839 and TMZ did not increase DNA damage compared to TMZ monotherapy.



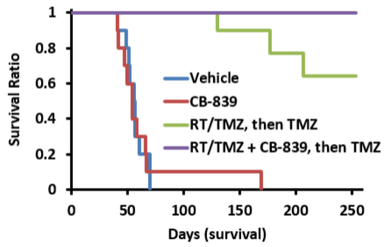
Conclusions

The addition of CB-839 to TMZ significantly reduces glioma aspartate and glutamate in an IDH1 mutant PDX glioma model without any impact on DNA damage.

Survival Studies in progress

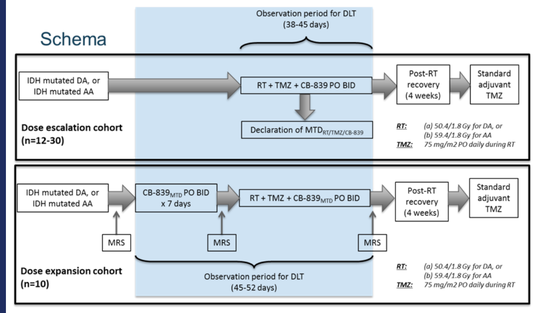
Survival studies are in progress to evaluate the efficacy of CB-839 in orthotopic GBM164 murine models.

At day 250, the addition of concurrent CB-839 to radiation/ temozolomide improves efficacy.



ETCTN trial in progress

NCI # 10218 (NCT03528642)



Support

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