The Clinical C/EBPβ Antagonist Peptide Lucicebtide Synergizes with Molecularly Targeted Therapies in GBM

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SAPIENCE THERAPEUTICS

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Abstract

Glioblastoma (GBM) is the most common and aggressive malignant brain tumor, with median overall survival (OS) following standard-of-care treatment of 15–17 months and 5-year survival of less than 10%. Despite extensive characterization of the genetic lesions driving GBM, there has been no improvement on the overall natural history of the disease, mostly due to the cellular and genetic heterogeneity of these tumors. CCAAT/Enhancer Binding Protein β (C/EBPβ) is a transcription factor identified as a master regulator of the mesenchymal transition in GBM. Lucicebtide (ST101) is a C/EBPβ antagonist peptide that was evaluated in a Phase 2 clinical study in patients with recurrent and newly diagnosed GBM (NCT04478279) and has shown durable responses in a subset of patients. We have previously demonstrated that lucicebtide anti-tumor activity is due to both direct cancer cell death and immune-activation within the tumor microenvironment. Here we investigated whether lucicebtide may synergize with targeted therapeutic strategies against genetic dependencies or immune checkpoints in GBM. We adopted a synthetic lethal CRISPR screen approach in which gain-of-function drivers that are mutated in more than 5% of GBM patients in publicly available datasets (EGFR, PDGFRA, PI3KCA, MDM2, MDM4, CDK4) were suppressed in the presence or absence of lucicebtide in three genetically characterized GBM lines. Our data identified that EGFR suppression by multiple independent sgRNAs was synthetic lethal in the presence of lucicebtide. We confirmed these findings by performing checkerboard assays of lucicebtide in combination with chemically distinct EGFR inhibitors, indicating that lucicebtide synergizes with EGFR inhibition (EGFRi) in GBM. We further characterized the mechanism of action of EGFRi-lucicebtide combination on known signal transducers of EGFR in the presence or absence of lucicebtide. Finally, we explored the relationship between transcriptional signatures of C/EBPβ activity and EGFR mutational profile in publicly available GBM datasets, identifying potential genetic biomarkers for prediction of maximal efficacy of this combination. These studies demonstrate the potential of lucicebtide to enhance the activity of molecularly targeted therapeutics such as EGFRi that are typically not effective when used as monotherapies in GBM. Biomarker analysis utilizing GBM genetics and signatures of C/EBPβ activity was performed to identify potential target populations likely to benefit from ST101 combinations.

Lucicebtide Mechanism of Action

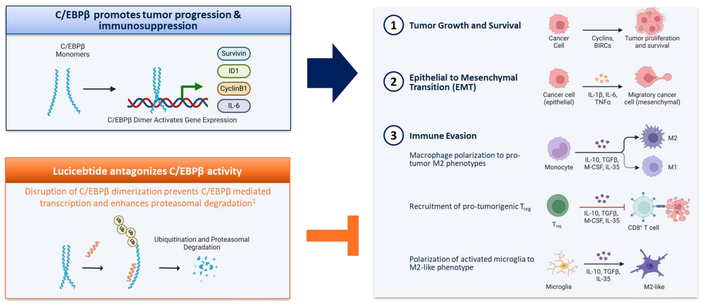


Figure 1. Response to lucicebtide impacts both the tumor cell and tumor microevironment (TME). C/EBPβ activation drives tumor cell proliferation and survival, and promotes mesenchymal transformation. Lucicebtide disrupts C/EBPβ dimerization, preventing C/EBPβ mediated transcription and enhancing its proteasomal degradation. The result is *1)* antagonism of oncogenic gene transactivation leading to selective tumor cell death (Darvishi et al, 2022); *2)* reduced mesenchymal transformation; and *3)* inhibition of C/EBPβ-driven immune evasion. Specifically, lucicebtide inhibits a transcriptional program that includes immunosuppressive molecules such as IL-6, CD206 and CD209 (DC-SIGN), resulting in potent repolarization of M2-type TAMs toward the immune active M1-like state in the TME (Scuoppo et al., 2025).

Results

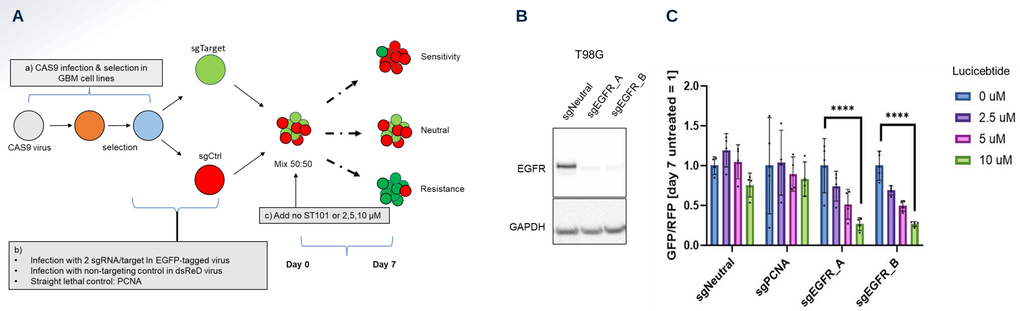


Figure 2. A CRISPR screen identifies EGFR inactivation as synthetic lethal with lucicebtide in GBM. A) Outline for synthetic-lethal screen in GBM lines. Cells were infected with CAS9-expressing lentiviruses and selected for blasticidin resistance (a). Once CAS9 expression was validated by Western Blot, cells were transduced with puromycin-resistance expressing viruses co-expressing the following sgRNAs: i) 2 unrelated sgRNAs for a GMB driver gene (*EGFR, PI3KCA,MDM2,MDM4,CDK4,PDGFR*) defined as gain-of-function found in at least 5% of cases in the TCGA GBM dataset (Brennan et al., 2013), tagged with EGFP; ii) a non-targeting control sgRNA, tagged with dsRED; and iii) a straight-lethal sgRNA targeting the essential gene PCNA (b). Selected pools were mixed 50:50 with dsReD-control and treated with the indicated lucicebtide concentrations or left untreated. Lethality was measured over 7 days (c). **B)** Western Blot confirms EGFR suppression in T98G GBM cells with no impact on GAPDH loading control**.** EGFR_A and EGFR_B indicate two non-overlapping sgRNA sequences. **C**) EGFP:dsRed ratio for the indicated pools after seven days at the indicated lucicebtide concentrations. Statistics, 2-way Anova, Student t-test n=4/group; ****p<0,0001).

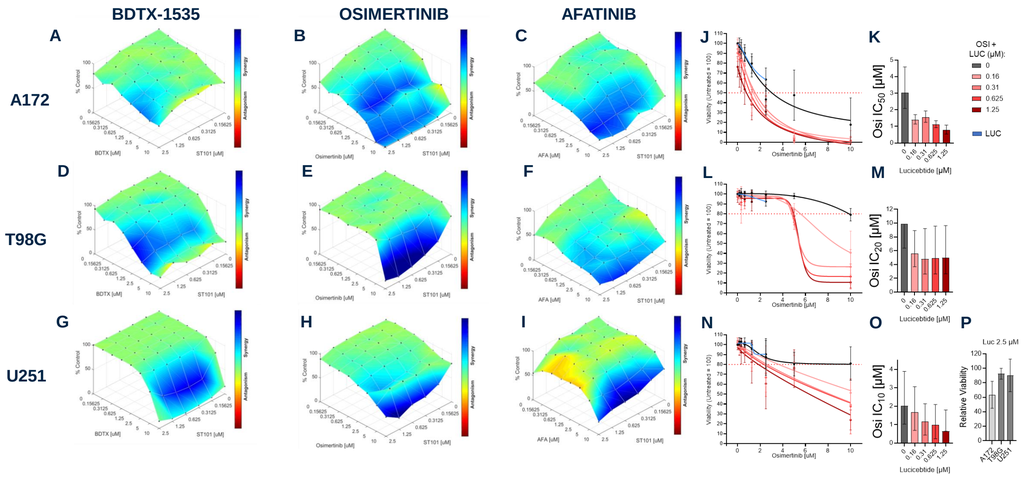


Figure 3. EGFR inhibitors (EGFRi) synergize with lucicebtide. A-I) Bliss surface models for three GBM lines (A172, T98G, U251) treated with lucicebtide and three chemically distinct EGFRis (BDTX-1535 (**A,D,G;** BDTX), Osimertinib (**B,E,H;** Osi), Afatinib (**C,F,I;** AFA)) at the indicated concentrations and combinations. Viability was assayed at 48hrs by Annexin V and Sytox Red assay. All points include 4 independent replicates. **J-O**) Dose-response curves and absolute inhibitory concentration (ICs) for Osi in combination with lucicebtide. IC was calculated for each cell line using the max effect reached in the Osi-only response (A172, IC50 (**J**); T98G, IC20 (**L**); and U251, IC10 (**U**)). For dose-response graphs, dotted lines represent IC50 (**J**, A172) IC20 (**L**,T98G) and IC10 (**U**,U251) levels. Lucicebtide-only response (Luc) is shown in cyan. Error bars represent 95% confidence intervals. **P**) Relative viability for lucicebtide at the max concentrations used in this assay for each cell line, reported as control for lucicebtide-only effect. Error bars represent standard deviations (n=4).

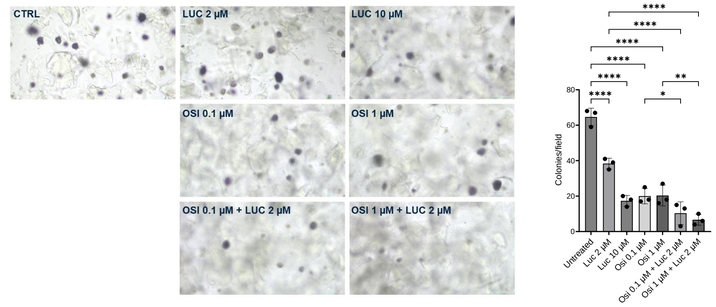


Figure 4. Lucicebtide increases Osimertinib suppression of GBM anchorage independent growth. A) Soft-agar assay of T98G cells seeded at 10,000/well in 6-well plate and grown in 0.4% agar for 2 weeks at the indicated drug concentrations. Colonies were stained with O/N with tetrazolium salts. Each field was counted as aggregate of 3 4x captures. Images represent median colony numbers for each condition. Osi, Osimertinib. Luc, Lucicebtide. **B)** Quantification of anchorage-independent growth at the indicated conditions. Lucicebtide 2 µM exposure reduces colony number by 3-fold compared to the corresponding Osi-only condition. Statistics, 1-way Anova T-test (Hom-Sidak Multiple hypothesis correction). ****p<0.001; **p<0.01; *p<0.05).

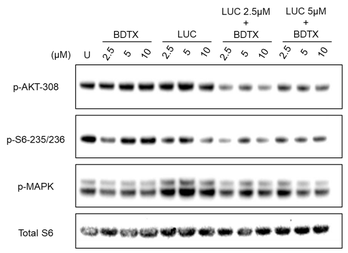


Figure 5. Synergistic suppression of AKT Ser-308 phosphorylation by EGFRi and lucicebtide. Western Blot for the indicated phospho-protein or total S6 for U251 cells untreated (U) or treated with the indicated concentrations of BDTX-1535 (BDTX), lucicebtide (LUC) or combination of BDTX and lucicebtide (LUC 2.5 µM and LUC 5 µM) for 1hr. The PI3K-responsive S-308 site is not impacted by either drug alone, while combination showed a ~3-fold suppression compared to untreated or single drug treatment. Similarly, S6 S235-S236 phosphorylation was decreased by the combination treatment. No significant suppression of MAPK was observed.

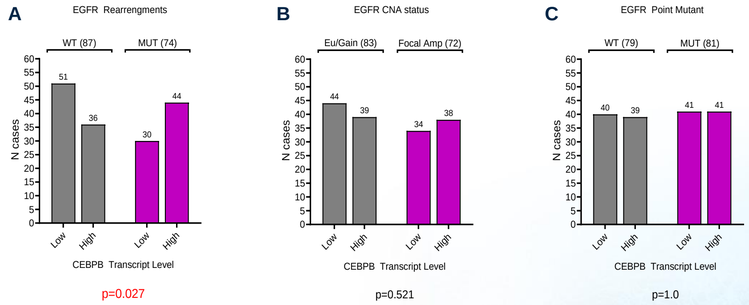
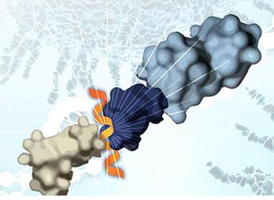


Figure 6. EGFR rearrangements, but not EGFR copy number or EGFR point mutations, correlate with high CEBPB expression in human GBM. Distribution of 160 GBM cases by CEBPB transcript level classified as "Low" or "High" according to the median CEBPB expression and by EGFR rearrangement status (**A**), copy number data (**B**) or EGFR point mutations (**C**) as reported in Brennan et al., 2013. Cases were considered harboring an EGFR rearrangement if EGFRvIII (deletion of exons 2-7: Δ2-7), EGFR Δ12-13, EGFR Δ14-15, EGFR Δ4, EGFR Δ25-27 were detected with allelic frequency greater than 0.01%. For copy number data (**B**) cases were classified as focally amplified (Focal Amp) or as Euploid or harboring low level non-focal gains (Eu/Gain). For point mutants cases were classified as mutated if allelic frequency greater than 0.01%. Statistic, Fisher T-test, as indicated.

Conclusions

1. CRISPR synthetic lethal screen identified genetic suppression of EGFR as synergistic with lucicebtide in GBM.
2. Combination of lucicebtide with three chemically distinct EGFR inhibitors results in synergy in 3 GBM lines in viability and anchorage-independent growth assays.
3. The activating phosphorylation of AKT (Thr308) and its downstream effector S6 are suppressed by EGFRi/lucicebtide combination.
4. In human GBM, high CEBPB expression correlates with presence of EGFR activating alleles (including but not limited to EGFRvIII variants).
5. These data support the combination of lucicebtide with EGFR inhibition to improve GBM responses and suggests the potential use of EGFR as an enrichement biomarker for patient selection.
6. Together with the recently published MOA data indicating lucicebtide promotes conversion of tumor-associated macrophages to pro-inflammatory M1 macrophages, combination strategies with lucicebtide can leverage both tumor- and microenvironment-driven anti-tumor activity.



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