C/EBPβ antagonist peptide, ST101, attenuates oncogenic gene transactivation in cancer cells to drive antitumor activity

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Introduction

Sapience Therapeutics is focused on discovering and developing peptide-based therapeutics to previously 'undruggable' targets for major unmet medical needs, particularly high mortality cancers.

ST101 is a peptide antagonist of C/EBPβ and is being developed for solid tumors and hematologic malignancies. The FDA/MHRA have accepted the Investigational New Drug (IND)/Clinical Trial Authorization (CTA) applications for ST101, enabling ST101 to move into a Phase 1/2 study in patients with advanced unresectable and metastatic solid tumors, with expansion cohorts in breast cancer, melanoma, prostate cancer and GBM. Enrollment for this trial is anticipated to commence in Q3 2020 in sites in the U.S. and U.K.

Abstract

Transcription factor dysregulation is common in cancer, resulting in aberrant gene expression that drives oncogenesis. Antagonism of oncogenic transcription factor activity, by disrupting essential protein-protein interactions needed for activation of downstream effector molecules or association with DNA, represents a powerful approach to target this previously 'undruggable' class of proteins. CCAAT/Enhancer Binding Protein Beta (C/EBPβ) is a transcription factor overexpressed in many cancers that regulates expression of factors that promote tumor survival, proliferation and inhibit differentiation. Here, we describe the anti-tumor activity of ST101, a cell-penetrating peptide antagonist of C/EBPβ. To demonstrate ST101 disruption of the interaction of C/EBPβ with co-factor activating transcription factor 5 (ATF5), a competition ELISA assay was performed. ST101 inhibited the interaction of C/EBPβ and ATF5 in dose-dependent manner, resulting in an IC_{50} of 25 nM. To demonstrate ST101 disruption of C/EBPβ phosphorylation and gene transactivation in cancer cells, western blot analysis and quantitative polymerase chain reaction (qPCR) were performed on U251 glioblastoma, MCF7 breast adenocarcinoma and A549 lung adenocarcinoma cells. Administration of ST101 resulted in a dose-dependent decrease in C/EBPβ activation, as evidenced by a decrease in Thr189 phosphorylation. Antagonism of C/EBPβ activity resulted in a dose-dependent decrease in mRNA expression of genes involved in survival (BCL2 and the baculoviral inhibitor of apoptosis factors BIRC3, BIRC5), inhibition of differentiation (Inhibitor of DNA binding proteins ID1, ID2 and ID3) and proliferation (cyclins CCNB1 and CCNA2 and cyclin-dependent kinases CDK1 and CDK2). Finally, in a mouse xenograft model, 25mg/kg ST101 administered three times per week for three weeks resulted in significant and sustained tumor growth inhibition in U251 subcutaneous tumors, both when ST101 administration was initiated early (day 2, 200 mm^3 tumors, p<0.05) or late (day 16, >500mm^3 tumors, p<0.05). These data demonstrate the therapeutic potential of systemic administration of ST101 and support clinical development of ST101 as a potent peptide therapeutic for a variety of solid tumor malignancies.

ST101 Mechanism of Action

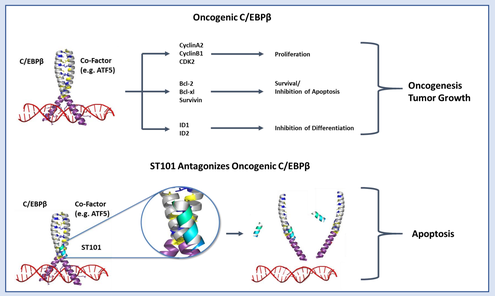


Figure 1: Mechanism of action of ST101. C/EBPβ drives tumor cell proliferation, survival and inhibits differentiation in many cell types. ST101 disrupts the interaction of C/EBPβ with cofactors such as ATF5, depriving cells of oncogenic signals they are dependent upon and resulting in selective tumor cell death.

Target Engagement

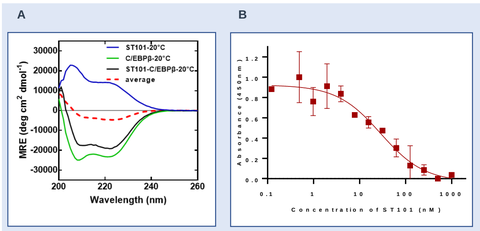


Figure 2: ST101 binds to C/EBPβ and inhibits the interaction with ATF5. A) Circular dichroism spectra data for the interaction of ST101 with target C/EBPβ. Spectra were measured at 20° at a total peptide concentration of 150 μM and presented as mean residue ellipticity (MRE). All experiments were performed in 10 mM potassium phosphate and 100 mM potassium fluoride (pH 7.0). The helical signature of ST101-C/EBPβ exceeds the average generated from the component peptides, indicating that a heterodimeric complex is preferentially formed. B) ELISA assay quantifying the antagonism of the interaction of ATF5 with C/EBPβ by ST101. Increasing concentration of ST101 was added to the plate-bound recombinant C/EBPβ prior to exposure to 1 nM ATF5, resulting in an IC_{50} of 25 nM.

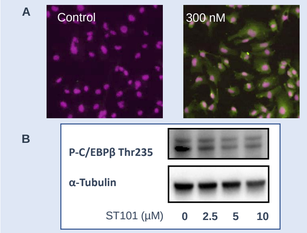
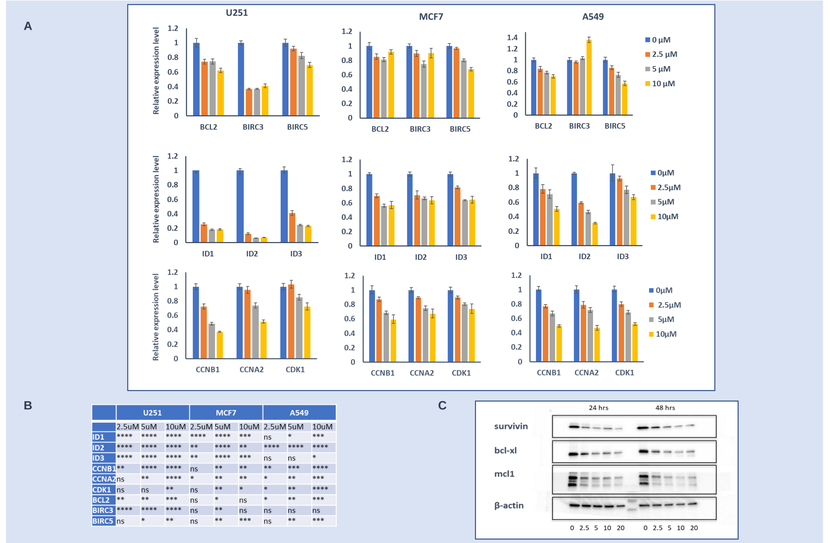


Figure 3: ST101 enters cells and antagonizes C/EBPβ activation. A) U251 cells were exposed to 300 nM ST101 for 5 minutes. Cell and nuclear penetration was assessed by IF staining using a rabbit polyclonal antibody against ST101. DAPI shown in purple and ST101 shown in green. B) Phosphorylation of C/EBPβ at Thr235 was assessed in U251 cells following exposure to ST101 (0-10 μM) for 24 hours. Western blot detection indicates dose-dependent decrease relative to α-tubulin expression.

ST101 In Vitro Activity

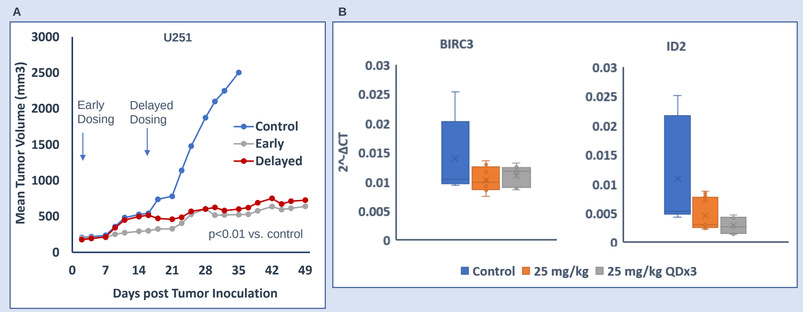


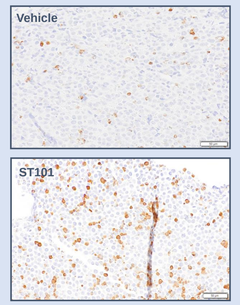
B

	U251			MCF7			A549		
	2.5uM	5uM	10uM	2.5uM	5uM	10uM	2.5uM	5uM	10uM
ID1	****	****	****	****	****	***	ns	*	***
ID2	****	****	****	**	****	**	****	****	****
ID3	****	****	****	**	****	***	ns	ns	*
CCNB1	**	****	****	ns	**	**	**	***	****
CCNA2	ns	**	****	*	**	**	*	**	***
CDK1	ns	ns	**	ns	**	*	*	**	****
BCL2	**	**	***	ns	*	ns	*	**	***
BIRC3	****	****	****	ns	**	ns	ns	ns	ns
BIRC5	ns	*	**	ns	**	***	ns	**	***

Figure 4: ST101 impacts C/EBPβ gene transactivation in vitro. A) Quantitative PCR analysis of genes involved in survival (BCL2, BIRC3, BIRC5); differentiation (ID1, ID2 and ID3); and cell cycle (cyclin B1, cyclin A2 and cyclin-dependent kinase 1). U251, MCF7 and A549 cells were exposed to ST101 (0, 2.5, 5 or 10 μM) for 24 hrs. Following RNA extraction, qPCR analysis was performed. Data represents log2 normalized expression (2^ΔΔCt) and standard error of mean. B) Statistical analysis of qPCR results in A. Student's t-test was used to identify statistically significant changes in gene expression after treatment with ST101 (ns: not significant; *p < 0.05; **p < 0.01; ***p < 0.001, ****p < 0.0001). C) Western blot analysis of Bcl-xl, Mcl-1 and Survivin protein expression in U251 cells 24 hrs post ST101 exposure (2.5-20 μM). Data are representative of at least two experiments. D) Densitometric analysis of Western blot results.

ST101 In Vivo Activity





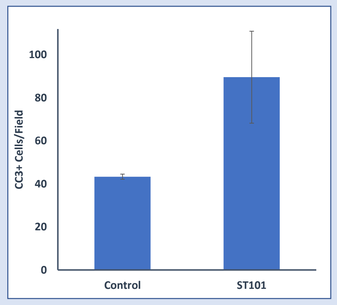


Figure 5: ST101 displays significant anti-tumor activity in U251 xenograft models. A) U251 GBM cells were implanted into subcutaneous flank of NOD-scid mice. ST101 administration (25 mg/kg administered three days per week by subcutaneous injection) was initiated on tumors with an average volume of 198 mm^3 (day 2 post inoculation; gray line), or alternatively, on tumors with an average volume of 516 mm^3 (day 16 post inoculation; red line). Tumor volume was monitored three times per week for the duration of the experiment. Individual tumor volumes were calculated as mm^3 = (length x width x height)/2. Data are plotted as mean tumor volume ± standard error of the mean. Wilcoxon matched-pairs signed rank test indicated significant difference in tumor growth between treated groups and control (p<0.01 for each; n=6 mice/group). B) Mice were inoculated as in A, and exposed to vehicle (blue) or ST101 (25 mg/kg) administered once (orange) or for three consecutive days (gray). Excised tumor samples were collected, and qPCR for BIRC3 and ID2 were performed. C) Alternatively, tumor fragments were collected following ST101 (25 mg/kg) administered for three consecutive days. Tissue were processed for cleaved caspase 3 (CC3) staining by immunohistochemistry. Apoptotic tumor cells were quantified, as determined by brown CC3 stain. Representative images of vehicle and ST101 treated tumors shown. D) Quantification of CC3+ staining. Data points represent mean ±SE for n=3-5/group.

Conclusions

- ST101 rapidly enters cells and the nucleus, binds C/EBPβ and inhibits interactions with co-factors.
- ST101 exposure results in significant decreases in gene expression of pro-survival factors (BCL2, BIRC3 and BIRC5), inhibition of differentiation factors (ID1, ID2, and ID3) and proliferative factors (cyclin B1, cyclin A2 and cyclin-dependent kinase 1).
- Significant tumor growth delay (TGD) was observed in U251 glioblastoma subcutaneous xenograft tumors (p<0.001) following ST101 administration in both 'early' and 'delayed' dosing groups.
- TGD was accompanied by a decrease in C/EBPβ gene transactivation (BIRC3 and ID2 data shown) by qPCR analysis and an increase in tumor cell apoptosis as determined by cleaved caspase 3 immunohistochemistry.
- ST101 is a promising therapeutic approach for many oncology indications.
- ST101 is Phase 1/2-ready, with IND accepted by FDA and CTA accepted by MHRA; anticipate enrolling first patients in Q3 2020.