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 to move into a Phase 1/2 study in patients with 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 2023 in sites in the U.S. and U.K.

Target Engagement

Transcription factor dysregulation is common in cancer, resulting in aberrant gene expression that drives oncogenesis. Agonists 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. COAT/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 inhibition of apoptosis. Here, we study in vitro and in vivo the antitumor activity of ST101, a cell-penetrating peptide antagonist of C/EBPβ. To demonstrate ST101 disruption of the interaction of C/EBPβ with cofactor 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 IC50 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 U51 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 Thr118 phosphorylation. Agonist 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, D2 and D3) and proliferation (cyclins CCNB1 and CCNB2 and cyclin-dependent kinase CDK1 and CDK2). Finally, in a mouse xenograft model, 525mg/kg ST101 administered three times per week for three weeks resulted in significant and sustained tumor growth inhibition in U51 subcutaneous tumors, both when ST101 administration was initiated early (day 2, 200 mm3 tumors, p<0.05) or late (day 16, +500 mm3 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 achieves its effect by competitively binding to C/EBPβ with selectivity such as ATF5, displacing the active oncogenic signals they are dependent upon and resulting in defective tumor cell growth.

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 (D1, D2, and D3) and proliferative factors (cyclin B1, cyclin A2 and cdk2/cyclin 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 window studies.
- TGD was accompanied by a decrease in C/EBPβ gene transactivation (BIRC3 and D2 data shown) by qPCR analysis and an increase in tumor necrosis factor-α expression by clean-up studies in immunohistochemistry.
- ST101 is a promising therapeutic approach for many oncologic indications.

ST101 is Phase 1/2 ready, with IND accepted by FDA and CTA accepted by MHRA: anticipating enrollment first patients in Q3 2020.