ST101, a Clinical CEBPβ Antagonist Peptide, Promotes an Immune-Active Tumor Microenvironment by Multiple Cellular Mechanisms

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SAPIENCE THERAPEUTICS

Abstract #2909

Abstract

CCAAT/Enhancer Binding Protein β (C/EBPβ) is a transcription factor that is an established driver of cellular transformation in several cancer types with poor prognosis, including glioblastoma (GBM), triple negative breast cancer (TNBC) and non-small cell lung cancer (NSCLC). These cancers' growth is often supported by an immunosuppressive (IS) tumor microenvironment (TME), characterized by high expression of tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and regulatory T cells (Tregs), and in the case of GBM, IS microglia. In addition to its cell-autonomous role of promoting survival and proliferation in cancer cells, C/EBPβ is also critical for IS M2 macrophage polarization, while its role in other TME populations is less understood.

ST101 is a peptide antagonist of C/EBPβ that is being evaluated in a Phase 2 clinical study in patients with recurrent and newly diagnosed glioblastoma (NCT04478279). Here we explored the impact of ST101 on IS TME cell populations and on activation of cytotoxic T cells in macrophage co-culture systems. ST101 increased the M1:M2 ratio up to 40-fold in hPBMC-derived macrophages cultured under conditions that typically drive M2 polarization. Further, ST101 promoted M2 repolarization to the M1 cell type, demonstrating that C/EBPβ is required for maintenance of the M2 program. When added to co-cultures of T cells with M2 macrophages, ST101 induced a 3-4-fold increase in intracellular IFNγ staining indicating enhanced T cell activation. Notably, in M1 cultures ST101 further enhanced expression of the immune activity marker CD80. Similar to its effect on M2 repolarization, ST101 induced activation of iPs-derived IS human microglia cells toward an M1-like program. ST101 also impacted IS cell populations in vivo, as demonstrated in an orthotopic TNBC model in which ST101 exposure was associated with a reduction of the TAM fraction, increased intratumoral M1/M2 ratios, reduction of Tregs, increase of the CD8:Treg ratio, and enhanced activity in combination with anti-PD-1. Similar observations were observed in clinical samples, where following ST101 exposure a significant decrease in expression of factors involved in M2 polarization, including CD209, SIGLEC5, and IL-24, and T cell suppression, including FoxP3 and inhibitory KIR proteins, were observed. Gene expression analysis suggests that ST101 induced an increase in the intratumoral CD8:Treg ratio, indicating a shift towards a more immune-active TME. Overall, these results provide novel evidence for the contribution of C/EBPβ to the expression of multiple IS cancer-associated cell types and support the use of ST101 to antagonize C/EBPβ and promote an immune-active TME. Further, these data provide rational for evaluating the clinical impact of ST101 in combinations with immune checkpoint inhibitors.

ST101 Mechanism of Action

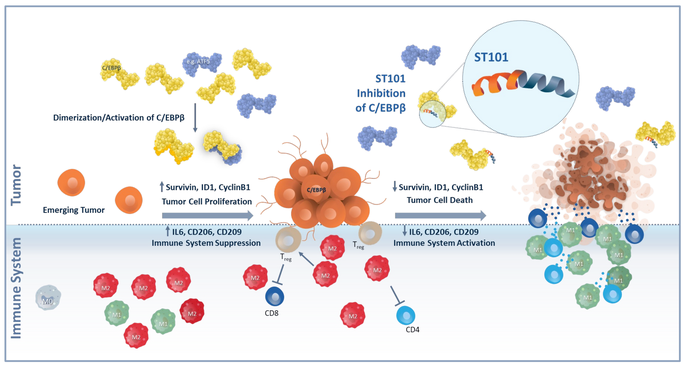

Figure 1: ST101 Anti-tumor Activity is enhanced by Immune Activation. *Top*, C/EBPβ activation in many cancers drives tumor cell proliferation, survival, and inhibits differentiation by regulating expression of targets including Survivin, CyclinB1 and ID transcription factors. ST101 disrupts C/EBPβ dimerization, preventing C/EBPβ mediated transcription and enhancing proteasomal degradation. The result is antagonism of oncogenic gene transactivation leading to selective tumor cell death. *Bottom,* ST101 inhibits a broad C/EBPβ-driven transcriptional program that includes immunosuppressive molecules such as IL-6, CD206 and CD209 (DC-SIGN). In the TME, ST101 targets multiple cell types including the M2-like TAMs and Tregs, resulting in activation of cytotoxic T cells and shifting the M2 TAM program toward the immune active M1-like state.

Results

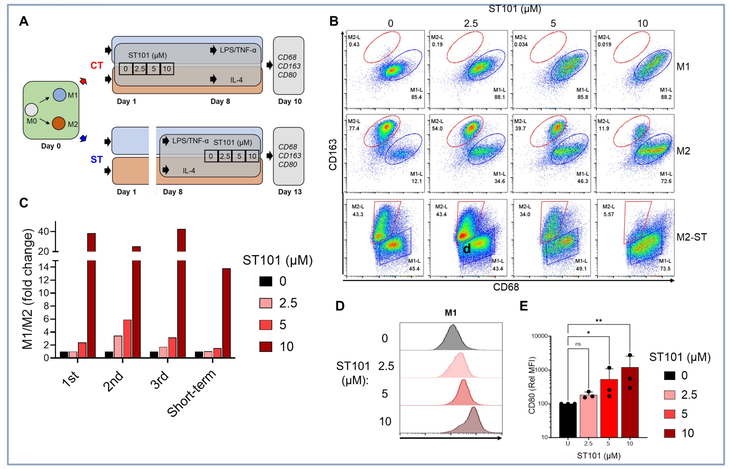

Figure 2: ST101 converts M2-like (M2) macrophages to M1-like (M1). A) M1 and M2 macrophage from hPBMCs were established by continuous treatment (CT) or short-term treatment (ST) protocols. **B**) Flow Cytometry plots at the indicated ST101 concentrations of M1-like ($CD68^{high}CD163^{low}$, blue shape) and M2-like ($CD68^{low}CD163^{high}$, red shape) cultures on Day 10 (top, M1; middle, M2) indicate ST101 prevents M2-like macrophage polarization. Day 13 (bottom, M2-ST) cultures indicate ST101 repolarizes M2-like macrophage to an M1-like phenotype. **C)** ST101 increases M1/M2 ratios in three unrelated donors on Day 10 from CT M2 cultures and Day 13 from ST M2 culture. Data are normalized to untreated. ST101 increases immune activation markers CD80 in M1 cultures on Day 10. **D**) Histogram plots and **E**) Relative CD80 median fluorescence intensity (MFI) at the indicated ST101 concentrations. Error bars represent standard deviations. Statistics represent 1-way ANOVA (n=3/group; *p<0.05; **p<0.01; ns, not significant).

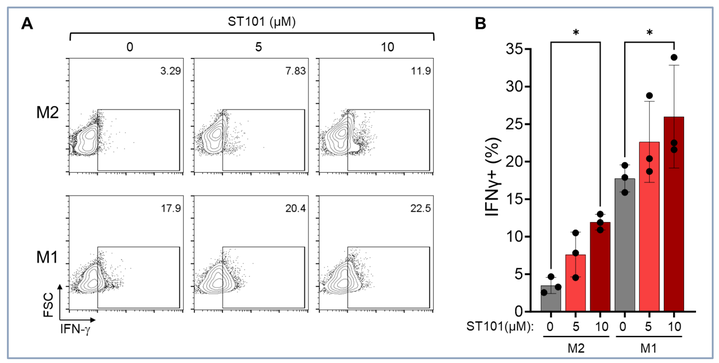

Figure 3: ST101-mediated M2-M1 conversion leads to T-cell activation. A) Representative flow-cytometry plots for T cell intracellular IFNγ staining in the indicated conditions (top, M2; bottom, M1; from left to right 0, 5 and 10 μM ST101). Cell frequency is shown in the top right corner**. B**) ST101 increases IFNγ frequency in M1 and M2 cultures at the indicated concentration. Error bars represent standard deviations. Statistics represent 1-way ANOVA (*p<0.05; n=3/group).

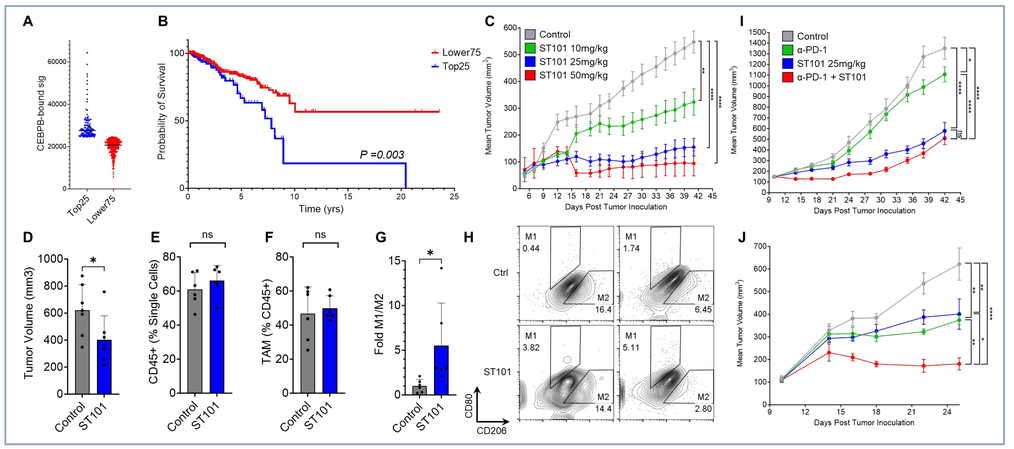

Figure 4. ST101 promotes M2-to-M1 repolarization in vivo and enhances anti-PD-1 activity. A) Distribution and (**B**) survival of TCGA HR-Negative Breast Cancer Samples according to a CEBPB-bound signature in Top Quartile (Top25, blue, n=124) vs. Lower Quartiles (Lower75, red, n=370). Statistics, Log-rank test. **C**) Tumor volumes of female Balb/c mice harboring orthotopic 4T1 TNBC tumors and treated with vehicle (gray) or with ST101 (green, 10mg/kg; blue 25 mg/kg; red, 50mg/kg). Statistics, 1-way ANOVA (**** p<0.0001; **p<0.01; n=7/group). **D**) Tumor volumes of mice treated with Vehicle (gray) or 25mg/kg ST101 (blue) and resected 16 days after start of treatment. Error bars represent standard deviation (*p<0.05, Student t-test, n=7/group). **E,F**) No change in fraction of CD45+ cells of total (E) or TAMs of CD45+ cells (**F**) in tumors treated with vehicle or ST101 (ns, not significant n=6/group). **G**) ST101 increases M1/M2 ratio of TAMs from 4T1 tumors. Data are normalized to control (*p<0.05, Student t-test, n=6/group). **H**) Flow cytometry plots of M1 ($CD80^{high}CD206^{low}$) and M2 ($CD80^{low}CD206^{high}$) TAMs from two control and ST101-treated tumors. **I,J)** Tumor volumes of Balb/c mice harboring 4T1 tumors treated with **I**) vehicle (gray) or with ST101 (25mg/kg, blue), anti-PD-1 (12.5 mg/kg, green) or combination (red). Statistics indicate 1-way ANOVA (**** p<0.001; ** p<0.01; * p<0.05; ns, not significant; n=10/group) for volumes at Day 42; or **J)** vehicle (gray) or with subpharmacologic ST101 (10mg/kg, blue), anti-PD-1 (12.5 mg/kg, green) or combination (red). Statistics, 1-way ANOVA (**** p<0.001; ** p<0.01; * p<0.05; n=7/group) for volumes at Day 25.

Clinical Data

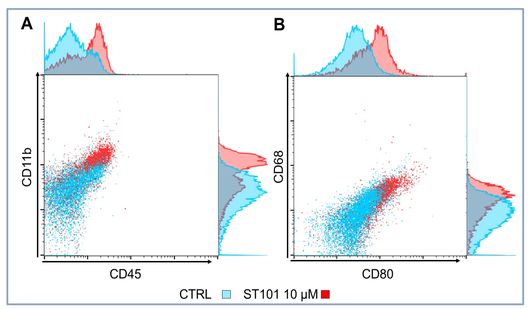

Figure 5: ST101 promotes M1-like markers in iPS-derived microglia. A) Flow cytometry plots for microglia cultures left untreated (Ctrl, cyan) or treated with 10 μM ST101 (red) and stained for the indicated markers. ST101 induces a shift toward macrophage identity ($CD11b^{high}$ $CD45^{high}$). **B**) CD80 and CD68 induction by ST101 exposure indicate polarization toward an M1-like phenotype. Experiments are representative of three replicates.

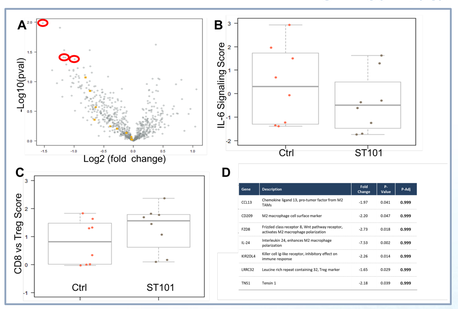

Figure 6. Clinical data indicates ST101 promotes an immune active TME. A) Differential gene expression profile (Nanostring) in 12 matched patient biopsy samples after receiving 4 mg/kg ST101 or greater. Yellow, IL-6 signaling genes; Red circle, M2 macrophage signaling genes. **B)** ST101 exposure results in a decreased IL-6 signaling score. **C**) Gene expression profiling indicates an increased CD8+:Treg ratio in tumors following ST101 exposure. **D**) Statistically significant differentially expressed genes cluster in immunosuppressive pathways.

Conclusions

- ST101 polarizes hPBMC-derived macrophages to the M1 identity and repolarizes established M2 macrophages to the M1 program. ST101 rescues CD8 T cell activation in M2/T cell mixed cultures as measured by IFNγ production. ST101 enhances M1 markers of immune activation and T cell engagement, and augments T cell activation in M1/T culture.
- ST101 enhances the activity of anti-PD-1 treatment in the syngeneic immunocompetent 4T1 TNBC model by increasing M1/M2 ratio in the TAM population.
- Gene expression analysis of clinical biopsies indicates that ST101 modulates the TME by suppressing genes required for M2 macrophage polarization, culminating in an enhanced CD8/Treg ratio.
- These data support a paradigm in which ST101 shifts the TME by inhibiting the M2 program, increasing the M1/M2 ratio in vivo and activating cytotoxic T cells in a previously immune-suppressive environment.
- ST101 represents a novel approach to enhance macrophage-driven anti-tumor activity and suggest its utility for combination strategies in cancers with poor response to immune checkpoint inhibition.

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