ST101, a peptide antagonist of novel I/O target CEBPβ, reprograms MDSC polarization and promotes an immunoactive tumor microenvironment

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Abstract LB236

Introduction & Abstract

ST101, a first-in-class antagonist of C/EBPβ, is currently being evaluated in the Phase 2 portion of an ongoing Phase 1-2 clinical study in patients with advanced unresectable and metastatic solid tumors (NCT04478279). ST101-101 is an open-label, Phase 1-2 dose-finding study designed to determine the safety, tolerability, PK, PD, and proof-of-concept efficacy of ST101 in patients with advanced solid tumors. ST101 has demonstrated clinical proof-of-concept with a mRANO-confirmed partial response in a patient with recurrent GBM, a durable RECIST 1.1-confirmed partial response (PR) in a patient with cutaneous melanoma and evidence of long-lasting stable disease in several additional patients.

CCAAT/Enhancer Binding Protein β (C/EBPβ) is a basic leucine zipper (bZIP) transcription factor that causes aberrant gene expression in many cancers. Upregulated or overactivated C/EBPβ drives oncogenesis by promoting tumor survival and proliferation and is a critical regulator of the immunosuppressive tumor microenvironment (TME). Specifically, C/EBPβ regulates macrophage differentiation, activating a transcriptional program driving macrophage polarization toward immunosuppressive M2-type myeloid-derived suppressor cells (MDSCs). Consistently, activation of C/EBPβ correlates with poor prognosis in several types of human cancer. Thus, targeting C/EBPβ to reprogram tumor-associated macrophages (TAMs) from the M2 toward the immune-promoting M1 phenotype represents an attractive strategy to enhance antitumor immunity. ST101 is a novel peptide antagonist of C/EBPβ dimerization that inhibits C/EBPβ-dependent gene expression. Here we evaluated the impact of ST101 on macrophage differentiation, cytotoxic T-cell activation, and in vivo anti-tumor activity. Primary human macrophages cultured from Peripheral Blood Mononuclear Cells (hPBMCs) were activated toward the M1 or M2 phenotype by LPS and IFNγ or IL-4, respectively. ST101 exposure dose-dependently suppressed expression of M2 markers (CD163, CD206) while inducing M1 markers (CD80, CD86) by flow cytometry and quantitative PCR, resulting in a 40-fold increase in the M1/M2 ratio without substantial impact on cell viability. Next, in co-cultures of T cells with M2 macrophage, ST101 exposure resulted in a 4-fold increase in T-cell activation compared to control M2/T cell co-cultures, as measured by intracellular IFN-γ staining. Importantly, ST101 did not suppress proliferation or activity of T cells cultured alone. Finally, in an orthotopic TNBC model in vivo, ST101 in combination with anti-PD-1 treatment enhanced anti-tumor activity compared to either single agent alone. The observed increase in tumor growth inhibition was accompanied by a reduced TAM fraction and increased intratumoral and peripheral M1/M2 ratios. ST101 is being evaluated in a Phase 1-2 clinical study in patients with advanced unresectable and metastatic solid tumors (NCT04478279). Initial gene expression analysis performed on 8 paired patient biopsies (prior to ST101 exposure and within 24 hrs of ST101 administration during cycle 2 of therapy) collected during dose escalation (4 mg/kg ST101 or greater) indicates a significant decrease in expression of multiple factors involved in M2 polarization, including CD209, SIGLEC5 and IL-24, and T cell suppression, including FoxP3 and inhibitory KIR proteins. The result is a decrease in intratumoral regulatory T cell (Treg) vs. TIL ratio, indicating a shift towards a more immunoactive TME. Overall, these results support a novel, macrophage-driven mechanism of action for ST101 as anticancer agent and suggest the exploration of ST101 in immune-oncology therapeutic strategies.

ST101 Mechanism of Action

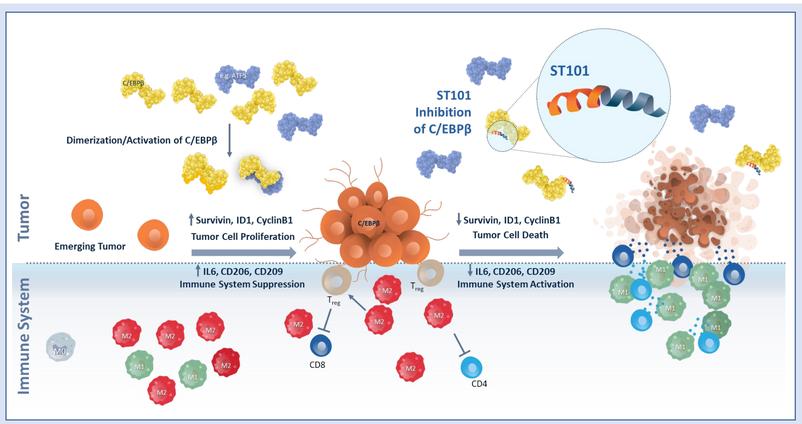

Figure 1: ST101 Anti-tumor Activity is enhanced by Immune Activation. *Top*, C/EBPβ overactivation in many cancer drives tumor cell proliferation, survival and inhibits differentiation by regulating multiple classes of targets including Survivin, CyclinB1 and the ID family of transcription factors. ST101 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 Tumor Associated Macrophages (TAMs) and regulatory T cells (Tregs), resulting in activation of cytotoxic T cells and shifting the M2 TAM program toward the immune active M1-like state.

Results

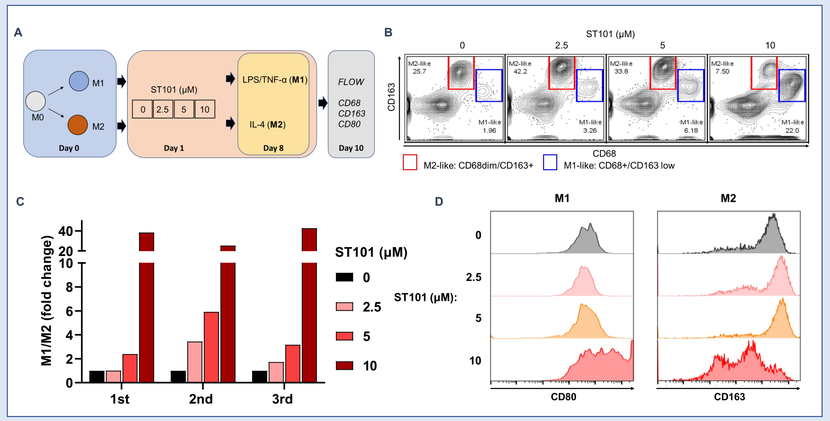

Figure 2: ST101 converts M2-like macrophages to M1-like. **A**) Experimental outline of M1 and M2 establishment from hPBMCs. **B**) Flow Cytometry plots at the indicated ST101 concentrations of M1 and M2 populations in M2 conditions. M2-like population (red) is gated as CD68dimCD163+; M1-like population (blue) is CD68+CD163low. **C**) HPBMCs M1/M2 population ratios for three donors at the in indicated ST101 concentrations. **C**) Histogram plots for CD80 in M1 conditions and CD163 in M2 conditions at the indicated concentrations of ST101.

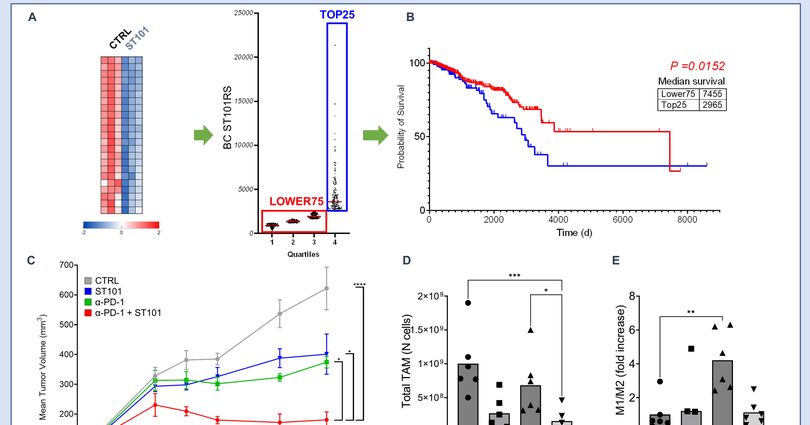

Figure 4. ST101 promotes response to anti-PD-1 therapy in vivo by multiple mechanisms. A) A 23-gene signature (1.5 fold-change, p<0.05,q-value) for ST101 response was identified in the MCF-7 BC line (untreated or treated with 5 μM ST101). B) HR-negative BC TCGA samples (n=420) were stratified based on their ST101 signature and their survival was compared (P-value represent Log-Rank statistics). **C**) Mean tumor volumes for the indicated treatment cohorts in the TNBC 4T1D orthotopic model (n=7/group,****p<0.0001; *p<0.05;1-way ANOVA) indicate enhanced anti-tumor activity in animals receiving anti-PD1 in combination with ST101. Error bars represent SEM. **D**) Total TAM ($CD11b^{low}$;$MHC\text{-}II^{+}$) were calculated by taking in account the total tumor cellularity. **E**) ST101 cohorts show a 4-fold increase in the M1/M2 ratio in the 4T1D-associated TAMs compared to controls (M1 gated $CD80^{high}$ $CD206^{low}$: M2, $CD80^{low}$ $CD206^{high}$ (*,p<0.05; **,p<0.01; ***,p<0.001; 1-way ANOVA).

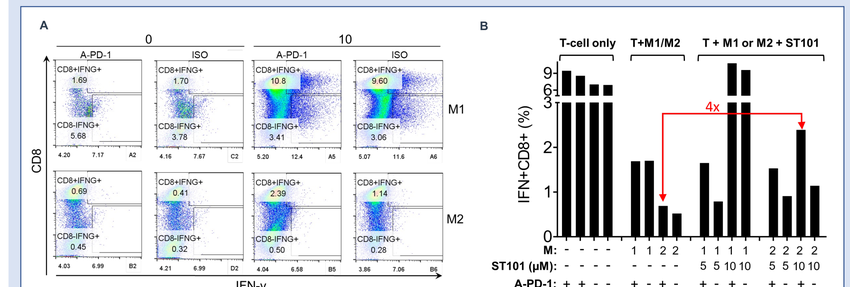

Figure 3: ST101 restores macrophage-induced CD8+ T cell activation in M2 suppressive conditions. A) CD8+ T cells were co-cultured for 3 days with M1 or M2 and left untreated (0) or treated with 10 μM ST101 (10) and anti-PD-1 (A-PD-1, BioXcell #SIM0003, 20 ng/mL) or IgG4 Isotype Control (ISO). Flow cytometry plots for CD8 and intracellular IFN-γ for the indicated conditions are shown. **B**) Frequency of IFN-γ producing CD8+ cells in the indicated conditions. T-cell only conditions were assessed in duplicate. The red arrow indicates a ~4x increase in IFN-γ producing T-cells in 10 μM ST101-treate cells compared untreated in M2 conditions.

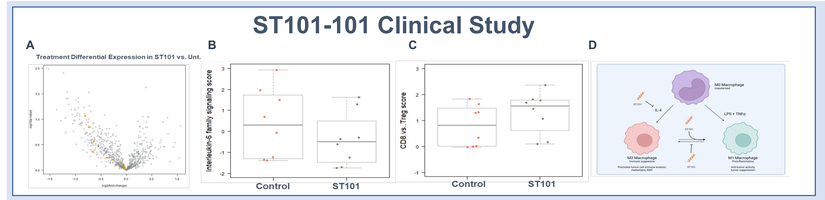

Figure 5. ST101 enhances the tumor immune microenvironment. Nanostring gene expression analysis was performed on patient biopsy samples collected prior to and post ST101 exposure (cycle 2). A) Differential gene expression profile in 12 matched patient biopsy samples after receiving 4 mg/kg ST101 or greater. Yellow highlighted genes belong to IL-6 signaling family. B) ST101 exposure results in a decreased IL-6 signaling score compared to tumors prior to ST101 exposure. C) Gene expression profiling indicates an increase in the CD8+ T cell to Treg ratio in patient tumors following ST101 exposure. D) Schematic of the impact of ST101 on macrophage polarization. In the presence of ST101, M2 macrophage polarization is inhibited, driving an increase in the M1/M2 ratio.

Conclusions

- hPBMC-derived M2 macrophages undergo a dose-dependent shift to the M1 identity following ST101 exposure in 3 independent donors, characterized by increased M1 markers CD80 and CD86 and decreased M2 marker CD163.
- ST101 rescues CD8 T-cell activation in macrophage/T cell mixed cultures as measured by IFN-γ production.
- Subtherapeutic ST101 enhances the activity of anti-PD-1 antibody in the syngeneic immunocompetent 4T1 TNBC model by reducing the total TAM and inducing a repolarization to the M1 identity in the TAM population.
- ST101 modulates the tumor immune microenvironment in patient biopsies by suppressing genes required for M2 macrophage polarization, culminating in an enhanced CD8/Treg ratio.
- These data support a model in which ST101 promotes a shift in the tumor microenvironment by inhibiting the M2 program in unstimulated precursor macrophages in vitro and in vivo, resulting in reduction of TAMs and an increase in the M1/M2 ratio in vivo, and activation of cytotoxic T cells in a previously immune-suppressive environment.