Anti-Tumor Activity of a TAM Kinase-targeting Compound in CT-26 Syngeneic Mouse Model

Abstract No. 4196
AACR Meeting 2017

Shenshen Lai, Rick Li, Paromita Raha, Yuxiang Hu, Zaihui Zhang, Jun Yan, Hong Zhang
SignalChem Lifesciences Corporation, Richmond, BC, Canada

INTRODUCTION

TAM family receptor tyrosine kinases, Tyro3, Axl and Mer, regulate a vast number of biological processes including cell proliferation, migration, the Epithelial-Mesenchymal Transition (EMT), and immune homeostasis. The expression levels of TAM kinases are found to correlate positively with poor prognostic outcome, metastasis and drug resistance in various types of human cancers. Recently, the TAM kinases have emerged as dual oncological therapeutic targets, owing to their tumor pro-survival and immunosuppressive activities. Mer mediates apoptotic cell removal by macrophages by suppressing pro-inflammatory M1 activity and promoting anti-inflammatory cytokine production by M2. Axl transcription level is reported to be correlated to resistance to checkpoint immunotherapy. Blocking the Axl/Mer-mediated immunosuppressive pathway can significantly enhance the therapeutic efficacy of immune checkpoint inhibitors. In our efforts to develop small-molecule inhibitors targeting the TAM kinases, we identified SLC-391 as one of the most promising preclinical candidates with potent activity towards both Axl and Mer. Studies in CT26 syngeneic mouse model revealed the activity of SLC-391 in reversing the immunosuppressive tumor microenvironment, and the potential of this compound as combination therapeutic agent with checkpoint inhibitor PD-1 antibody.

POTENCY AND SELECTIVITY

Table 1. The IC_{50} values of SLC-391 against the TAM family kinases in radiometric activity-based assays.

Code	MW	cLogP	AXL	TYRO3	MER
			IC_{50} (nM)		
SLC-391	<400	<1	9.6	42.3	44.0

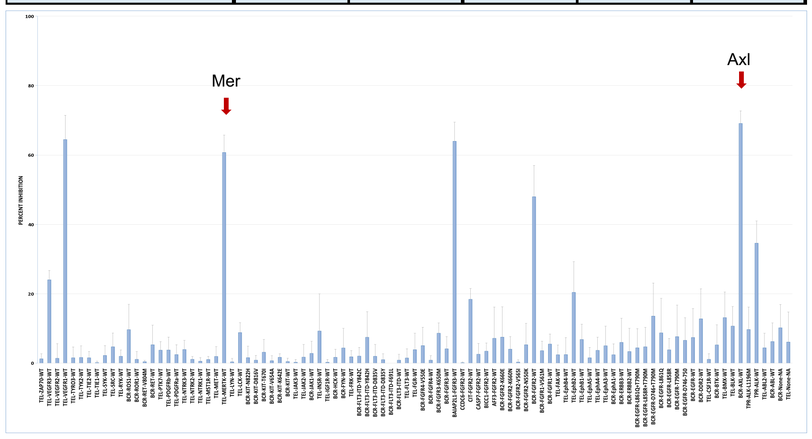

Figure 1. Selectivity profile of SLC-391 in Ba/F3 cell-based assay. The selectivity of SLC-391 was evaluated in a panel of 93 unique human tyrosine kinases individually expressed in a common lymphoid cell line (mouse Ba/F3 cells). Each cell line is dependent upon activity of the recombinant kinase for survival; inhibition of kinase activity leads to cell death, which is monitored via ATP concentration using CellTiter-Glo (Promega). At 100 nM, SLC-391 showed strong inhibition towards Axl, Mer, as well as VEGFR and FGFR.

DIRECT TUMOR GROWTH INHIBITION

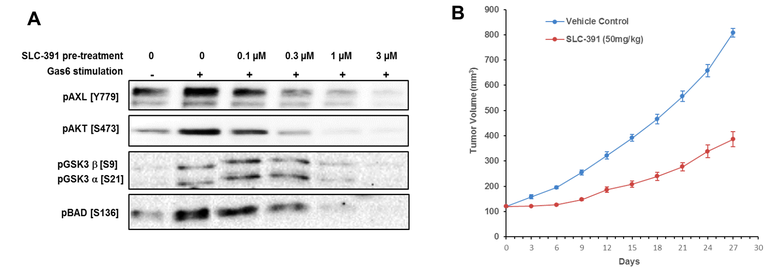

Figure 2. Direct tumor growth inhibition of SLC-391 in A549 non-small cell lung cancer cells. (A) SLC-391 inhibits Axl phosphorylation and downstream signaling in a dose-responsive manner. The IC_{50} of SLC-391 in inhibiting A549 cell proliferation *in vitro* was determined to be 0.7 μM in ^3H thymidine incorporation assay. (B) In an A549 xenograft model, SLC-391 demonstrated 52% tumor growth inhibition when compared to that of the vehicle control group on Day 28 at a dose of 50 mg/kg, b.i.d.

PHARMACODYNAMICS IN A CT26 MODEL

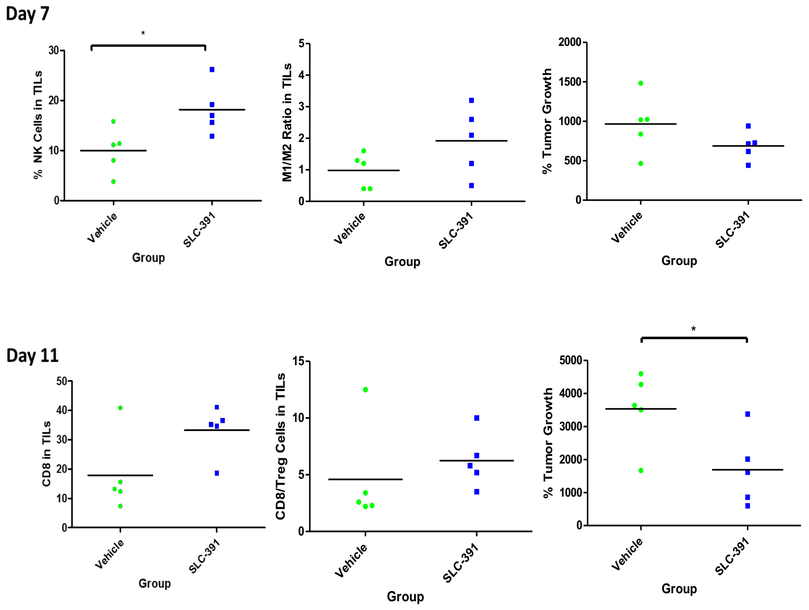

Figure 3. Pharmacodynamics study of SLC-391 in a CT26 syngeneic mouse model. Tumor infiltrating lymphocytes were isolated and analyzed 7 or 11 days after initial dosing with SLC-391 (50 mg/kg, q.d.). Increased number of NK cells and ratio of M1/M2-polarized macrophages were detected on Day 7 in the treatment group relative to the vehicle control, followed by the rise of CD8+ T cells and CD8+ T/Treg ratio on Day 11. This is indicative of sequential engagement and stimulation of pro-inflammatory innate immune response and adaptive immune response. Significant tumor growth inhibition was also observed by the end of the study.

COMBINATION WITH ANTI-PD-1

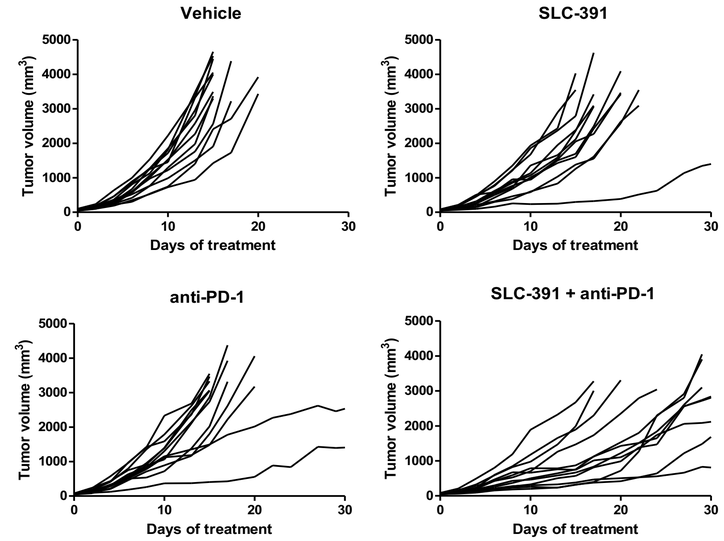

Figure 4. Efficacy study of SLC-391 in combination with an anti-PD-1 antibody in a CT26 syngeneic mouse model. Individual tumor growth curved were plotted for each treatment group.

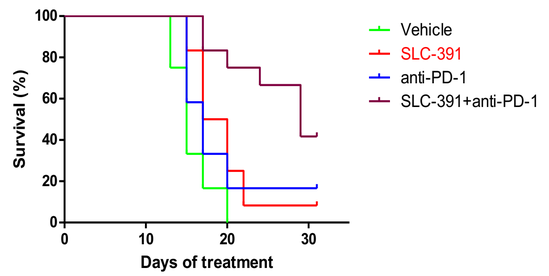

Figure 5. Overall survival of each group over 32 days of treatment. A synergistic inhibition effect on tumor growth was observed when the anti-PD-1 insensitive CT26 model was treated with a combination of SLC-391 and an anti-PD-1 antibody.

SUMMARY

- **As a TAM-kinase targeting compound, SLC-391 is potent against Axl and Mer in biochemical and Ba/F3 cell-based assays.**
- **SLC-391 directly inhibits A549 cell proliferation and tumor growth in a xenograft mouse model through down-regulation of Axl signaling pathways.**
- **The anti-tumor activity of SLC-391 is mediated by directly inhibiting tumor growth as well as reversing the immunosuppressive tumor microenvironment.**
- **SLC-391 demonstrates a synergistic effect in tumor growth inhibition and overall survival in combination with an anti-PD-1 antibody in a CT26 syngeneic mouse model.**