Activity of the TAM kinase-targeting compound, SLC-391, is mediated by the engagement of the immune system in CT-26 syngeneic mouse model

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INTRODUCTION

Over-expression of TAM family receptor tyrosine kinases, including Tyro3, Axl and Mer, has been found to correlate positively with poor prognostic outcome, metastasis and drug resistance in numerous types of human cancers. Aside from the role in cell growth and survival, TAM kinases have long been recognized for their immunosuppressive activity as well. Axl is reported to contribute to the resistance of checkpoint immunotherapy. Mer mediates the polarization of macrophages by promoting anti-inflammatory cytokine production in tumor microenvironment. Blocking the Axl/Mer-mediated immunosuppressive pathway is expected to significantly enhance the therapeutic efficacy of immune checkpoint inhibitors. SLC-391, one of the several identified TAM-targeting small-molecule inhibitors, displays a relatively strong activity against Axl, as evidenced by the reductions of phosphotransferase activity in radiometric biochemical assay and the level of Axl Y779 phosphorylation in the cell-based assay. While the compound only demonstrated minor impact on the proliferation of CT26 murine colon carcinoma cells *in vitro*, strong tumor growth inhibition was observed in CT26 syngeneic mouse model, which is mediated through sequential engagement and stimulation of pro-inflammatory innate immune response and adaptive immune response. Furthermore, a combination of SLC-391 with an anti-PD-1 antibody significantly prolonged the survival in this CT26 syngeneic mouse model.

ACTIVITY OF SLC-391 IN CT26 CELLS

Table 1. Potency of SLC-391 in activity-based biochemical assays and ^{3}H-thymidine incorporation cell-based assays.

	Biochemical Assay IC_{50} (nM)			Cell Proliferation IC_{50} (μM)		
	AXL	TYRO3	MER	A549	4T1	CT26
SLC-391	9.6	42.3	44.0	0.63	1.05	~10

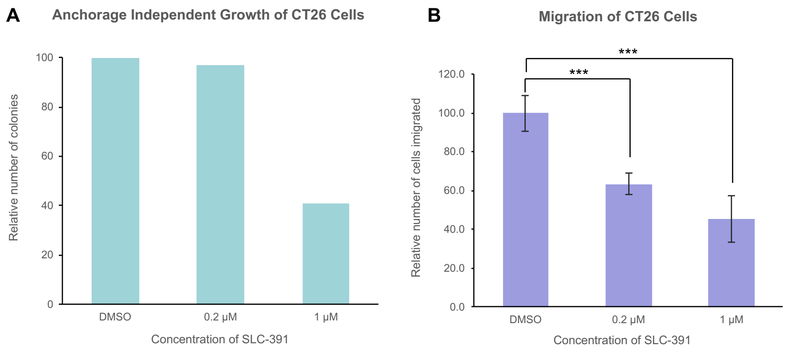

Figure 1. SLC-391 inhibits the anchorage independent growth and migration of CT26 cells *in vitro*. (A) Anchorage independent growth of CT26 cells in presence or absence of SLC-391. Number of colonies was reduced by more than 50% with 1 μM of SLC-391 over 7 days of incubation in soft agar. (B) Migration of CT26 cells from transwell permeable supports over 24 hours. Low concentration of SLC-391 was able to suppress the migration of cells significantly ($p<0.0001$).

DIRECT INHIBITION OF PHOSPHO-AXL

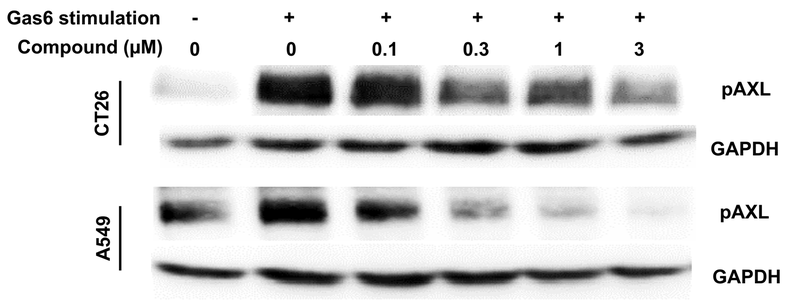

Figure 2. Direct inhibition of AXL activation by SLC-391 in CT26 and A549 cells. Cells were starved overnight, pretreat with various concentrations of SLC-391 for 1 hour before stimulated by 5 nM recombinant Gas6 for 15 minutes. SLC-391 inhibited the phosphorylation of activatory Y779 of Axl in a dose-responsive manner in both cell lines.

PHARMACODYNAMICS IN A CT26 MODEL

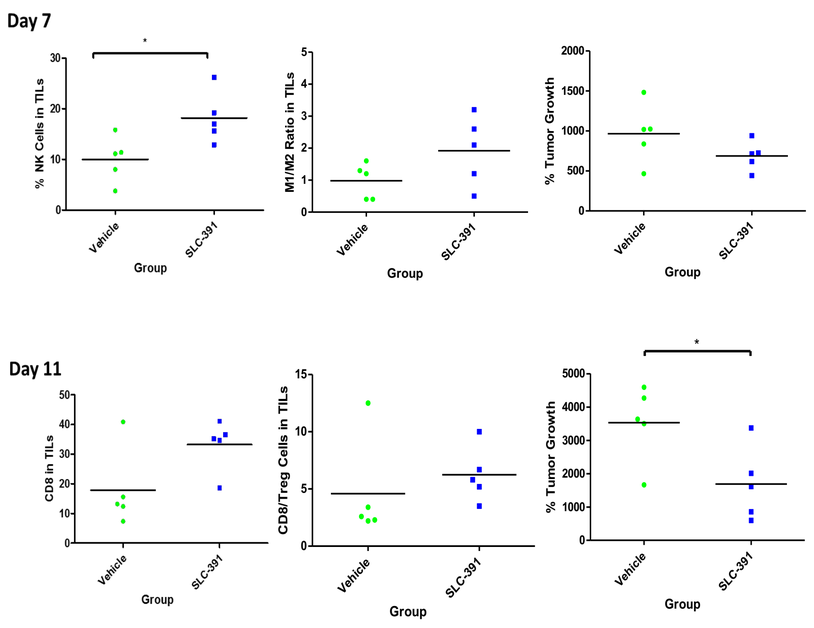

Figure 3. Pharmacodynamics study of SLC-391 in a CT26 syngeneic mouse model. Tumor infiltrating lymphocytes were isolated and analyzed 7 or 11 days after initial dosing with SLC-391 (50 mg/kg, q.d.). Increased number of NK cells and ratio of M1/M2-polarized macrophages were detected on Day 7 in the treatment 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 ($p<0.05$).

COMBINATION WITH ANTI-PD-1

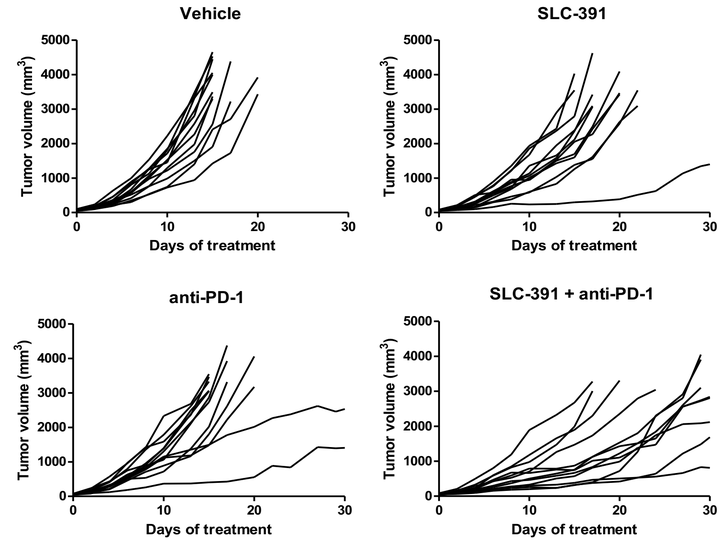

Figure 4. Efficacy study of SLC-391 in combination with an anti-PD-1 antibody in a CT26 syngeneic mouse model. Individual tumor growth curve were plotted for each treatment group.

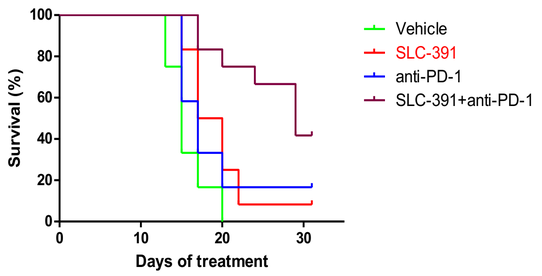

Figure 5. Overall survival of each group over 32 days of treatment. A synergistic inhibition effect on tumor growth was observed when the anti-PD-1 insensitive CT26 model was treated with a combination of SLC-391 and an anti-PD-1 antibody.

SUMMARY

- **SLC-391 is potent against Axl in biochemical activity-based assay and cell-based assays.**
- ***In vitro*, SLC-391 inhibits anchorage independent growth and migration, but not anchorage dependent proliferation of CT26 murine colon carcinoma cells.**
- **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.**
- **The anti-tumor activity of SLC-391 is at least in part mediated by reversing the immunosuppressive tumor microenvironment in CT26 colon carcinoma model.**