AXL Inhibitors Promote Anti-Tumor Immunity through Modulation of Macrophage Polarization

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INTRODUCTION

Polarization of tumor-associated macrophages (TAMs) to classic pro-inflammatory M1 or alternatively activated M2 types plays an important role in establishing tumor microenvironment and determining therapeutic responses. M2 macrophages contribute to tumor progression by producing anti-inflammatory cytokines and suppressing anti-tumor immunity. AXL receptor tyrosine kinase has recently emerged as a dual therapeutic target in oncology, due to its function in tumor growth, survival and metastasis, as well as immunosuppressive activity. SLC-391, a selective small molecule inhibitor for AXL, displays high potency against numerous cancer cell lines through inhibition of AXL/P38/AKT-dependent cell proliferation and survival in vitro. Additionally, this compound was also found to alter the cytokine profile expressed in THP1-derived M2 macrophages. In a co-culture system consisting of THP1-derived M2 and A549 non-small cell lung cancer cells, SLC-391 targeted AXL activity and suppressed epithelial mesenchymal transition (EMT). This observation is supported by increased ratio of M1/M2-polarized TAMs and inhibition of tumor growth from mice treated with SLC-391 in a CT-26 murine colon carcinoma syngeneic model, considering CT26 cells are not sensitive to SLC-391 in cell-based proliferation assay. In summary, in addition to direct inhibition of tumor cells, SLC-391 also appears to promote anti-tumor immunity through modulation of TAMs.

Table 1. Potency of two AXL inhibitors in activity-based biochemical assays and 4'-hydroxymidine incorporation cell-based assays.

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<th>Biochemical Assay IC50(nM)</th>
<th>Cell Proliferation IC50 (µM)</th>
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<td></td>
<td>AXL</td>
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*SLC-531 is a reference compound against AXL.

Figure 1. Differentiation of THP1-derived macrophages. (A) THP1 monocytes were differentiated into macrophages by PMA, followed by stimulation with LPS and IFNγ, or IL-4 and IL-13 into M1 or M2 subtypes, respectively. (B) West blot confirmed upregulation of AXL and differential signaling pathways in M1 and M2 cells.

Figure 2. Modulation of cytokine profile in M1 and M2 macrophages by AXL inhibitors. Levels of pro-inflammatory cytokine CXCL-10/IP-10 (A) and anti-inflammatory cytokine IL-10 (B) were measured by ELISA. A549 cells induced M2 phenotype. AXL inhibitors promoted production of CXCL-10 and suppressed production of IL-10 in M2 macrophages and M2-A549 co-culture. Final concentration was 1 µM for both compounds. (n ≥ 5, *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001).

Figure 3. Gas6 is produced by M2 macrophages and induces epithelial mesenchymal transition (EMT) of A549 tumor cells. (A) Level of Gas6 increased significantly in M2 comparing to M1 96 hours after induction, which was delayed by SLC-391. (B) Gas6 level was further elevated in A549-M2 co-culture. (C) and (D) EMT of A549 cells can be induced by Gas6 treatment and M2 co-culture, featured by increased vimentin and decreased E-cadherin 24 hours after initial treatment or co-culture. This process was suppressed by SLC-391, but not by SLC-531.

Figure 4. Pharmacodynamics study of SLC-391 in a CT26 syngeneic mouse model. Increased ratio of M1/M2-polarized macrophages (A) and NK cells (B) in tumor infiltrating lymphocytes were observed in animals treated with SLC-391 (50 mg/kg, q.d.) on Day 7. Elevated CD8+ cell level (C) and significant tumor growth inhibition (D) and was also observed on Day 11.

SUMMARY

- SLC-391 directly inhibits AXL/P38/AKT pathway and A549 cell proliferation in vitro.
- AXL compounds inhibit THP1-derived M2 by promoting production of pro-inflammatory cytokine CXCL-10 and suppressing production of anti-inflammatory cytokine IL-10 in vitro.
- Gas6-AXL pathway is actively involved in tumor cell-macrophage interactions and epithelial mesenchymal transition (EMT), which is directly targeted by SLC-391.
- SLC-391 promotes pro-inflammatory M1 macrophages in tumors and leads to overall tumor growth inhibition in vivo.