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Drug Resistance & Modifiers
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AB801 is a Potent and Selective AXL Inhibitor That Demonstrates Significant Anti-Tumor Activity In Combination With Standard of Care Therapeutics

Susan L. Paprcka, Subhasree Sridhar, Armon Goshayeshi, Eugene Park, Suan Liu, Ruben Flores, Lauren Rocha, Dillon Miles, Manjunath Lamani, Sean Cho, Ning Wang, Yihong Guan, Saranya Chandrasekar, Ritu Kushwaha, Salema Jafri, Angelo Kaplan, Enzo Stagnaro, Lisa Seitz, Janine Kline, Ester Fernández-Salas

Arcus Biosciences, Inc.; 3928 Point Eden Way, Hayward, CA 94545, USA

Overview

- The tyrosine kinase receptor AXL is overexpressed in a variety of cancer, stromal, and select immune cells in tumors, and has been implicated in resistance to chemotherapy, targeted therapies, and immunotherapies
- AB801 has been recently described as a highly potent and selective small molecule AXL inhibitor[1]
- This poster describes the preclinical evaluation of AB801 in *in vitro* & *in vivo* models

AXL Signaling Supports Therapeutic Resistance in Tumors

- AXL expression correlates with proliferation, migration/ invasion, and epithelial-mesenchymal transition (EMT) in cancer cells. Overexpression results in drug resistance and attenuated immune responses.[2,3]
- Activation of AXL can occur in a ligand-dependent manner via its ligand, growth arrest specific protein 6 (Gas6)
- AXL can also signal independently of Gas6, via homodimerization or heterodimerization with other receptor tyrosine kinases, leading to activation
- AXL phosphorylation initiates signaling cascades that promote cancer cell proliferation, survival, migration, EMT, and an immunosuppressive microenvironment

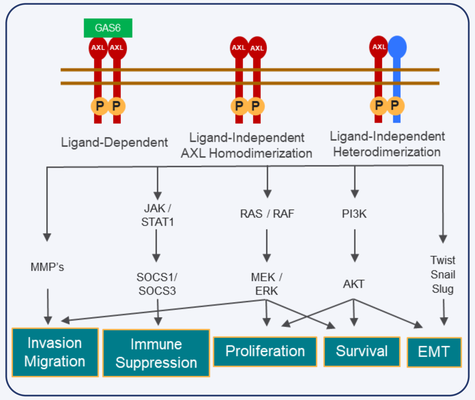

Figure 1. Ligand-dependent AXL signaling is mediated by binding of its ligand Gas6 leading to receptor dimerization and subsequent phosphorylation. Ligand-independent homo- or hetero-dimerization can also occur, resulting in AXL phosphorylation. Phosphorylated AXL signals through various pathways that promote cancer cell survival and proliferation as well as immunosuppression.

High AXL Expression Is Associated With Resistance to TKI Therapies

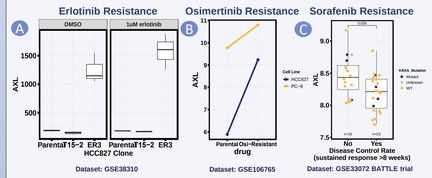

Figure 2. TKI resistance is associated with high expression of AXL. High AXL expression is correlated with lack of response to TKI inhibition *in vitro* to A) Erlotinib and B) Osimertinib as well as lack of clinical response to C) Sorafenib in NSCLC patients.

Osimertinib Drug-Tolerant PC-9 Cells Upregulate AXL Expression

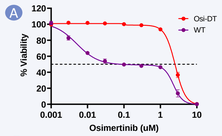

Figure 3. Increased AXL expression is observed in Osimertinib drug-tolerant (Osi-DT) compared to wild type (WT) NSCLC PC-9 cells. EGFR^{mut} PC-9 cells were cultured in the presence of 1 μM Osimertinib for 1.5 months to generate Osi-DT cells. A) Viability was evaluated by Cell Titer Glo after 72 hrs of exposure to Osimertinib B) Increased AXL surface expression in Osi-DT cells evaluated by flow cytometry

AB801 Potently Inhibits AXL Phosphorylation

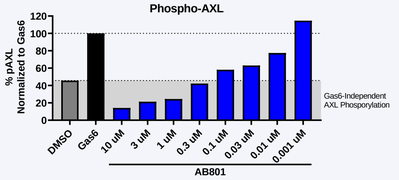

Figure 4. AB801 inhibits both ligand-dependent & ligand-independent AXL phosphorylation in a concentration-dependent manner. Ligand-independent AXL phosphorylation is noted by the grey shaded area at levels of phospho-AXL (pAXL) detected in the DMSO control. Notably, no Gas6 was detected in the media of DMSO treated cells. H1299 NSCLC cells were treated with AB801 for 1 h. Followed by 800 ng/mL hGas6 added for 15 min before cells were collected. Cell lysates were used to run an ELISA (AXL capture antibody & pan-phosphotyrosine detection antibody) using the Meso Scale Discovery (MSD) platform.

AB801 In Combination With Osimertinib Causes Significant Tumor Regression in PC9 EGFR^{mut} NSCLC Xenografts

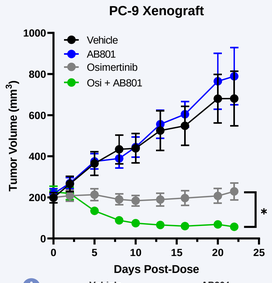

Figure 5. AB801 in combination with EGFR-specific inhibitor osimertinib (osi) results in significant tumor regression of EFGR^{mut} NSCLC PC-9 xenografts vs. single agent osi. Nude mice were injected subcutaneously with 1x10^6 PC-9 cells. Treatments, n=9 per group, started when tumors reached ~200 mm^3. No significant differences in tumor growth were observed with single agent AB801 treatment. osi was dosed orally at 2.5 mg/kg QD and AB801 was dosed orally at 30 mg/kg BID.

Statistical significance was calculated using a mixed-effects model with multiple comparisons. p = 0.0192 for osi vs. osi + AB801. Points represent the mean ± SEM.

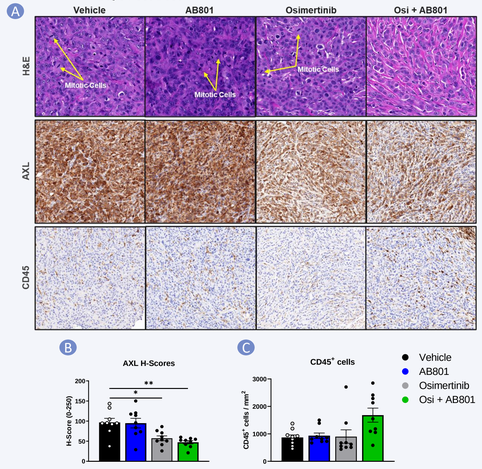

Figure 6. IHC analysis of PC-9 xenograft tumors shows lower tumor cell density, decreases in AXL levels, and a trend in increased infiltration in tumors treated with osimertinib + AB801. A) Representative H&E images (20x magnification) show that single-agent treated tumors have densely-packed tumor nuclei and mitotic cells and less extracellular space compared to those treated with a combination of osi + AB801. Tumors were stained for human AXL and murine CD45 (n=9 per group). B) AXL expression was quantified using HALO-calculated H-scores. Significant reductions in AXL expression were observed in tumors treated with osi alone & in combination with AB801. C) Immune infiltration analysis, number of CD45$^+$ cells per mm^2 of tumor, showed that multiple tumors treated with the osi + AB801 combination displayed greater immune infiltration. Statistical significance was calculated using a mixed-effects model with multiple comparisons. Dots represent individual tumors, bars are the mean, and error bars are ± SEM.

AB801 In Combination With Sunitinib Significantly Reduces Tumor Growth in 786-O ccRCC Xenografts

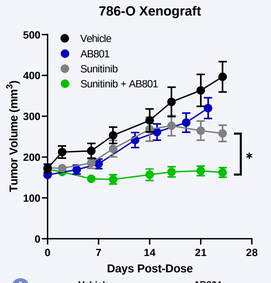

Figure 7. AB801 in combination with multi-TKI sunitinib significantly reduces tumor volume of ccRCC 786-O xenograft tumors compared to single agent sunitinib. Nude mice were injected subcutaneously with 1x10^6 786-O cells. Treatments, n=10 per group, were initiated when tumors reached ~175 mm^3. No significant differences were observed with AB801 single agent. Sunitinib was dosed orally at 40 mg/kg QD and AB801 was dosed orally at 30 mg/kg QD.

Statistical significance was calculated using a mixed-effects model with multiple comparisons. p = 0.0116 for sunitinib vs. sunitinib + AB801. Points represent the mean ± SEM.

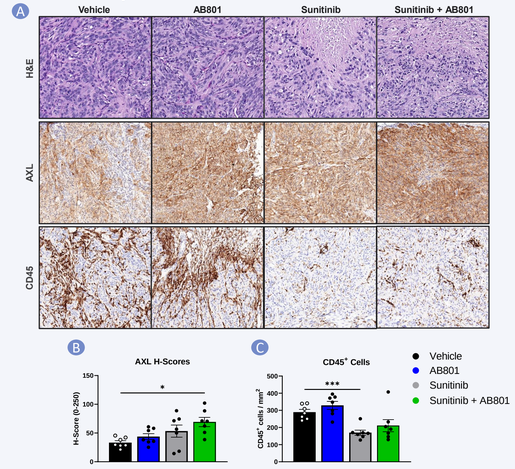

Figure 8. IHC analysis of 786-O xenograft tumors shows increased necrosis and greater AXL levels in tumors treated with sunitinib + AB801. A) Representative H&E images (20x magnification) show that tumors from mice treated with sunitinib alone or sunitinib + AB801 have increased areas of necrosis compared to those treated with Vehicle or AB801 alone. Tumors were stained for human AXL and CD45 (n=7 per group). B) AXL expression was quantified using HALO-calculated H-scores. Significant increases in AXL expression were observed in tumors treated with sunitinib + AB801 combination. Multi-TKI's, like sunitinib, increase AXL expression that is further stabilized by AXL inhibition, resulting in increased AXL levels. C) Immune infiltration, determined by the number of CD45$^+$ cells per mm^2 of tumor, was significantly decreased in tumors treated with sunitinib, likely due to the promiscuous activity of sunitinib leading to immune cell death. AB801 in combination with sunitinib did not show significant differences. Statistical significance was calculated using a mixed-effects model with multiple comparisons. Dots represent individual tumors, bars are the mean, and error bars are ± SEM.

AB801 Treatment Sensitizes Cancer Cells to Chemotherapy & Immunotherapy

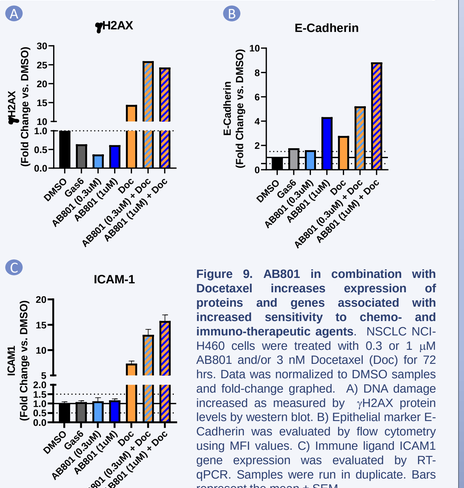

Figure 9. AB801 in combination with Docetaxel increases expression of proteins and genes associated with increased sensitivity to chemo- and immuno-therapeutic agents. NSCLC NCI-H460 cells were treated with 0.3 or 1 μM AB801 and/or 3 nM Docetaxel (Doc) for 72 hrs. Data was normalized to DMSO samples and fold-change graphed. A) DNA damage increased as measured by γH2AX protein levels by western blot. B) Epithelial marker E-Cadherin was evaluated by flow cytometry using MFI values. C) Immune ligand ICAM1 gene expression was evaluated by RT-qPCR. Samples were run in duplicate. Bars represent the mean ± SEM.

Summary

- Resistance to TKI therapies is associated with high AXL expression in vitro and in patients
- AB801 inhibits both Gas6-dependent and independent AXL phosphorylation in a concentration-dependent manner
- *In vivo*, AB801 in combination with TKI therapies significantly reduces tumor volume, cell mitosis, and increases necrosis
- AB801 was well-tolerated as a single agent & in combination with TKI therapies *in vivo*
- AB801 in combination with docetaxel upregulates proteins and genes that will increase responses to chemotherapy and immunotherapy

References

1) Miles, D. *et al. EORTC-NCI-AACR* ***2022*** Abstract 290
2) Zhu, C. *et al. Mol. Cancer* **2019**, 18:153.
3) Son, H-Y. *et al. Front. Oncol.* **2021**, 11:756225

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Susan L. Paprcka spaprcka@arcusbio.com
Ester Fernández-Salas esalas@arcusbio.com