2023 AACR MEETING
Experimental and Molecular Therapeutics
Novel Antitumor Agents 2
Abstract 518
Board Number 18

AB801 is a Highly Potent and Selective AXL Kinase Inhibitor that Demonstrates Significant Anti-Tumor Activity

Susan L. Paprcka, Armon Goshayeshi, Suan Liu, Ruben Flores, Lauren Rocha, Jhansi L. Leslie, Dillon H. Miles, Corinne N. Foley, Shiwei Qu, Manjunath Lamani, Srinivas Paladugu, Nidhi Tibrewal, Ada Chen, Joseph Kulusich, Stefan Garrido-Shaqfeh, Patricia Fabila, Salema Jafri, Anuja Devarajan, Samantha Schwager, Eugene Park, Angelo Kaplan, Ester Fernández-Salas

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

OVERVIEW

- The tyrosine kinase receptor AXL is highly expressed in a variety of cancer, stromal, and select immune cells in tumors, and has been implicated in resistance to chemotherapy, targeted therapies, and immunotherapies
- Our team has discovered and characterized AB801, a novel 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

- High expression of AXL 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

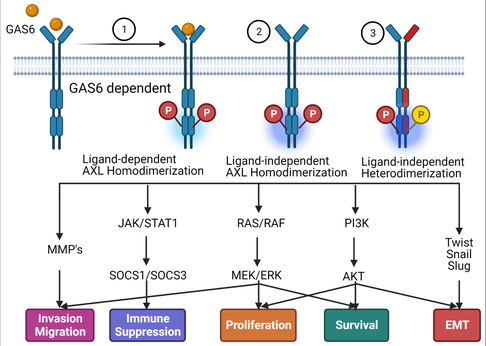


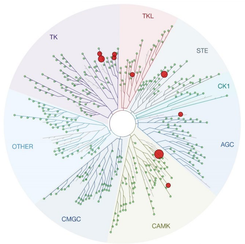
Figure 1. Ligand-dependent AXL signaling is mediated by binding of its ligand GAS6 leading to receptor dimerization and subsequent phosphorylation. Ligand-independent homo- or hetero-dimerization can also occur, resulting in AXL phosphorylation. Phosphorylated AXL signals through various pathways that promote cancer cell survival and proliferation as well as immunosuppression. Schematic created with BioRender.com.

AB801 Medicinal Chemistry

	Assay	AB801	Bemcentinib[4] (BGB324, R428)
biochemical	hAXL HTRF IC_{50} (700 μM ATP)	1.75 ± 1.3 nM (n=5)	10.5 ± 1.78 nM (n=4)
	mAXL HTRF IC_{50}	0.43 ± 0.20 nM (n=3)	7.21 ± 1.26 nM (n=4)
	AXL K_i	0.024 ± 0.004 nM	0.25 ± 0.19 nM
	Fold selectivity over hMERTK / hTYRO3 (enzyme K_i over AXL K_i)	860x / 1400x	130x / 400x
cellular	pAXL ELISA IC_{50} (serum-free media)	17 ± 3.1 nM (n=5)	270 ± 1.4 nM (n=7)
	pAXL ELISA IC_{50} (100% serum)	68 ± 1.4 nM (n=7)	1,100 ± 2.0 nM (n=6)

Table 1. Characterization and comparison of AB801 and Bemcentinib: Kinase activity of AXL was determined using a HTRF KinEASE-TK kit (CisBio) in the presence of 700 μM ATP. *Ki* values of AXL, MERTK, and TYRO3 were determined using Cheng-Prusoff analysis. Dose-dependent inhibition of AXL activation was determined using pAXL ELISA in HEK293T cells transiently transfected with Nanoluc-tagged AXL. pAXL assay was carried out in either serum-free media or 100% human serum.

Pan-Kinase Selectivity of AB801



Kinase	K_d	$K_{d,Kin}/K_{d,AXL}$
AXL	0.093	1
DRAK1	4.4	47
HPK1	26	280
TRKA	31	330
RPS6KA4	130	1,400
ACVR1	170	1,800
TRKB	300	3,000
MAST1	>1000	>10,000

Figure 2. AB801 is very selective for AXL. AB801 was tested against a broad non-mutant kinase panel (403 kinases total) to determine any other potential off-target liabilities. Using a qPCR-based competition binding experiment, AB801 showed <35% of control activity (indicating stronger binding) against 7 other kinase targets, excluding MERTK and TYRO3. Follow-up K_d values were determined through qPCR-based competition binding dose response.

AB801 Inhibits Ligand-dependent and Ligand–independent AXL Phosphorylation

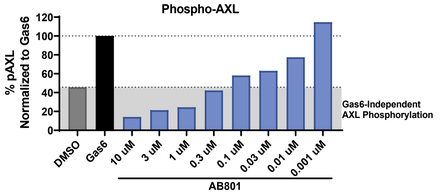
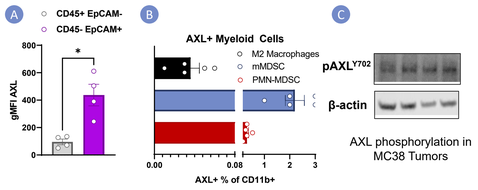
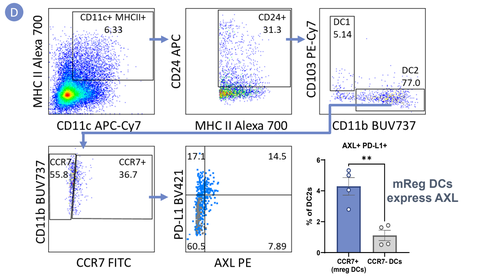


Figure 3. AB801 inhibits ligand-dependent & ligand-independent AXL phosphorylation. Ligand-independent AXL phosphorylation is noted by the grey shaded area at levels of phospho-AXL detected in the DMSO control. H1299 NSCLC cells were treated with AB801 for 1 h. Followed by 800 ng/mL hGas6 added for 15 min before cells were collected. Cell lysates were used to run an ELISA (AXL capture antibody & pan-phosphotyrosine detection antibody) using the MSD platform.

AXL Pathway Is Activated in MC38 Tumors

AXL is Expressed in Cancer and Myeloid Cells in MC38 Tumors





MC38 Tumors Are Infiltrated by TCF+ CD8 T Cells

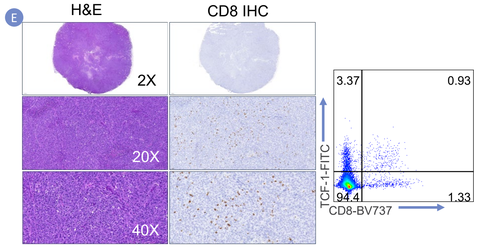
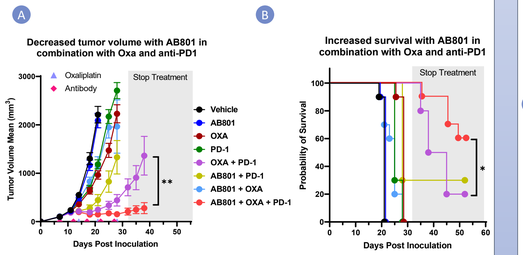


Figure 4. AXL expression is present in cancer cells and in the myeloid compartment in MC38 tumors. Tumors (300-450 mm^3) were used to characterize populations expressing AXL. A) AXL expression is higher on cancer cells compared to CD45+ cells. AXL-PE on Live/CD45+/EpCAM- lymphocytes and Live/CD45-/EpCAM+ cancer cells (gated on live cells). B) AXL+ cells in the myeloid compartment (parent gating: Singlets/Live/CD45+/CD11b+) including M2 Macrophages (F4/80+,Ly6C/CD206+), mMDSC (Ly6C+,F4/80+ or -), and PMN-MDSC (Ly6G+ & Ly6Cint). C) Western blot of MC38 tumors showing pAXL indicative of pathway activation. D) Representative gating for mregDCs: Singlets/Live Dump- (SiglecF, Ly6G, TcrB, CD19)/CD45+. Final panel shows Isotype control (grey) for PD-L1-BV421 and AXL-PE overlayed (teal). Double positive AXL+,PD-L1+, CCR 7+ mregDCs or CCR7- DCs as a percent of DC2s. E) Representative H&E and CD8 IHC and flow plot from MC38 tumors demonstrating that tumors are infiltrated by CD8 T cells that express TCF-1.

AB801 in Combination with Oxaliplatin & αPD-1 Significantly Decreases Tumor Volume and Increases Survival in the MC38 Model



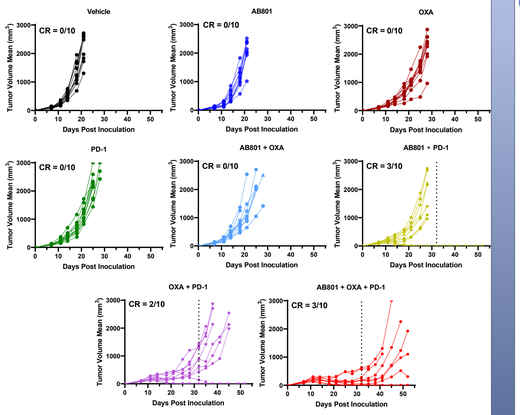


Figure 5. AB801 in combination with Oxaliplatin (OXA) and anti-PD-1 (PD-1) results in significantly lower tumor volumes and increased survival vs double agent OXA + PD-1. C57BL/6 mice were injected subcutaneously with $1x10^6$ MC38 cells. Treatments, n=10 mice per group, started when tumors reached ~100 mm^3. OXA was dosed IP at 10 mg/kg Q7DX4, anti-PD-1 or isotype control were dosed IP 10 mg/kg Q5D, and AB801 was dosed orally at 30 mg/kg BID. A) Combined tumor volume for each treatment group. No significant differences in tumor growth were observed with single agent AB801 treatment. Statistical significance was calculated using a mixed-effects model with Dunnett's multiple comparisons test. p = 0.0118 for triplet vs. OXA + PD-1. Points represent the mean ± SEM. B) Survival proportions, animals with tumors greater than 2000 mm^3 were deemed to have reached the clinical end point. Significance was determined by Mantel-Cox test. p= 0.0419 for triplet vs OXA + PD-1. C) Spider plots showing tumor volume for each individual animal and the number of complete regressions (CR) per treatment. Hashed lines denote end of treatment.

AB801 Enhances Chemosensitivity by Increasing DNA Damage

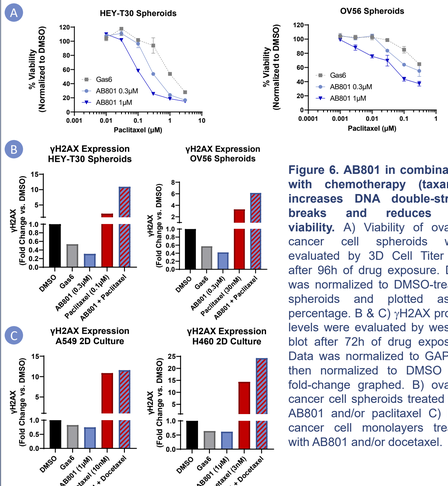


Figure 6. AB801 in combination with chemotherapy (taxanes) increases DNA double-strand breaks and reduces cell viability. A) Viability of ovarian cancer cell spheroids were evaluated by 3D Cell Titer Glo after 96h of drug exposure. Data was normalized to DMSO-treated spheroids and plotted as a percentage. B & C) γH2AX protein levels were evaluated by western blot after 72h of drug exposure. Data was normalized to GAPDH, then normalized to DMSO and fold-change graphed. B) ovarian cancer cell spheroids treated with AB801 and/or paclitaxel C) lung cancer cell monolayers treated with AB801 and/or docetaxel.

SUMMARY

- AB801 exhibits excellent potency and selectivity in functional biochemical and cell-based assays. AB801 possesses an improved profile over previous AXL inhibitors
- AB801 displays excellent pan-kinase selectivity profile, thus limiting off-target liabilities. The lowest K_d ratio is for DRAK1 (47-fold), with all other kinases being >100-fold
- The AXL pathway is activated in MC38 tumors with AXL detectable on both tumor cells and in the myeloid compartment.
- AB801 in combination with Oxaliplatin and α-PD-1 significantly decreases tumor volume and increases survival in the MC38 tumor model
- AB801 enhances taxane chemosensitivity of multiple cancer cell lines in 2D and 3D culture by increasing DNA damage

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
4) Data generated by Arcus. Bemcentinib purchased from Synnovator

Contact

Ester Fernandez-Salas esalas@arcusbio.com
Susan Paprcka spaprcka@arcusbio.com