Targeting AXL sensitizes non-small cell lung cancer to ATR inhibitors by enhancing replication stress

#3205

[1]Kavya Ramkumar, [2]Pan Tong, [1]You-Hong Fan, [1]David Peng, [1]John V. Heymach, [1]Don L. Gibbons, [2]Jing Wang and [1]Lauren A. Byers.

[1]Department of Thoracic/Head & Neck Medical Oncology, [2]Department of Bioinformatics & Computational Biology, The University of Texas MD Anderson Cancer Center, Houston, TX

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Background

- Primary and acquired drug resistance remains a major clinical challenge for patients with advanced stage non-small cell lung cancer (NSCLC).
- AXL has been identified as a key determinant of resistance to chemotherapy, radiation and targeted therapies including DNA damage repair inhibitors (Zhang et al. 2012, Byers et al. 2013, Skinner et al. 2017, Sen et al. 2017).
- AXL mediates epithelial-mesenchymal transition (EMT), immune escape and DNA damage repair (Asiedu et al. 2014, Aguilera et al. 2017, Balaji et al. 2016).

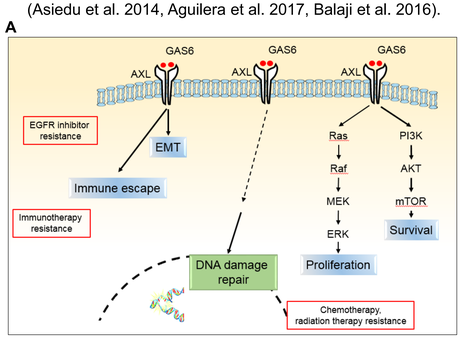


Figure 1. AXL is a key determinant of resistance to multiple therapies. (A) AXL, a TAM family RTK, has been implicated in intrinsic and acquired resistance to chemotherapy, radiation, targeted therapies as well as immunotherapy. (B) AXL overexpression has also been previously identified as a biomarker of resistance to the inhibition of WEE1, another RSR kinase, in small cell lung cancer (C) AXL knockdown causes DNA damage and impairs the efficiency of homologous recombination-mediated DNA damage repair.

Hypothesis

AXL plays a unique role in regulating replication stress and, therefore, targeting AXL will sensitize NSCLC cells to treatments targeting the replication stress response pathway.

Methods

Cell viability assays: A panel of 25 NSCLC cell lines were screened against single agent AXL inhibitor (BGB324) and ATR inhibitors (AZD6738, VX-970) in a 5-day cell proliferation assay.

Reverse phase protein array (RPPA): Expression of 200 phospho- and total proteins were determined in a panel of 115 NSCLC cell lines and used to determine biomarkers of drug sensitivity.

Immunofluorescence microscopy: H1299 cells plated in a 24-well plate were treated with BGB324 and/or VX-970 for 24 h. Permeabilized and fixed cells were stained with anti-γH2Ax (Ser139) antibody and Alexa Flour 546 secondary antibody. Nuclear DNA was counter-stained with DAPI.

Cytometric analysis: H1299 cells treated with BGB324 and/or VX-970 for indicated durations were stained with (i) γH2Ax and PI to analyze distribution of γH2Ax in the different cell cycle phases, and (ii) Annexin V and PI to detect induction of apoptosis.

Results

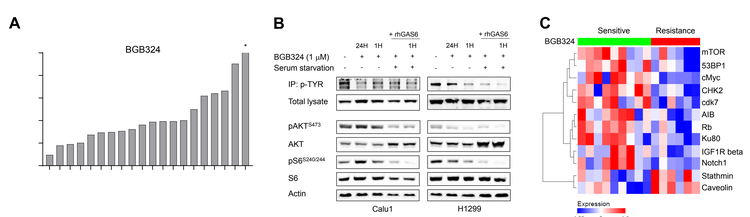


Figure 2. AXL inhibitor BGB324 shows modest single agent activity in a panel of NSCLC cell lines. (A) Bar graph shows IC_{50} values (μM) for the selective AXL inhibitor BGB324 in a panel of NSCLC cell lines. * denotes IC50 greater than the highest concentration tested (9.61 μM). (B) BGB324 potently inhibits AXL phosphorylation and its downstream signaling mediators (pAKT and pS6) even at non-cytotoxic concentrations. (C) Heatmap shows an enrichment of cMYC as well as proteins related to DNAPKCs DNA repair pathway (53BP1, KU80) in NSCLC cell lines sensitive to BGB324 ($p<0.05$).

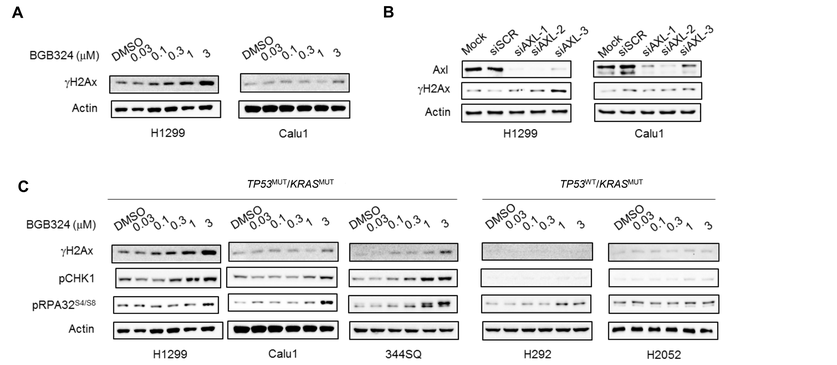


Figure 3. AXL inhibition results in DNA damage and activates the ATR-CHK1 axis. (A,B) AXL inhibition using selective inhibitor BGB324 (A) as well as AXL knockdown (B) induces γH2Ax, a marker of DNA damage. (C) Treatment with BGB324 induces a replication stress response, as seen by a dose-dependent increase in ATR-mediated CHK1 phosphorylation, RPA32 hyper-phosphorylation and γH2AX accumulation, more significantly in the p53-deficient NSCLC cell lines as compared to the p53-WT cells.

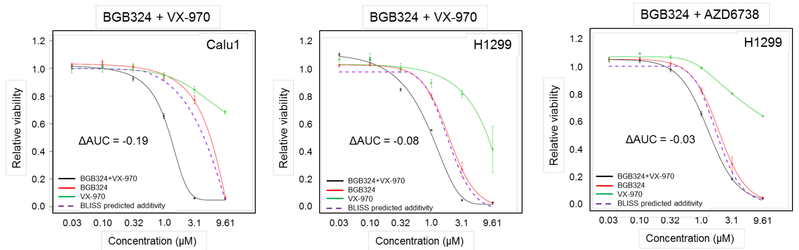


Figure 4. AXL inhibition sensitizes NSCLC cells to ATR inhibitors. Dose-response curves in Calu1 and H1299 cells treated with AXL inhibitor (BGB324), ATR inhibitor (VX-970 or AZD6738) or their combination. A greater than predicted additive effect of the drug combination (ΔAUC) using the BLISS model was observed.

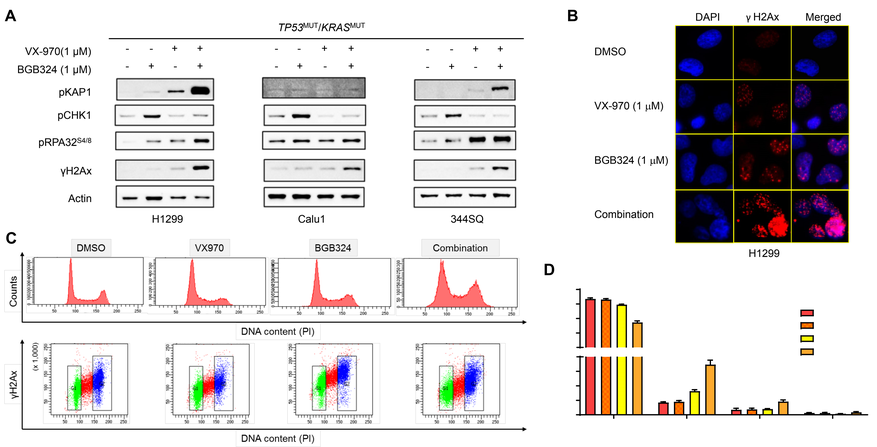


Figure 5. Combination of AXL and ATR inhibitors causes significant DNA damage, prolonged G2/M arrest and apoptotic cell death. (A) Combination of BGB324 and VX-970 or AZD6738 caused significant DNA damage. (B) Nuclear localization of γH2Ax in asynchronous H1299 cells 24 h post-treatment shows increased double strand breaks. (C) Representative bivariate dot plots show relative accumulation of γH2AX in the G2/M phase of cell cycle at 24 h post-drug treatment in p53-deficient H1299 cells. (D) FACS analysis of Annexin V/PI staining shows increase in percentage of cells in early apoptosis at 72 h post-treatment with the drug combination.

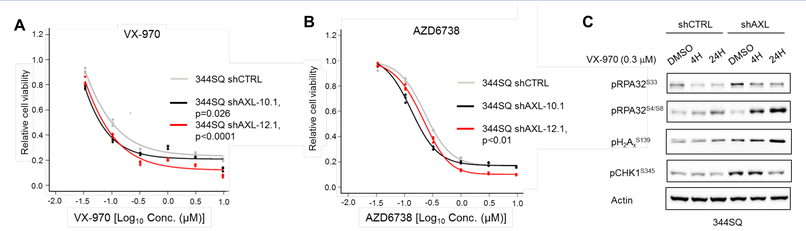


Figure 8. AXL knockdown sensitizes GEMM-derived NSCLC cell line to ATR inhibition. Knockdown of AXL sensitized GEMM-derived KrasLA1/+; p53R172HΔG (KP) NSCLC cell line 344SQ to VX-970 (A) and AZD6738 (B). Relative cell viabilities at all doses in shCTRL and shAXL cells were compared by paired t-test. Overall P-values are indicated. (C) Significant increase in pRPA32S4/8 and pH2Ax in response to ATR inhibition suggests increased fork instability in AXL knockdown cells.

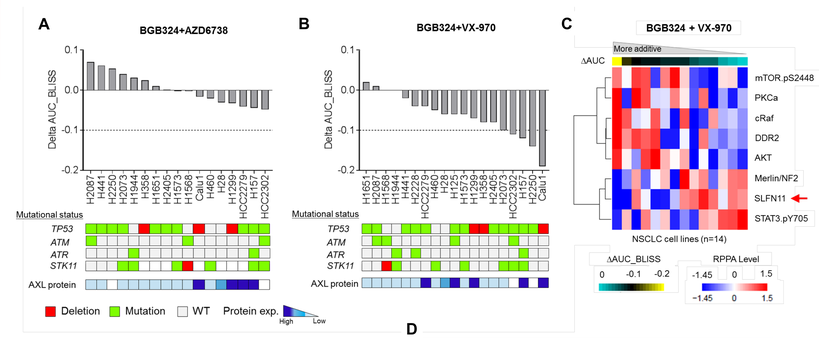
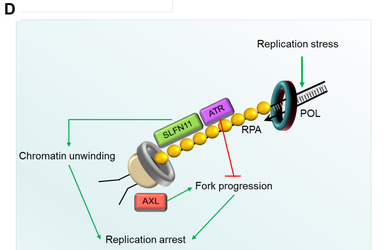


Figure 9. Cell lines with low SLFN11 are more susceptible to AXL/ATR co-targeting. (A, B) Bar graph depicts BLISS ΔAUC values for AXL and ATR inhibitor combination across a panel of NSCLC cell lines. IC50 values of single agent BGB324 ranges from 1-6 μM. (C) Heatmap of proteins significantly ($p<0.05$) correlated with the ΔAUC values for BGB324/VX-970 combination. (D) SLFN11 is a marker of sensitivity to AXL/ATR co-targeting. Adapted from Murai et al., 2018.



Conclusions

- AXL inhibition induces DNA damage and activates a replication stress response. AXL/ATR co-targeting resulted in synergistic cell death.
- Low SLFN11 expression predicts resistance to DNA damaging agents such as cisplatin and PARP inhibitors, which can be overcome by AXL-ATR co-targeting.
- *Future directions*: To elucidate the role of AXL in preserving replication fork stability during replication stress.

Translational relevance

- A novel and unexpected role for AXL in replication stress response, which can shed light on the implication of AXL upregulation in response to multiple anticancer treatments contributing to such pan-treatment resistance.
- AXL-ATR inhibitor combination can be useful in targeting platinum- and PARP inhibitor-resistant SLFN11-low tumors.

Acknowledgements

This work was supported by: The LUNGevity Foundation, NIH/NCI NIH/NCI R01-CA207295, The University of Texas SPORE in Lung Cancer (5P50 CA070907), MDACC CCSG Grant (P30CA01667), MDACC Physician Scientist Award, AstraZeneca, and through generous philanthropic contributions to The University of Texas MD Anderson Lung Cancer Moon Shot Program. BGB324 was generously provided by BergenBio.