AXL targeting enhances lymphocyte-mediated cytotoxicity of lung cancer cells

Salem Chouaib[1,2], Stéphane Terry[1], Stéphanie Buart[1], Agnete Engelsen[1], Gro Gausdal[3], James Lorens[4], Jean Paul Thiery[1], Fathia Mami-Chouaib[1]. [1]Gustave Roussy, Inserm U1186, Villejuif, France. [2]Thumbay Research Institute of Precision Medicine, Gulf Medical University, Ajman, United Arab Emirates. [3]BerGenBio, Bergen, Norway. [4]University of Bergen, Bergen, Norway

BACKGROUND

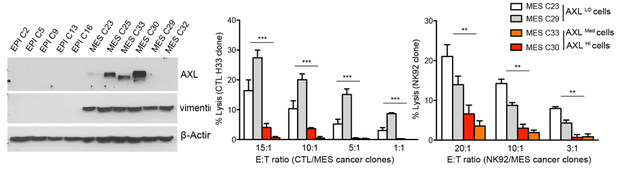
- As immunotherapies are now used to treat a large proportion of NSCLC patients, defining mechanisms of immune resistance is critical.
- Immune resistance may arise from extrinsic or intrinsic mechanisms of immune resistance. Previous work showed that acquisition of a more mesenchymal (MES) phenotype after EMT can be associated with an increased propensity to resist cytotoxic T lymphocytes (CTL) and Natural killer (NK) cells attacks (Terry S. et al. OncoImmunology 2017).
- AXL, a member of the TAM receptor tyrosine kinase family is widely expressed human cancers and increasingly recognized for its role in drug resistance and immune suppression.
- In this study, we asked whether AXL could impact on tumor resistance to cytotoxic immune effectors such as NK cells and Cytotoxic T Lymphocytes (CTLs).

METHODS

- Mesenchymal (MES) and Epithelial (EPI) cancer cell clones with null to high expression of AXL deriving from NSCLC IGR-heu model cells were used in this study.
- Gene expression was assessed by qRT-PCR, Western Blot, Flow Cytometry analysis, or SurePrint G3 Gene Expression Microarrays.
- Inhibition of AXL was performed using bemcentinib (BGB324) from BerGenBio company and gene expression analysis was performed on drug or vehicle-treated cells.
- The cytotoxic activity of effector (immune) cells to target (cancer) cells was measured by a conventional 4-h ^{51}Cr-release assay in round-bottomed 96-well plates

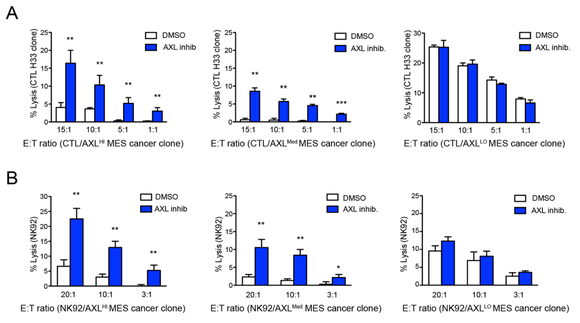
RESULTS

Selective expression of AXL in MES lung carcinoma cells is associated with resistance to cell-mediated cytotoxicity



(Left) various cancer clones with EPI or MES phenotypes were analyzed for AXL expression. (Right) MES cancer clones expressing Low (Lo) to high (HI) AXL expression were analyzed for susceptibility to CTLs or NK-mediated lysis

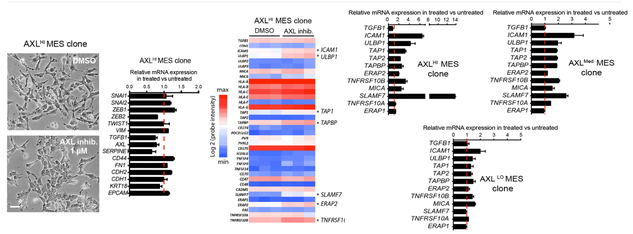
Treatment of AXL^{HI} MES and AXL^{Med} lung carcinoma clones with AXL inhibitor results in increased susceptibility to NK and CTL-mediated lysis



MES cancer clones with Low (Lo) to high (HI) AXL expression were treated with bemcentinib (1 µM) or DMSO for 72h, after which they were analyzed for their susceptibility to CTLs (A) or NK (B) -mediated lysis

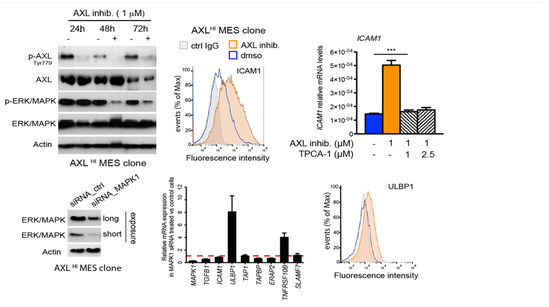
CONCLUSION

Treatment of the AXL expressing MES carcinoma clones with the AXL inhibitor is accompanied by perturbation of certain NK-receptors-ligands



(Left) AXL^{HI} MES cancer cells were treated with Bemcentinib or DMSO for 72h and analyzed for morphological changes, expression of EMT-related, and immune related genes (middle). Genes such as ICAM1 and ULBP1 were found upregulated upon treatment with bemcentinib. (Right) qRT-PCR validation in various MES cancer clones.

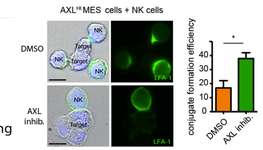
AXL inhibition led to increased expression of ICAM-1 and ULBP1 in conjunction with NF-κB activity and inhibition of MAPK

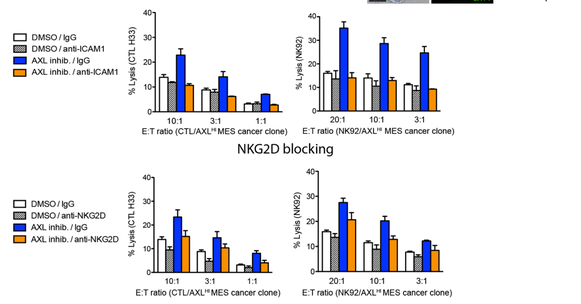


(Top panels) bemcentinib treatment of AXL^{HI} MES cancer cells inhibited ERK phosphorylation, increased surface expression of ICAM1, which is blocked by treatment with the IKK/NF-κB inhibitor TPCA-1 (Sigma). (Bottom panels) Reduction of ERK/MAPK expression after siRNA targeting upregulated ULBP1 expression.

Targeting the ICAM1/LFA-1 and ULBP1/NKG2D axes impairs bemcentinib's effect on cancer cell susceptibility to killing

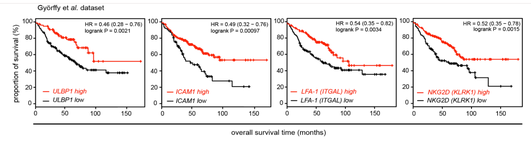
bemcentinib treatment of AXL ^{HI} MES cancer cells increased the number of cell contacts (conjugates) between cancer cells and immune cells





AXL ^{HI} MES cancer cells pretreated with bemcentinib were cocultured with an CTL or NK clones for 4h in the presence or absence of anti-ICAM-1 (Top) or anti-NKG2D (bottom) (Biolegend). % of cancer cells lysis was assessed using ^{51}Cr-release assay.

Both ICAM1/LFA-1 and ULBP1/NKG2D axes are associated with better survival in a NSCLC dataset



Model

The results suggest that increased AXL expression in mesenchymal lung cancer clones correlates with increased cancer cell intrinsic resistance to both NK and CTL-mediated killing, and that small molecule AXL targeting can sensitize these cells to cytotoxic lymphocyte-mediated killing in a manner involving a novel molecular network comprising NF-κB activation, increased ICAM1 expression, and upregulation of ULBP1 expression coupled with MAPK inhibition. These results support AXL targeting to optimize immune response in NSCLC.