AXL as a therapeutic target in STK11 mutant NSCLC

#3245

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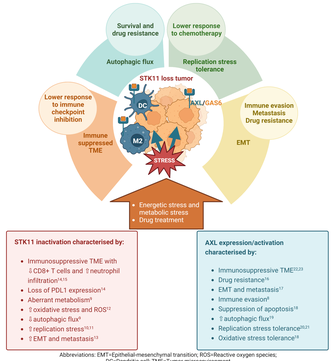
AACR ANNUAL MEETING 2023, April 14-19, 2023
Orange County Convention Center Orlando, Florida

INTRODUCTION

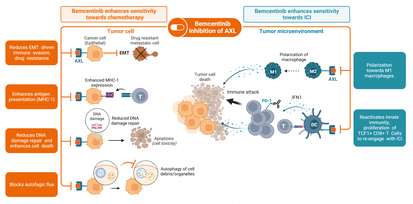
Serine-threonine kinase 11 (STK11/LKB1) is a tumor suppressor, and loss of STK11 protein contributes to tumorigenesis. Mutations in the STK11 gene (STK11m) are present in ~ 20% of NSCLCs,[1,2] and STK11 is one of the most frequently mutated genes in NSCLC. Inactivation of STK11 is associated with a poorer prognosis, irrespective of treatment modality and represents a sub-group of patients with high unmet need.[3] AXL, a member of the TAM family of receptor tyrosine kinases, is activated in response to inflammation, hypoxia, cellular stress or drug treatment.[4-8] Cancer cells use the AXL pathway to sense stress, triggering molecular mechanisms to ensure survival or escape from the toxic environment (Figure 1). AXL is expressed in both tumor cells, where it enhances survival and drug resistance, and in innate immune cells, such as dendritic cells (DC) and macrophages, where AXL drives immune suppression (Figure 1). The phenotypic characteristics (high energetic and metabolic stress) of a tumor with inactivated STK11 are likely to promote AXL activation in both tumor cells and/or innate immune cells of the tumor microenvironment (TME) (Figure 1). The selective AXL inhibitor bemcentinib targets key survival and resistance mechanisms within the tumor and restores the anti-tumor phenotype of innate immune cells. Specifically, inhibition of AXL in DCs has been shown to increase immune check point inhibition responses in preclinical models of STK11 mutant NSCLC (Figure 2).[22]

Inactivation of STK11 is found in approximately 30% of lung adenocarcinomas as a result of either mutations in the STK11 gene (STK11m) or other non-mutational mechanisms.[2] The Kaufman STK11 loss signature,[2] is a 16 gene classifier for identifying tumors lacking STK11 activity. In the current work, we have applied the Kaufman STK11 loss signature to publicly available data sets (TCGA/CPTAC-3) as well as the BerGenBio trial in 2L NSCLC (BGBC008) to better understand the characteristics and mutational status of these tumors. This signature identifies KEAP1 mutations as strongly associated with STK11 loss. Differential gene expression analysis confirms that mutations in STK11 and KEAP1 have strongly overlapping transcriptional consequences. AXL expression was explored in NSCLC tumors in correlation to STK11m and STK11 loss. Transcriptional changes following AXL inhibition by bemcentinib in a STK11m/KEAP1m NSCLC cell line were mapped to further strengthen the rationale for targeting NSCLC patients characterized by STK11 loss with the AXL inhibitor bemcentinib.



Abbreviations: EMT=Epithelial-mesenchymal transition; ROS=Reactive oxygen species; DC=Dendritic cell; TME=Tumor microenvironment

Figure 1. AXL is a key driver of survival and drug resistance in NSCLC tumors with STK11 loss



Abbreviations: ICI=Immune checkpoint inhibition; EMT=Epithelial-mesenchymal transition

Figure 2. The AXL inhibitor bemcentinib targets key survival and resistance mechanisms of STK11m NSCLC

DATA SOURCES

Clinical Trial NCT03184571 (BGBC008)(†)
- 90 evaluable patients 2L+ NSCLC, 50 with available transcriptomic data.
- Small molecule AXL inhibitor bemcentinib in combination with pembrolizumab.
- RNAseq, whole exome sequencing, AXL IHC, PD-L1 IHC.

Patients from Haukeland University Hospital Bergen REC 45562(‡)[24]
- 119 Stage III/IV NSCLC patients.
- 28% first-line, 72% chemo-refractory 2L+.
- Whole exome sequencing, AXL IHC, PD-L1 IHC.

Public data from Genomic Data Commons (GDC) Data Portal
- 662 lung adenocarcinoma cases with transcriptomic and mutation information.
  - 458 TCGA-LUAD: primary, newly diagnosed, untreated patients.
    - "The results published here are in whole or part based upon data generated by The Cancer Genome Atlas managed by the NCI and NHGRI. Information about TCGA can be found at https://cancergenome.nih.gov, dbGaP accession phs000178.v11.p8, retrieved Aug 2022.
  - 204 CPTAC-3: primary, newly diagnosed, untreated patients.
    - "Data used in this publication were generated by the National Cancer Institute Clinical Proteomic Tumor Analysis Consortium. (CPTAC), dbGaP accession phs001287.v16.p6, retrieved Aug 2022.

Patients from Li *et al*, 2022(*)[22]
- 62 NSCLC cases, known STK11 mutation status, AXL IHC.

METHODS

- AXL IHC staining
  - Staining with 7E10 antibody (Thermo) or C89E7 (Cell Signaling). AXL positive defined as tumor H-score >5 or ≥1% AXL positive immune cells per tumor area.
- PD-L1 IHC staining with 22C3 pharmDx, scored by TPS.
- Whole-exome and mRNA sequencing of FFPE patient samples from BGBC008 and REC 45562 were performed by Almac, Inc.
- Differential gene expression analysis of GDC data using DESeq2, with pathway analysis by Metascape.[26]
- Kaufman STK11 loss score
  - RNAseq count data normalized, variance stabilized and batch-corrected using DESeq2 and limma,[27,28] adjusted by subtraction of mean. Kaufman score calculated as average expression of 16 gene set.[2] Published cut-off at 0.2 captures over 95% of STK11 mutations documented in GDC dataset.
- Circos circle plots: Outer arcs identify each gene list. Inner arcs: dark orange color represents genes that appear in both lists; light orange color represents genes that are unique to that gene list. Purple lines link same genes shared by both gene lists. Blue lines link genes that fall into the same (statistically significant) ontology term.
- Wordclouds generated from GO terms by ranking words by significance of the pathway and frequency.
- GSEA analysis of RNA seq data from A549 cells (STK11m/KEAP1m/KRASm/p5) treated with DMSO or 1 µM bemcentinib for 24 hrs (n=3). Count reads were variance-stabilizing transformed using DESeq2 before GSEA analysis.[29,30]

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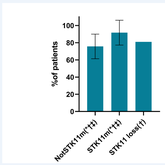
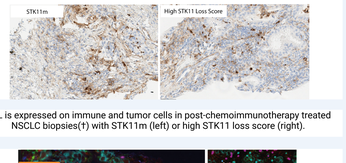
RESULTS

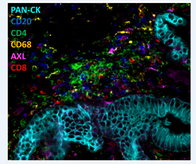
AXL is expressed in tumor and immune cells in NSCLC patients with or without STK11 loss



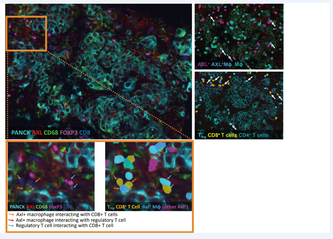
AXL quantification of IHC from three studies: Li *et al.*, 2022(*),[22] BGBC008(†) and REC 45562(‡).[24] Bars represents AXL positive staining (Tumor H-score >5 or ≥1% immune cells expressing AXL).



AXL is expressed on immune and tumor cells in post-chemoimmunotherapy treated NSCLC biopsies(†) with STK11m (left) or high STK11 loss score (right).

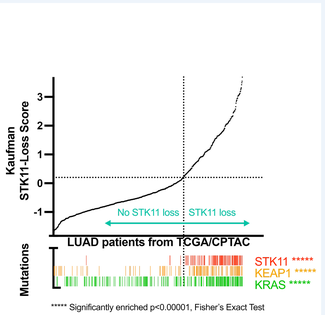


AXL is expressed on tumor associated immune cells and cancer cells in STK11m patient.



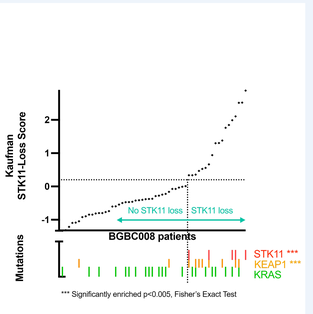
Tumor infiltrating AXL+ M2 macrophages observed to interact with Tregs and CD8+ T cells in responding patient pretreatment biopsy.[32]

Mutations in STK11 and KEAP1 and low PD-L1 expression are associated with a STK11 loss signature



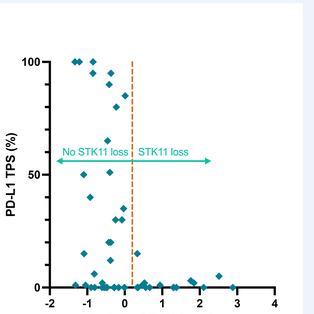
***** Significantly enriched p<0.00001, Fisher's Exact Test

GDC dataset: Patients with the STK11 loss signature are significantly more likely to carry STK11, KEAP1 or KRAS mutations.



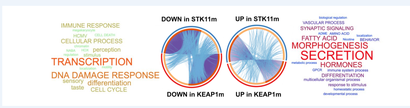
*** Significantly enriched p<0.005, Fisher's Exact Test

BGBC008 dataset: Patients with the STK11 loss signature are significantly more likely to carry STK11 or KEAP1 mutations.



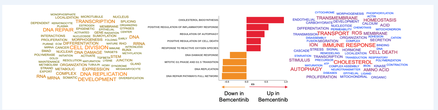
BGBC008 dataset: Patients with STK11 loss signature are significantly more likely to have low PD-L1 expression (Spearman r=-0.38, p=0.006). These findings are similar to the low PD-L1 expression levels associated with STK11 and KEAP1 mutated patients from the Keynote 189 study.[3]

Mutations in STK11 and KEAP1 promote similar transcriptional consequences in NSCLC patients



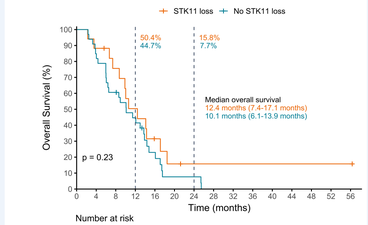
- Comparison of differentially-expressed genes from patients carrying STK11m or KEAP1m to patients not carrying the corresponding mutation show a large number of common transcriptional pathways, especially among down-regulated genes.
- Down-regulation of immune response and DNA damage response characteristic of SKT11 tumors.

Bemcentinib modulates pathways involved in tumorigenic features of STK11m NSCLC

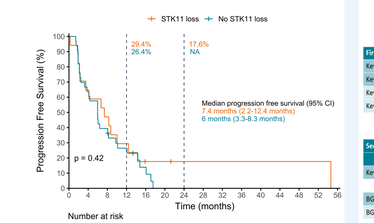


Inhibition of AXL in A549 NSCLC cells (STK11m/KEAP1m) with bemcentinib upregulates inflammatory response signatures, modulates autophagy, and down-regulates pathways required for survival in response to replication stress and DNA damage (see also AACR Poster 6206).[33]
A549 cells (STK11m/KEAP1m/KRASmv/p53wt) treated with bemcentinib or DMSO. Center: Selected enriched terms (GSEA). Left/Right: Word clouds of enriched terms (Right, blue/red: enriched in bemcentinib treated; Left, green/orange: enriched in control).

Bemcentinib in combination with pembrolizumab in 2L+ NSCLC has similar activity when STK11 is present and absent



Comparison of Overall Survival BGBC008
No significant difference between STK11 loss and no STK11 loss.



Comparison of Progression Free Survival BGBC008
No significant difference between STK11 loss and no STK11 loss.

Bemcentinib + pembrolizumab combination compares favorably to existing therapies in 2L NSCLC

	pemetrexed-platinum		Pembrolizumab + pemetrexed-platinum	
First Line†	OS	PFS	OS	PFS
Keynote 189 STK11 WT	12.4 (7.6-25.0)	4.7 (4.6-5.3)	22.5 (20.1-NR)	10.4 (8.2-14.0)
Keynote 189 KEAP1 WT	12.2 (7.6-25.0)	4.7 (4.5-5.3)	23.5 (20.4-NR)	10.4 (8.2-14.0)
Keynote 189 STK11 mutated	8.4 (6.5-NR)	4.8 (4.5-8.6)	17.4 (4.7-NR)	6.1 (3.6-9.2)
Keynote 189 KEAP1 mutated	8.7 (6.6-NR)	4.8 (4.6-8.8)	13.3 (7.5-NR)	5.1 (3.7-11.1)

STK11/KEAP1 mutations reduce survival irrespective of treatment

Pembrolizumab provides survival benefit to all groups

	Pembrolizumab monotherapy	
Second Line +	OS	PFS
Keynote 189 control crossover[25*]	6.9 (4.8-10.5)	2.8 (2.5-5.9)
	Bemcentinib + Pembrolizumab	
BGBC008 No STK11 loss	10.1 (6.1-13.9)	6.0 (3.3-8.3)
BGBC008 STK11 loss	12.4 (7.4-17.1)	7.4 (2.2-12.4)

Bemcentinib provides similar clinical outcomes

*On study crossover of placebo plus pemetrexed-platinum group (n=84) to pembrolizumab monotherapy

Clinical outcomes from the Phase 2, 2L+ metastatic NSCLC study (BGBC008) investigating the combination of bemcentinib and pembrolizumab indicate that patients with STK11 loss have similar clinical outcomes to non-STK11 loss patients.

CONCLUSIONS

- STK11/KEAP1 mutations are transcriptionally similar and share a common signature for STK11 loss.
- AXL is a recognized driver of key features characterizing STK11 inactivated tumors such as an immune suppressed TME and tumor drug resistance and metastasis.
- AXL is expressed in over 80% of NSCLC tumors that show STK11 loss/mutation.
- STK11 loss due to mutations in the STK11 gene (STK11m) or other mechanisms such as KEAP1m is associated with low level of PD-L1 expression and poorer outcomes.
- Clinical outcomes from the Phase 2, 2L+ metastatic NSCLC study (BGBC008) investigating the combination of bemcentinib and pembrolizumab indicate that patients with STK11 loss have similar clinical outcomes to non-STK11 loss patients, providing a rationale to target NSCLC with mutations in either STK11 or KEAP1 with an AXL inhibitor.
- The clinical utility of targeting AXL in patients with STK11 mutation will be further explored in a global, open-label Phase 1b/2a trial of bemcentinib with SOC (pembrolizumab, pemetrexed and carboplatin) in 1L advanced/metastatic non-squamous NSCLC patients [NCT05469178].

ACKNOWLEDGEMENTS

This study is in collaboration with Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA.
Thanks to all patients and their families, investigators and study coordinators who participated in and supported this study.

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