**Multi-omic characterization of *KRAS/STK11/KEAP1* co-mutant non-small cell lung cancer (NSCLC) displays a unique metabolic profile and therapeutic vulnerabilities.**

Utsav Sen1, Charles Coleman2,3, Andrew Elliott4, Ari Vanderwalde4, Balazs Halmos5, Patrick Ma5, Dan Hasson2,3, Nishant Gandhi4, **Triparna Sen1,2.**

**Affiliation**

1 Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, 10029, USA.

2 Tisch Cancer Institute, Mount Sinai, New York, NY, 10029, USA.

3 Tisch Cancer Institute Bioinformatics for Next Generation Sequencing (BiNGS) core, Icahn School of Medicine at Mount Sinai, New York, NY, 10029, USA.

4 Caris Life Sciences, Phoenix, AZ, USA.

5 Montefiore Medical Center, Albert Einstein College of Medicine, New York, NY, USA.

6 Penn State Milton S. Hershey Medical Center, Hershey, PA, USA.

**Background:** *KRAS*-mutant NSCLC with co-occurring loss-of-function mutations in *STK11* and *KEAP1* are remarkably aggressive and unresponsive to chemo- and immunotherapy. Novel therapeutic strategies are urgently needed to improve outcomes for patients with *KRAS/STK11/KEAP1* co-mutant NSCLC (KSK). We interrogated the transcriptomic landscape using a large real-world dataset of NSCLC to identify therapeutic vulnerabilities that may help guide treatment selections in *KSK*.

**Method**: *KRAS* mutant NSCLC clinical samples (N=7210) were tested with NextGen Sequencing (592-gene panel or whole exome sequencing) and RNA (whole transcriptome sequencing) at Caris Life Sciences (Phoenix, AZ). Specimens were stratified into *KRASMUT/STK11MUT/KEAP1MUT* (KSK; N=698), *KRASMUT/STK11MUT/KEAP1WT* (KS; N=786), *KRASMUT/STK11WT/KEAP1MUT* (KK; N=466), and *KRASMUT/STK11WT/KEAP1WT* (K; N=4536). Additionally, an *in vitro* CRISPR screen, bulk RNA sequencing, and phospho-kinase arrays were performed in *KRAS/STK11/KEAP1* co-mutant models.

**Results:** *KEAP1* mutations (mOS: KK=7.83m, KSK=7.23m) were strongly associated with poor OS compared to *STK11* mutations (mOS: KS=17.6m). Pathways significantly upregulated in KSKclinical samples included fatty acid metabolism and redox pathways. KSK clinical samples had significant overexpression of genes involved in ferroptosis evasion and metabolism like *SLC7A11*(KSK/KK=1.28, KSK/KS=4.82, KSK/K=10.24; all q<0.01)and *SCD1* (KSK/KS=1.19, KSK/K=1.24; both q<0.01)compared to single mutants or wild-type groups.

CRISPR/Cas9-based genetic screening identified *SCD1* as a potential therapeutic target in the KSK cell lines. *SCD1* inhibition led to global metabolomic changes in KSK cells, including key pathways involved in lipid and glucose metabolism. Moreover, *KSK* co-mutant cells have a significantly higher expression of *SLC7A11,* an amino acid transporter that enables cystine uptake and its subsequent conversion to cysteine.Consequently, *KSK* co-mutant cells are most resistant to cysteine depletion in the mediaas compared to single mutants or NTC cells. SCD1 inhibition causes a decrease in SLC7A11 expression exclusively in *KSK* co-mutant cells. Finally, pharmacological inhibition of SCD1 significantly reduced the viability of KSK cells and caused significant tumor regression in KSK syngeneic mouse models.

**Conclusion:** We highlight the importance of the *SCD1-SLC7A11* axis in regulating unique metabolic and ferroptosis evasion pathways in *KRAS/STK11/KEAP1* co-mutant NSCLC. The study data furthers the understanding of ferroptosis in NSCLCand the potential to translate SCD1 inhibitors and ferroptosis inducers in NSCLC clinical trials.