3056: *KRAS* Mutations Shape an Oxidative, Immune-Cold Tumor microenvironment Driving Chemo-immunotherapy Resistance in advanced NSCLC

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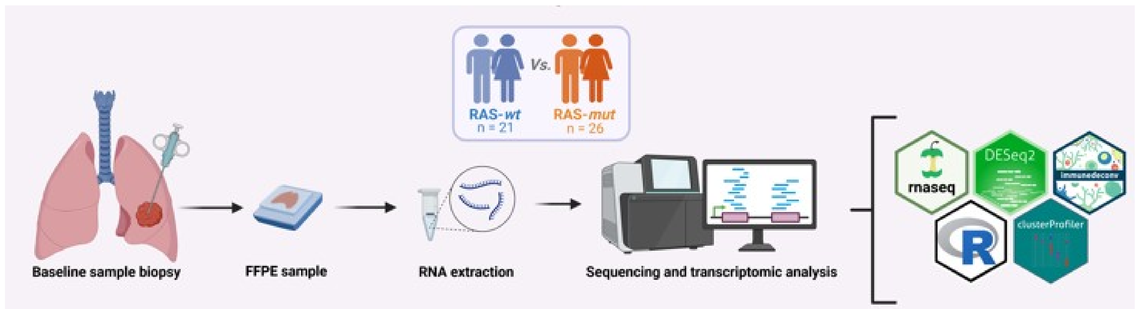
INTRODUCTION

- Despite improved outcomes with checkpoint inhibitors (CPI) and Precision Medicine approaches, **NSCLC** is still the **leading cause of death by cancer.**
- **~ 30% of NSCLC harbor *KRAS*** mutations (G12C the most common and the only one with approved molecular-matched therapy).
- Beyond genomic alterations, the **tumor microenvironment** has emerged as a **critical determinant of treatment resistance**.
- **Oxidative stress** has been shown to **modulate CPI efficacy** through altered PD-L1 expression and immune cell infiltration but the the precise **immunometabolic interplay** within the TME of *KRAS*-mutated tumors **remains to be elucidated.**

CONCLUSION

- Our data suggest that ***KRAS* mutations** in NSCLC **shape a metabolically reprogrammed and immunosuppressive TME** that may drive resistance to ChT-IO.
- Targeting **oxidative stress and immunomodulatory pathways**—such as those involving ***EDN1***—may represent **promising therapeutic strategies** to overcome resistance and improve outcomes in *KRAS*-driven NSCLC.

METHODS



Prospective collection of samples from **47 patients**:
- **Advanced NSCLC**
- **Treatment naïve,** candidates to receive ChT-IO
- Included **KRAS-mut and KRAS-wt** cases (oncogene-addicted cases excluded)

→ RNA extracted from samples with ≥30% tumor cellularity and processed using the TruSeq RNA Access Kit (Illumina), followed by paired-end sequencing on the NovaSeq 6000 platform

→ Transcriptomic data pre processed via nf-core/rnaseq. Differential gene expression analysis conducted using DESeq2, with downstream gene set enrichment analysis (GSEA). Immune landscape inference using CIBERSORT and ESTIMATE algorithms.

→ Associations between gene expression, immune composition, and clinical outcomes were evaluated using Spearman correlation, Cox multivariate modeling, and Kaplan-Meier analysis.

RESULTS

Patients with ***KRAS*-mutant tumors** had significantly **shorter PFS** (p 0.006) (Fig 1). Lower *CD274* expression was associated with worse prognosis (p 0.005) and was balanced between groups (p 0.355). Conversely, a **KEAPness signature** assessment (Scalera et al CCR 2024) **replicates survival curves**, highlighting the importance of this pathway (Fig 2). Comparing the *KRAS*-mutant vs the *KRAS*-wt samples, there were no differences in the KEAPness signature expression (Fig 3A), but **patients with poor response** to treatment showed **increased expression of *NFE2L2*** gene (Fig 3B). Transcriptomic analysis underlined 83 differentially expressed genes between *KRAS*-mutant and *KRAS*-wt tumors, with 11 showing |log2FC| > 1 (Fig 4). **GSEA** demonstrated a **marked downregulation of immune-related pathways**—including antigen processing, interferon signaling, and T cell-mediated cytotoxicity—**in *KRAS*-mutant tumors** (Fig 5). The **CIBERSORT immune deconvolution** highlights a **general downregulation of the immune components** in the ***KRAS*-mutant** samples (Fig 6). Overexpressed in *KRAS*-mutant tumors (log2FC = 1.16; p 0.026), ***EDN1*** was the gene **most negatively correlated with CD8+ T cell infiltration** (Fig 7), with **known roles in ROS metabolism and TGFβ-mediated immunosuppression.** EDN1 is **differentially expressed** (p 0.026) (Fig 7A) and its **higher expression** is related with **lower survival** (p 0,001) (Fig 7B).

Figure 1. Kaplan Meier curves with 95% Confidence Intervals showing progression-free survival (PFS) stratified by RAS mutation status.

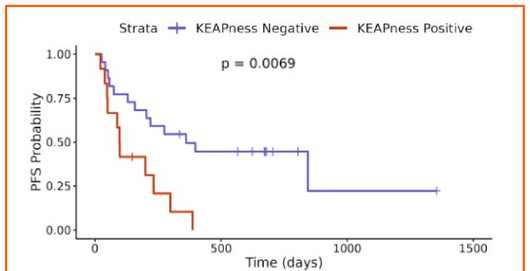


Figure 2. Kaplan Meier curves showing progression-free survival (PFS) stratified by the expression of a KEAPness gene signature.

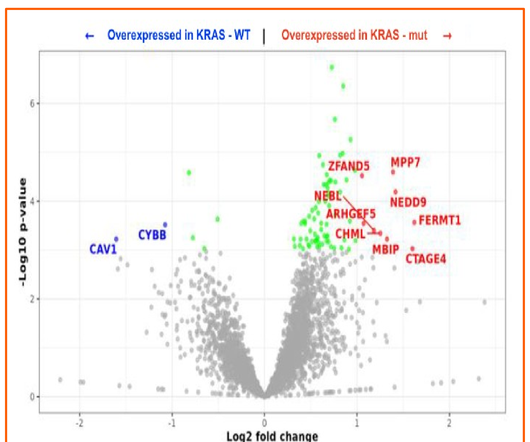


Figure 4. Volcano plot of differential gene expression analysis between KRAS-mutant and KRAS-wt tumors. Genes with adjusted p value <0.05 are showed in color.

Figure 3. Violin plots showing the differential expression of a KEAPness signature in the KRAS-mut vs KRAS-wt samples (A), and the differential expression of the NFE2L2 gene in the responder vs non-responder population (B).

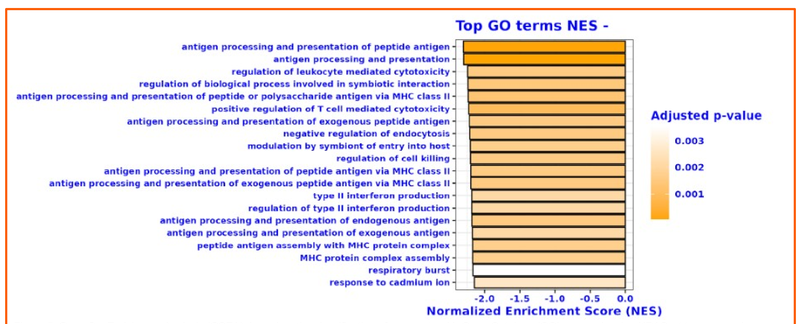


Figure 5. Gene Set Enrichment Analysis (GSEA) based on the classification of gene sets with Gene Ontology (GO) terms, showing KRAS-wt tumor samples present higher antigen processing and presentation processes, as well as MHC II, IFN type 2 production and T cell-mediated cytotoxicity

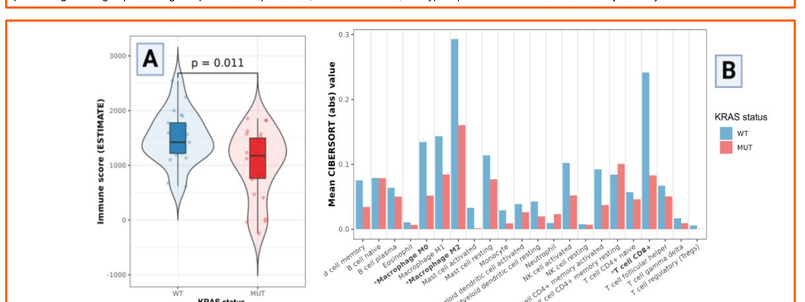


Figure 6. Results from immune cell deconvolution highlighting a general dowregulation of immune components in the KRAS-mutant samples. A) Immune infiltration score values as scored by ESTIMATE algorithm . B) CIBERSORT analysis between KRAS mutant and wt samples, showing a significant change in T cell CD8+ proportion (P value < 0.05).

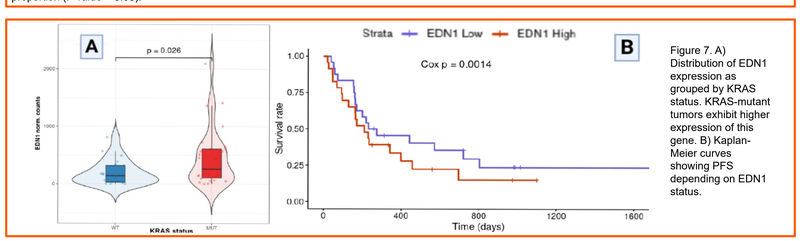


Figure 7. A) Distribution of EDN1 expression as grouped by KRAS status. KRAS-mutant tumors exhibit higher expression of this gene. B) Kaplan-Meier curves showing PFS depending on EDN1 status.

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