Increased immunotherapy sensitivity is associated with damaging IFN-γ pathway mutations in NSCLC

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HIGHLIGHTS

Non-synonymous mutations in members of the interferon gamma pathway associate with better outcomes after treatment with checkpoint inhibition in non-small cell lung cancer. This association is robust when tested with multivariable models, permutation testing and externally validates in the real-world Caris Life Sciences (CARIS) dataset.

INTRODUCTION

Baseline disruption of the interferon gamma (IFN-γ) pathway enhances tumor immune elimination post-checkpoint blockade in murine models.[1] In multiple solid tumor types, mixed-effects meta-analysis has shown a significant increase in responses to immunotherapy with IFN-γ pathway mutations, including in multivariable models incorporating high versus low tumor mutational burden (TMB).[2]

However, an extensive analysis of multiple co-variables and outcomes has not been conducted. In the Stand Up to Cancer-Mark Foundation (SU2C-MF) NSCLC clinical cohort, we found that non-synonymous mutations in IFN-γ pathway genes (n=26, 8%) associated with better outcomes following PD-(L)1 inhibitors (ICI), including improved radiological responses, progression-free survival, and overall survival (OS) (AACR 2024).[3,4]

This association remains significant in multivariable models and using different permutation tests.[4] Here, we present key and updated findings from SU2C-MF NSCLC cohort, but crucially aimed to validate these results in an independent dataset and examine predictive versus prognostic significance.

METHODS

In both cohorts, non-synonymous mutations were defined as truncating (frameshift indels, non-sense and splice-site), in-frame indels and missense mutations.

In the SU2C-MF NSCLC cohort, pathway mutations were non-synonymous mutations in IFN-γ pathway genes (*IFNGR1*, *IFNGR2*, *JAK1*, *JAK2*, *STAT1*, *IRF1* & *IRF9).* Response outcomes was defined using RECIST v1.1, categorizing complete/partial responses as R and stable/progressive disease as NR, and linear percentage change in target lesions (CTL) was collated. These response data were analyzed using additive logistic (LogR) and linear regression (LinR), respectively. Overall survival (OS) and progression-free survival (PFS) were analyzed with Cox models. The multivariable model included TMB (log), PD-L1 tumor proportion score (PD-L1) (log), prior platinum therapy, age at diagnosis, biological sex, smoking status, smoking pack years (log), first-line immune checkpoint inhibition, histology, monotherapy vs. combination immunotherapy, and initial tumor stage.

50,000 random genesets were created to be within 33% of the coding sequence (CDS) length of the IFN-γ pathway geneset. The curveball algorithm, iterated 10,000 times, generates shuffled data to model the effect of the tumor-specific mutational burden.[5]

The Caris Life Sciences (CARIS) dataset includes NSCLC patients with standard-of-care biopsies prior to immune checkpoint treatment. Survival outcomes were tracked using claims. IFN-γ pathway mutation was defined as non-synonymous mutations in the genes available from CARIS's curated whole exome sequencing data (WES) and deep panel of 592 cancer-associated whole-genes: *IFNGR1*, *JAK1*, *JAK2* & *IRF1*. Cox proportional-hazards models (CoxPH) assessed OS from ICI initiation. Co-variables, in the multivariable models, had to be dichotomized: age (<65/≥65), PDL1 TPS (0%/>0%), smoking history (non-smoker/ever smoker), and continuous, including TMB, by median.

RESULTS

In the SU2C-MF NSCLC cohort, 309 patients had evaluable whole-exome sequencing data, treatment with PD-(L)1 inhibitor regimens, and matched radiological responses (121 R [39%], 188 NR [61%]); 227 had matched change in target lesions (CTL) data. 26 from 309 (8%) patients had non-synonymous mutations in the IFN-γ pathway (Fig. 1) [*IFNGR1* (n=3); *IFNGR2* (n=1); *JAK1* (n=6); *JAK2* (n=8); *STAT1* (n=3); *IRF1* (n=1); *IRF9* (n=3); and *JAK2* & *IRF9* (n=1)].

Significant relationships were observed between IFN-γ pathway mutation and both response (LogR: OR 0.17, p=2.0x10^{-4}* *(Fig. 1)*; LinR: OR 0.70, p=4.8x10^{-4}*) and survival (PFS: p=0.0053*; OS: p=0.044*). Multivariable models incorporating TMB confirmed the significance of IFN-γ pathway mutation in response and these relationships were clearer in the comprehensive, multivariable models (LogR: OR 0.24, p=0.045*; LinR: p=0.0079*; PFS: p=0.082; OS: HR 0.15, p=0.0039*) *(Fig. 2* & *3)*.

IFN-γ pathway mutation remains significantly more associated with response (p=0.0044*) and CTL (p=0.0053*) when compared with 50,000 random genesets. Furthermore, IFN-γ pathway mutation remains significantly associated with response (p=0.0002*) and CTL (p=0.0004*) when using 10,000 iterations of the curveball algorithm to control for the influence of TMB.

In the CARIS dataset, among 1070 patients with pretreatment mutation data, ICI treatment, and OS data, 88 (8%) had IFN-γ pathway mutations. Mutation was associated with significantly improved OS (HR 0.63, p=0.028*). Median OS for patients with disrupted vs. intact pathways was 60.0 months (95% CI: 27.6-NA) vs. 26.2 months (95% CI: 20.5-29.7) (*Fig. 4*).

Clinical multivariable CoxPH models incorporating TMB and PD-L1 TPS confirmed pathway mutation as independently significant (HR 0.63, p=0.043*) *(Fig. 5*); this persisted when including sex, age and smoking (OS: HR 0.66, p=0.05). Biological models, including TMB, PD-L1 TPS, and immune biomarkers (CXCL9, CD8A, GZMA, PRF1, IFNG), supported survival benefits (HR 0.65, p=0.046*). Correlation coefficients between pathway disruption and covariates were low (e.g., IFNG, 0.005; PD-L1 TPS, -0.04; TMB, 0.14).

To assess prognostic effects, in the CARIS dataset, we evaluated analogous patients with NSCLC who had pretreatment WES, no prior or concurrent immunotherapy, but were treated with chemotherapy (mutated: n=15; intact: n=143). Pathway mutation did not associate with OS [univariable: HR 1.64, p=0.42 (*Fig. 6*); multivariable with TMB and PD-L1: HR 1.86, p=0.22].

Similarly, TCGA lung data (mutated: n=40; intact: n=902) also failed to suggest a prognostic impact on either PFI [univariable: HR 0.95, p=0.85*;* multivariable - continuous TMB, histology, stage, purity, age and sex: HR 0.99, p=0.98] or OS [univariable: HR 0.96, p=0.88 *(Fig. 7);* multivariable - continuous TMB, histology, stage, purity, age and sex: HR 1.86, p=0.70].

CONCLUSIONS

In the SU2C-MF and CARIS NSCLC cohorts, non-synonymous IFN-γ pathway mutation predicted improved ICI outcomes, independently of TMB and other variables, but was not prognostic. IFN-γ pathway disruption could guide immunotherapy stratification in NSCLC.

Figure 1: In SU2C-MF NSCLC, IFN-γ Pathway Mutation Associates with Greater Radiological Response to Immune Checkpoint Inhibition

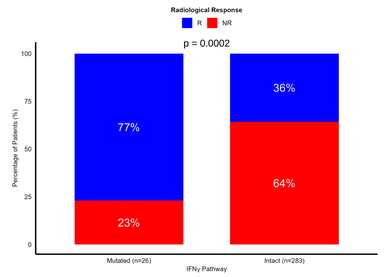


Figure 2: In SU2C-MF NSCLC, IFN-γ Pathway Mutation Associates with Improved Overall Survival in Final Multivariable Cox Models

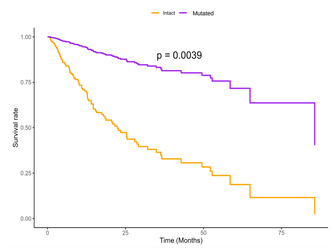
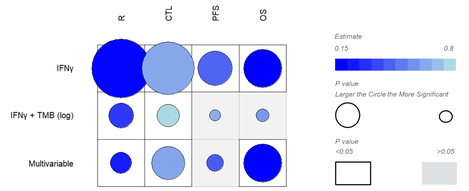


Figure 3: In SU2C-MF NSCLC, IFN-γ Pathway Mutation Significantly Associates with Improved Outcomes in Regression Models of Response and Cox Models of Survival



^ - Figure adapted from the Output of CARIS' Database

Figure 4: In CARIS NSCLC, IFN-γ Pathway Mutation Associates with Improved Overall Survival for Patients Treated with Immune Checkpoint Inhibition ^

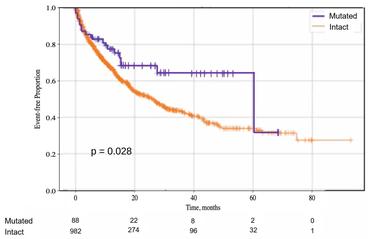


Figure 5: In CARIS NSCLC, IFN-γ Pathway Mutation Associates with Improved Overall Survival in Multivariable Models ^

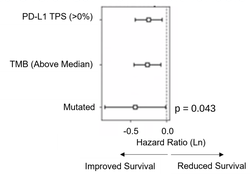


Figure 6: In CARIS NSCLC, IFN-γ Pathway Mutation has No Effect on Overall Survival for Patients Treated with Chemotherapy ^

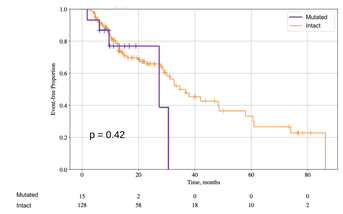
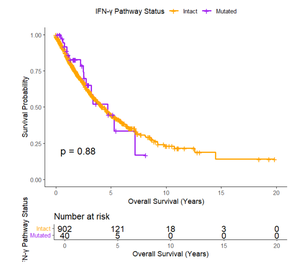


Figure 7: In the TCGA lung cohort, IFN-γ Pathway Mutation has no Association with Overall Survival



FURTHER WORK

We are assessing the functional significance of the mutations to define a biological mechanism for this strong statistical association.

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