# TNFSF14 (LIGHT) Co-Stimulation in Combination with Dual TIGIT/PD1 Checkpoint Inhibition (CPI) Induces Anti-Tumor Immunity in a CPI Acquired Resistance (AR) Model that Shares Transcriptional Similarities to Lung Cancer Patients who Acquire Resistance to CPI

SHATTUCK LABS

#1697

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## Abstract

More than half of non-small cell lung cancer patients who initially benefit from PD-1/L1 checkpoint blockade later develop progressive disease, known as acquired resistance (AR). Very little is understood about the underlying mechanisms of AR to checkpoint inhibitors (CPI). With increasing CPI use across a large number of tumor types, the proportion of cancer patients with CPI-AR is increasing, and an improved understanding of the mechanisms which drive CPI-AR may aid in the discovery of therapeutics to reverse AR. Preclinical tumor models which mimic CPI-AR in humans are needed.

We generated a murine CPI-AR tumor model by serially passaging CT26 colon cancer cells *in vivo*, followed by the excision and *ex vivo* expansion of the tumors that failed to respond to PD1 antibody therapy. This was repeated until passaged tumors no longer responded to anti-PD1 treatment. Transcriptomes of CT26/WT (wild type) and CT26/AR tumors were sequenced either under normal culture conditions, or following 24-hour exposure to IFNγ, to assess interferon responsiveness. Paradoxically, CPI-AR tumors maintain a baseline state of type I/II IFN gene hyperactivation and increased expression of genes involved in MHC class I mediated antigen presentation/processing and Jak/Stat signaling pathways. Additionally, upon challenge with IFNγ, CPI-AR tumors are unable to up-regulate gene expression for PD-L1 (Cd274), Irf1, Stat1, Stat2, Tap1, β2m, and other key pathways typically up-regulated by IFN stimulation.

The transcriptomes of CT26/AR tumors and human non-small cell lung cancer (NSCLC) biopsies from patients who developed acquired resistance to checkpoint inhibition were strikingly similar. In particular, the comparison demonstrated a similar dysregulation of IRF1, STAT1, STAT2, and other genes involved in type I/II IFN responsiveness, Jak/Stat signaling, and the antigen processing machinery. These data suggest that the CT26/AR model could be useful in identifying therapies and/or combinations that may have unique activity and address this unmet need.

TIGIT is an immune cell expressed checkpoint molecule that is up-regulated downstream of PD1. To date, clinical responses to TIGIT blockade have been isolated to anti-PD-1 naïve tumors expressing high-levels of PD-L1, and dependent upon co-administration of a PD-1/L1 blocking antibody. Thus, extending the utility of TIGIT blockade to either CPI-AR or PD-L1 low tumors requires additional investigation. We developed a bi-functional **TIGIT-Fc-LIGHT** fusion protein that is capable of simultaneously blocking checkpoint activity through the TIGIT:PVR axis, stimulating adaptive/innate immune activation in T/NK cells via LIGHT:HVEM, and also inducing a proinflammatory tumor microenvironment (TME) via LIGHT:LTβR interactions. Due to the observed upregulation of Ltbr and Pvr gene expression in the CT26/AR tumor model, we hypothesized that **TIGIT-Fc-LIGHT** could have unique anti-tumor activity both alone or in combination with anti-PD-1/L1 antibodies.

We found that **TIGIT-Fc-LIGHT** delayed tumor progression and extended survival of mice bearing CT26/WT and /AR tumors in a CD8+ T cell and NK cell dependent manner; which may be due to the abundant expression of HVEM on TIL isolated from these animals. Consistent with emerging clinical data, combined TIGIT + PD1 antibody therapy showed little activity in the CT26/AR model. Together, the data presented here has uncovered unexpected conservation of transcriptional dysregulation in CPI-AR murine and human tumors, and also identified a bifunctional **TIGIT-Fc-LIGHT** fusion protein that demonstrated efficacy in this setting.

## Generation of Anti-PD1 Acquired Resistance Model (AR)

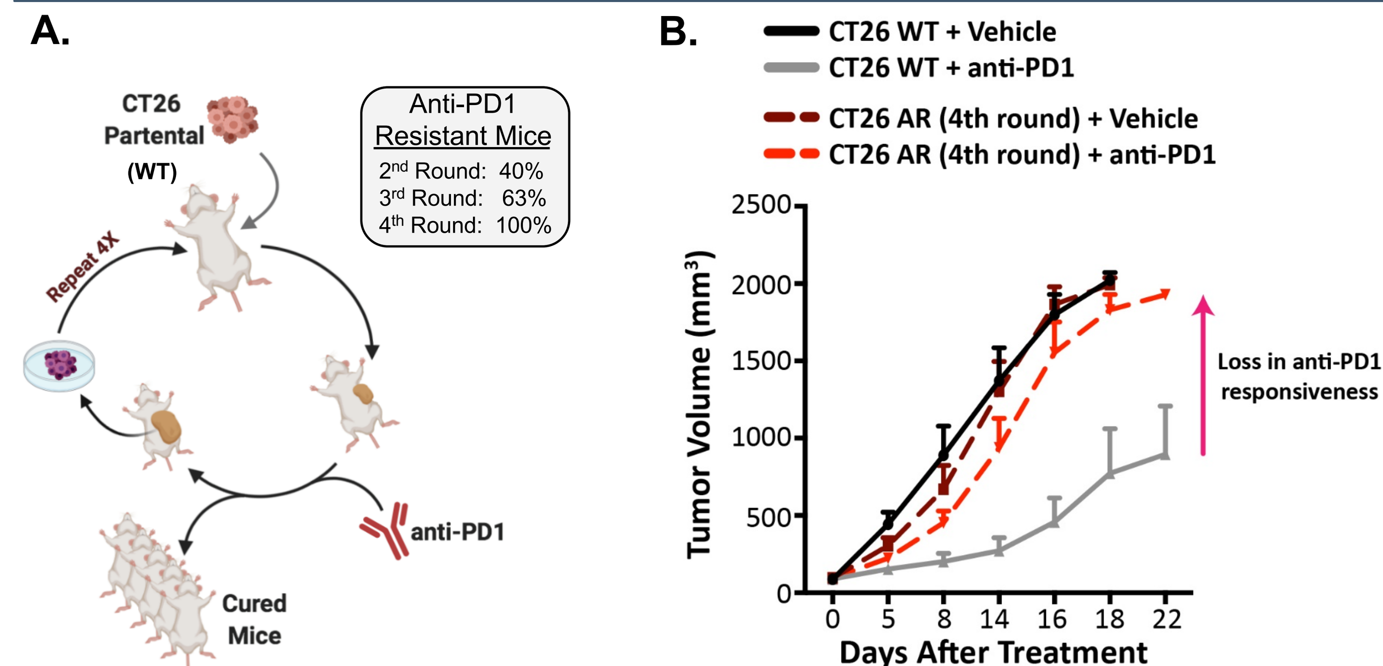


**Figure 1. Generation of anti-PD1 acquired resistance model (CT26/AR).** (A) Mice with established WT CT26 colon tumors were treated with anti-PD1 therapy. Tumors were excised from non-responsive mice, expanded *ex vivo*, inoculated into new recipient mice, and given another course of anti-PD1. (B) This process was repeated for a total of four rounds, until there were no remaining mice sensitive to anti-PD1 (shown are average tumor growth curves). Two distinct tumor clones isolated from the 2nd round and four distinct tumor clones isolated from the 4th round, are described throughout this poster.

## Paradoxical Transcriptional Hyperactivity and IFN Pathway Upregulation in Check Point Inhibitor Acquired Resistance Tumors

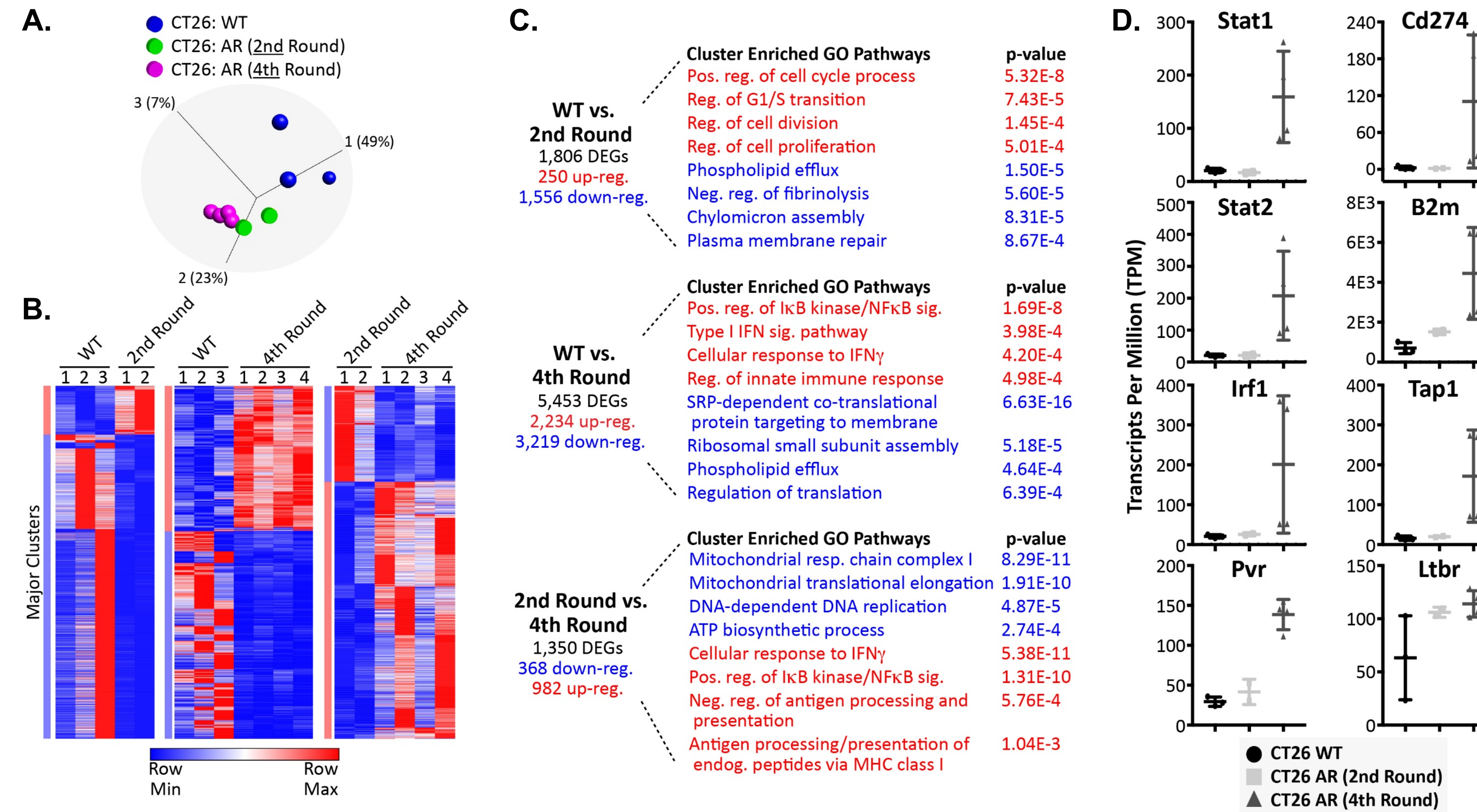


**Figure 2. RNA-seq analysis of CT26/WT and CT26/AR.** (A) PCA spatial analysis of three CT26 parental replicates, two '2nd round' clones, and four '4th round' clones. (B) DESeq was used to determine differentially expressed genes (DEGs) between WT and '2nd round', WT and '4th round', and '2nd round' and '4th round'. Shown are the transcripts per million (TPM) values displayed as row min/max, and hierarchical clustering identified two major clusters. (C) The number and directionality of DEGs are shown, along with gene ontology associations from each gene set (GO PANTHER). (D) TPM values of example genes demonstrate a progressive increase in expression upon *in vivo* anti-PD1 selective pressure.

## Acquired Resistance Tumors Have Dysfunctional Responses to IFNγ Stimulation *Ex Vivo*

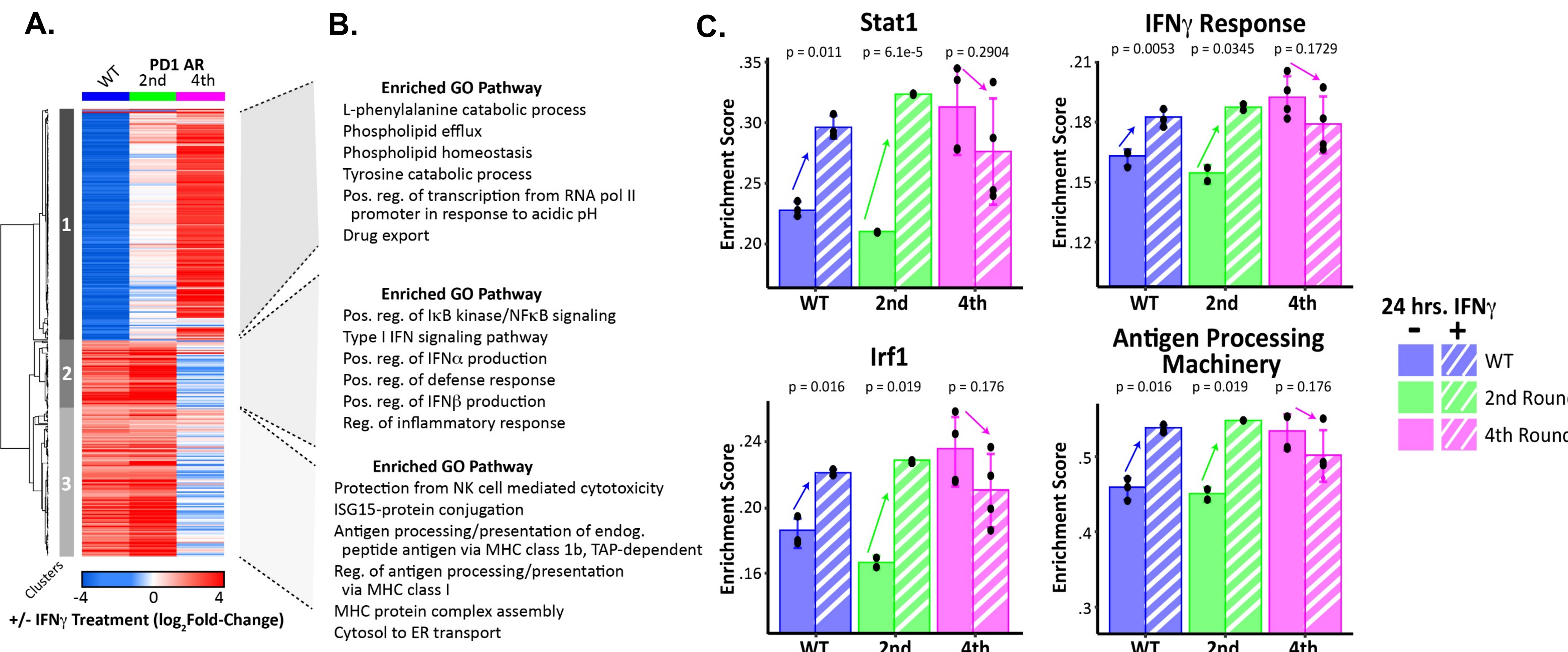


**Figure 3. RNA-seq analysis of IFN responsiveness in CT26/WT and CT26/AR.** (A) Transcriptomes from CT26 WT, '2nd round', and '4th round' AR cells cultured for 24 hours with 20 ng/mL IFNγ were assessed by RNA-seq. The DEGs identified in WT CT26 in response to IFNγ were plotted in the heatmap, and the corresponding responsiveness of '2nd round' and '4th round' cells to IFNγ at these genes are shown. (B) Hierarchical clustering identified three major clusters of genes, and the pathways associated with these genes (GO PANTHER) are shown. (C) Comparison of significance of change in gene set Enrichment Scores in WT CT26, '2nd round', and '4th round' AR cells following IFNγ treatment.

## NSCLC Patients that Develop CPI Acquired Resistance also Dysregulate Transcription of IFN Pathway Genes

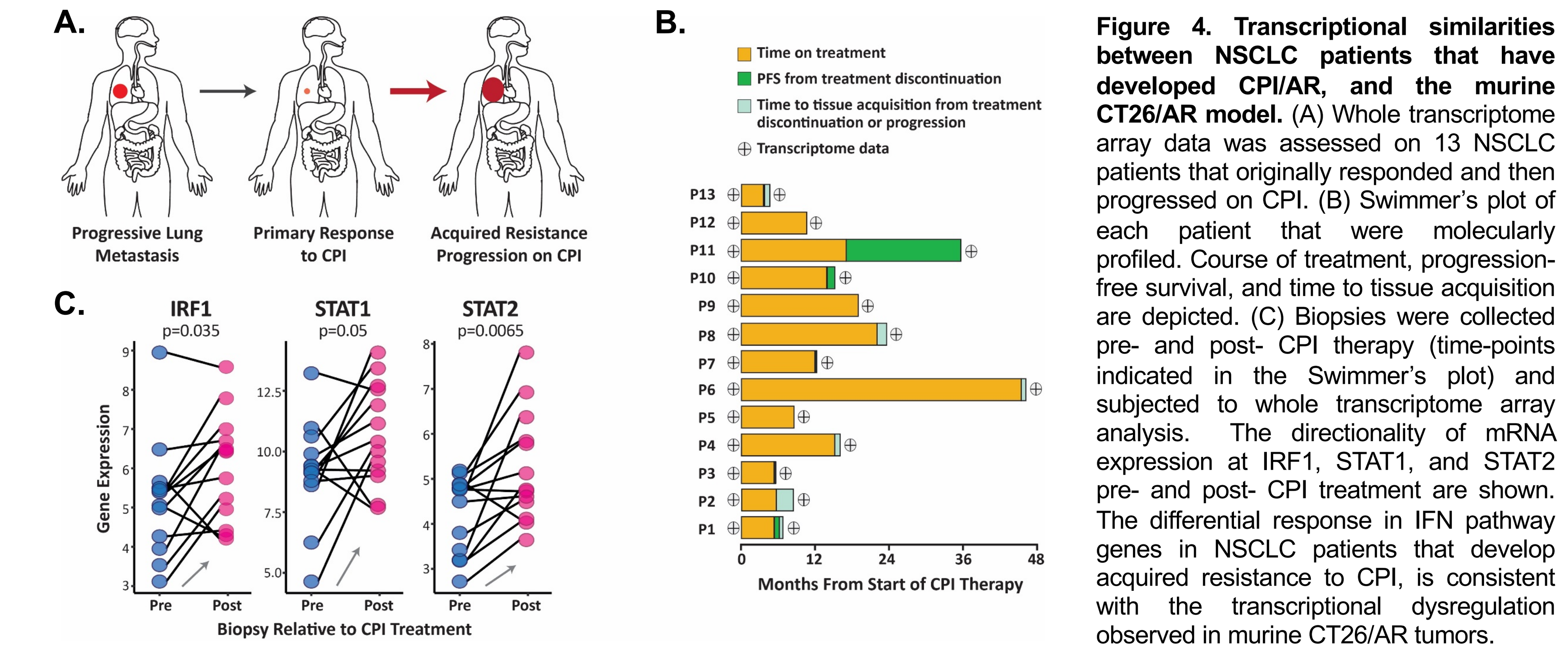


**Figure 4. Transcriptional similarities between NSCLC patients that have developed CPI/AR, and the murine CT26/AR model.** (A) Whole transcriptome array data was assessed on 13 NSCLC patients that originally responded and then progressed on CPI. (B) Swimmer's plot of each patient that were molecularly profiled. Course of treatment, progression-free survival, and time to tissue acquisition are depicted. (C) Biopsies were collected pre- and post- CPI therapy (time-points indicated in the Swimmer's plot) and subjected to whole transcriptome array analysis. The directionality of mRNA expression at IRF1, STAT1, and STAT2 pre- and post- CPI treatment are shown. The differential response in IFN pathway genes in NSCLC patients that develop acquired resistance to CPI, is consistent with the transcriptional dysregulation observed in murine CT26/AR tumors.

## A Bi-Functional TIGIT-Fc-LIGHT Fusion Protein is Highly Active in Both CPI-Responsive and CPI-Acquired Resistance Tumor Models

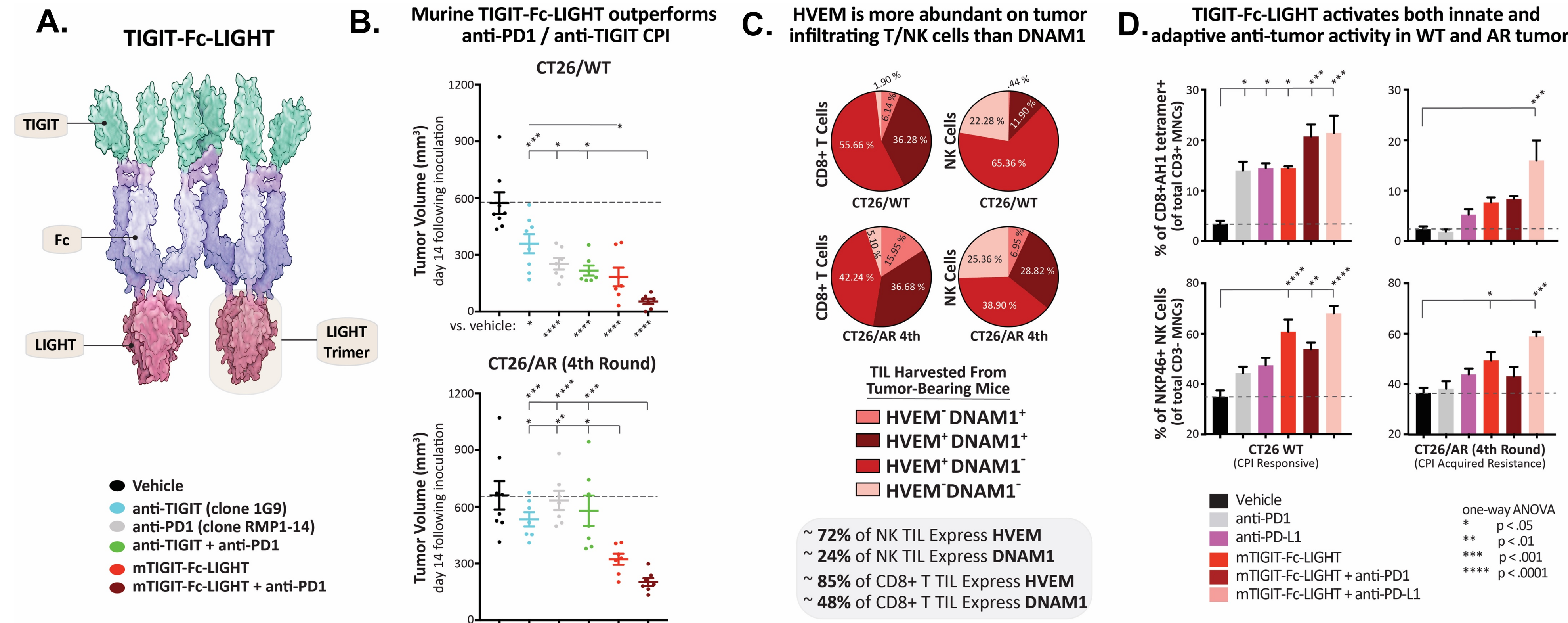


**Figure 5. *In vivo* anti-tumor activity of TIGIT-Fc-LIGHT.** (A) TIGIT-Fc-LIGHT exists as a hexamer with two functional sets of LIGHT trimers linked to six individual TIGIT extracellular domains. (B) Mice bearing WT or AR mutant CT26 cells were treated with anti-TIGIT, anti-PD1, the antibody combination, anti-PDL1 (clone 10F.9G2), mTIGIT-Fc-LIGHT, or mTIGIT-Fc-LIGHT + anti-PD1 or anti-PDL1 (Drugs given via IP injection on days 7,10,13; 100ug per antibody and 200ug of mTIGIT-Fc-LIGHT). Shown are the mean tumor volumes between treatment groups on day 14 after tumor inoculation. (C) Tumors were excised from a cohort of mice on day 14, dissociated, and HVEM / DNAM1 expressing T and NK cells were assessed by flow cytometry. (D) Tumors were excised from another cohort of mice on day 14, dissociated, and the percent of antigen-specific (AH1+) CD8 T cells and NK cells in both WT and AR mutant CT26 were assessed by flow cytometry.

## Conclusions and Acknowledgements

- A preclinical model of checkpoint acquired resistance (CT26/AR) revealed a state of transcriptional hyperactivity, associated with inverted sensitivity to IFN-signaling, antigen processing machinery, and other key cellular functions.
- NSCLC patients that developed acquired resistance and progress on CPI, displayed similar transcriptomic and phenotypic signatures as the murine AR model.
- A **TIGIT-Fc-LIGHT** bi-functional fusion protein that is able to simultaneously block TIGIT:PVR checkpoint signaling and activate both innate and adaptive anti-tumor immunity, retained activity in this resistance model and outperformed dual checkpoint blockade with anti-PD1 +/- anti-TIGIT.
- We would like to thank Shannon Tsai from the University of North Carolina at Chapel Hill for bioinformatics support and patients with cancer that play a brave and essential role in the development of novel immunotherapies.

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