THE TEMPORAL CONTRIBUTION OF INTERFERON-γ IN DRIVING T-CELL EXHAUSTION AND RESPONSE TO IMMUNE CHECKPOINT BLOCKADE

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Background Immune checkpoint blockade (ICB) therapies have revolutionized treatment for cancer patients, yet minority do not respond, highlighting the importance of understanding therapeutic resistance. Interferon-gamma (IFNγ) drives protective T cell responses and augments anti-tumor immunity yet also promotes T cell exhaustion during tumor progression. Such dichotomy exhibited by IFNγ impacts immunotherapeutic responses. However, whether IFNγ continues to modulate immune responses on exhausted T (TEx) cells remains unanswered. Our lab has been extensively studying lineage plasticity of TEx cells by lineage-tracing lymphocyte activation gene-3 (LAG3)-expressing cells in the context of anti-tumor responses. We hypothesize that understanding the temporal expression profile of IFNγ on TEx cells will help address mechanistic insights into diverse responses to ICB.

Methods To evaluate the pleiotropic role of IFNγ, we established two unique murine models, one assessing temporal tamoxifen induced global transcriptional expression of Ifnγ on Lag3-expressing cells (Lag3iCreERT2 IfnγYFP Rosa26Rosa26LSL-tdTomato). This allows the immune cell-specific contribution of IFNγ on progenitor TEx and terminal TEx cells to be assessed. The second model temporally induces genetic deletion of Ifnγ on Lag3-expressing cells (Lag3iCreERT2 IfnγL/L Rosa26Rosa26LSL-tdTomato). Melanoma (B16F10) and adenocarcinoma (MC38) models were used to evaluate tumor growth and survival kinetics, TEx cell profile and response to ICB therapy.

Results Assessing the transcriptional profile of Ifnγ shows that IFNγ is expressed by LAG3-expressing cells as early as D7, a progenitor TEx state, with NK and NKT cells as major contributors. Expression of IFNγ is maintained through a terminally exhausted state (D23), with CD8+ T cells as the major IFNγ-producing cells. Our Lag3iCreERT2 IfnγL/L Rosa26Rosa26LSL-tdTomato model system, allows to examine the pleiotropic effects of IFNγ in early immune responses as well as T cell exhaustion (early vs terminal). Early temporal deletion of IFNγ (D5–D7) potentiates tumor growth in MC38 model with no survival advantage with anti-PD1. This suggests that during initial antigen exposure, IFNγ is necessary for reinvigoration of anti-tumor response and deletion of Ifnγ augments T cell exhaustion. However, with later time-point deletion (D11–D13), we observed 50% survival advantage with anti-PD1 ICB, which suggests modulating tumor microenvironment in a time-dependent manner is the key to augmenting ICB response.

Conclusions This study highlights the distinct temporal response patterns and exhaustion profile with deletion of Ifnγ from pre-exhausted to terminally exhausted Lag3+ cells. IFNγ production at early time points (D5–D7) is a key mediator of anti-tumor immunity while the deletion of Ifnγ at terminal points (D11–D13) highlights better ICB response.

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