IL-6 contributes to the suppression of T and NK cell anti-tumor activity in EGFR-mutant NSCLC

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Introduction

- Patients with NSCLC with activating mutations in epidermal growth factor receptor (EGFR) receive clinical benefit from treatment with EGFR tyrosine kinase inhibitors (TKIs)
- The majority of these patients will acquire resistance which can be mediated by various mechanisms including secondary *EGFR* mutations such as T790M, *MET* amplification, or EMT.
- Anti-PD-1/PD-L1 immune checkpoint-blockade demonstrated clinical benefit in NSCLC patients. However, among patients with EGFR-mutant NSCLC, response rates to immunotherapy are minimal.
- Previous studies show that IL-6 is a critical mediator of EGFR-TKI resistance. Thus, we sought to investigate the impact of IL-6 on anti-tumor immunity in EGFR-mutant NSCLC.

Hypothesis

Given the immunosuppressive role of IL-6, we hypothesized that IL-6 in part mediates the immunosuppressive phenotype responsible for EGFR-TKI resistant NSCLC's marginal response to anti-PD-1/PD-L1 therapy through altering the tumor infiltrating immune cell populations and modulating their cytotoxic potential.

References

1. Engelman, *et al. Clin Cancer Res* **14**, 2895-2899 (2008).
2. Nilsson, *et al. Sci Transl Med* **9** (2017).
3. Lee et al., *JAMA Oncology (*2018).

Results

Acquired EGFR-TKI resistance is associated with increased levels of IL-6

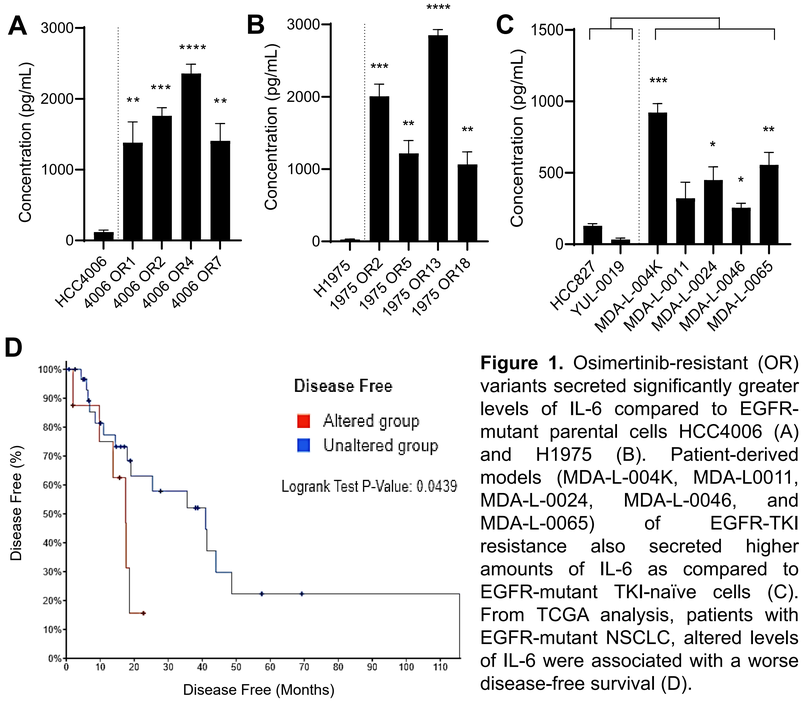

Figure 1. Osimertinib-resistant (OR) variants secreted significantly greater levels of IL-6 compared to EGFR-mutant parental cells HCC4006 (A) and H1975 (B). Patient-derived models (MDA-L-004K, MDA-L0011, MDA-L-0024, MDA-L-0046, and MDA-L-0065) of EGFR-TKI resistance also secreted higher amounts of IL-6 as compared to EGFR-mutant TKI-naïve cells (C). From TCGA analysis, patients with EGFR-mutant NSCLC, altered levels of IL-6 were associated with a worse disease-free survival (D).

Depletion of IL-6 increases overall survival and number of infiltrating lymphocytes in EGFR mutant GEMMs

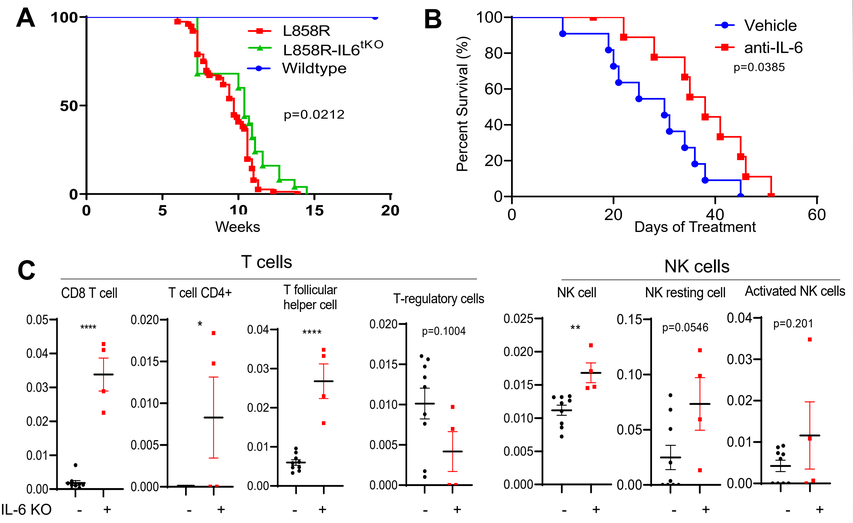

Figure 2. Survival analysis of EGFR^{L858R} GEMMs and EGFR^{L858R} GEMMs crossed with an IL-6 knockout mouse showed that knockout of IL-6 significantly increased overall survival (A). EGFR-mutant NSCLC tumors treated with mouse anti-IL-6 blocking antibody significantly increased overall survival of mice (B). Knockout (KO) of IL-6 resulted in increased NK cell populations and the CD8 T cell populations but a decreased T-regulatory and T follicular helper cell populations (C).

EGFR-independent TKI resistant tumors display mesenchymal, immunologically cold phenotype and secrete IL-6

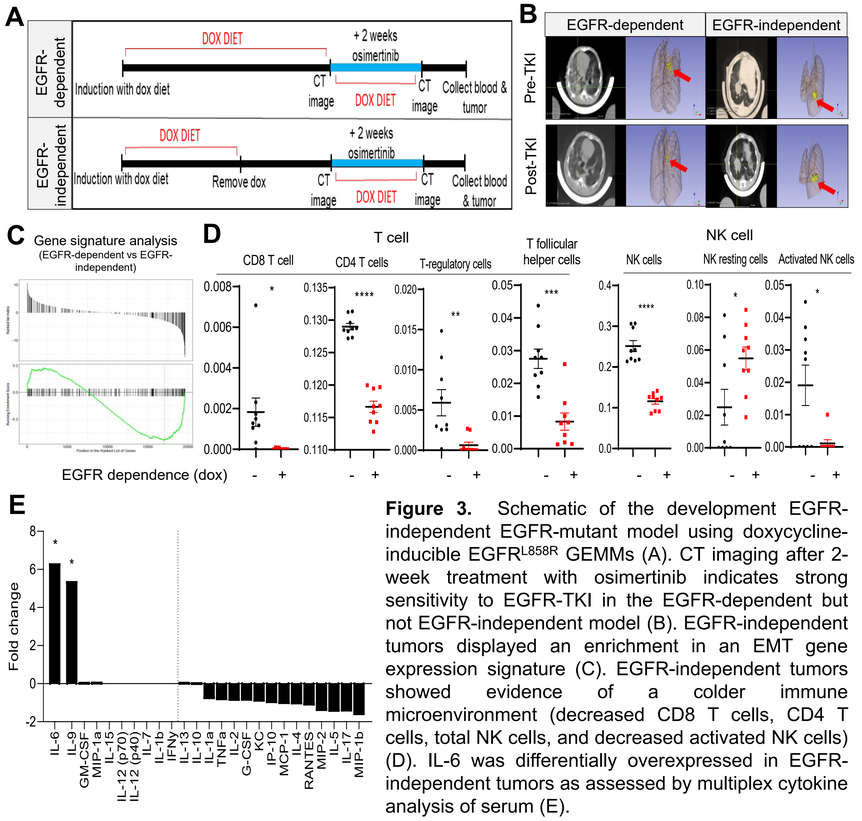

Figure 3. Schematic of the development EGFR-independent EGFR-mutant model using doxycycline-inducible EGFR^{L858R} GEMMs (A). CT imaging after 2-week treatment with osimertinib indicates strong sensitivity to EGFR-TKI in the EGFR-dependent but not EGFR-independent model (B). EGFR-independent tumors displayed an enrichment in an EMT gene expression signature (C). EGFR-independent tumors showed evidence of a colder immune microenvironment (decreased CD8 T cells, CD4 T cells, total NK cells, and decreased activated NK cells) (D). IL-6 was differentially overexpressed in EGFR-independent tumors as assessed by multiplex cytokine analysis of serum (E).

IL-6 suppresses the activation of NK cells in the EGFR-mutant microenvironment

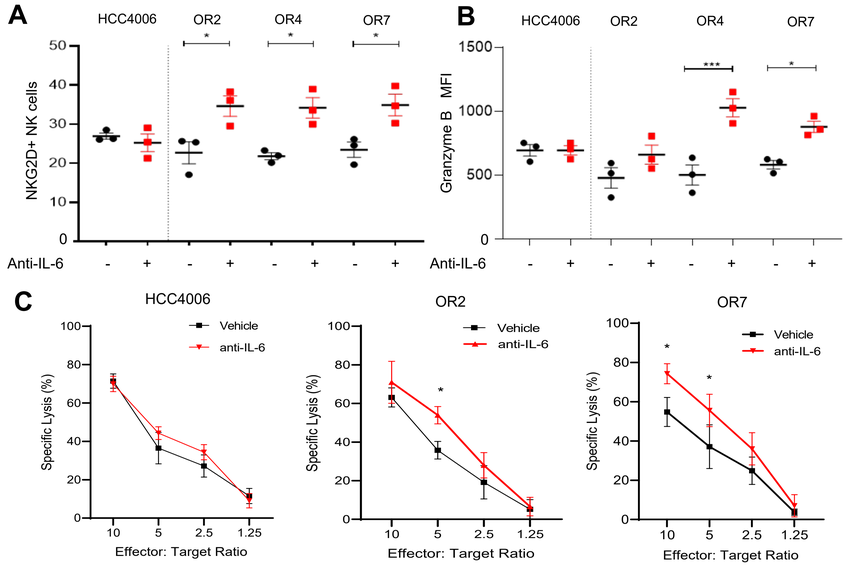

Figure 4. Blockade of IL-6 increased NKG2D (A) and Granzyme B (B) expression in the NK cells co-cultured with EGFR-mutant NSCLC tumor cell lines. Blockade of IL-6 in the EGFR-TKI refractory cells co-cultured with NK cells increased NK cell-mediated killing (C).

Blockade of IL-6 induces T cell activity in EGFR-mutant NSCLC microenvironment

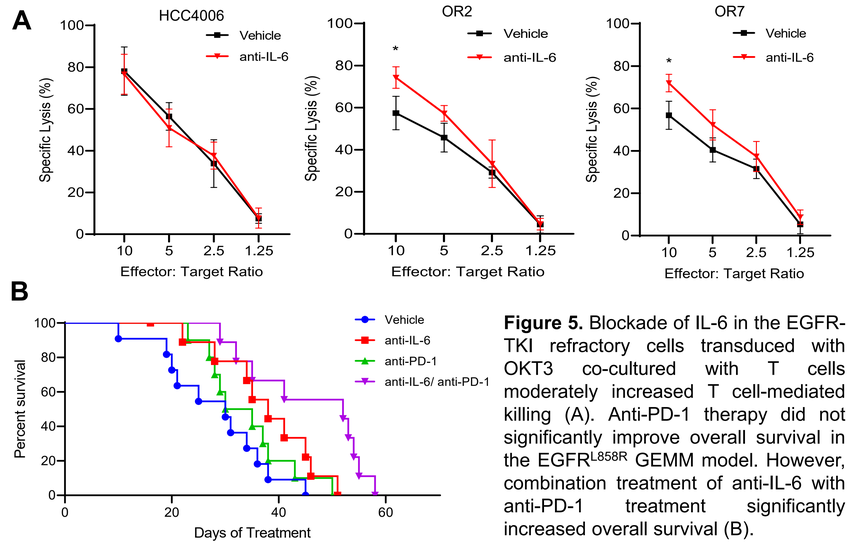

Figure 5. Blockade of IL-6 in the EGFR-TKI refractory cells transduced with OKT3 co-cultured with T cells moderately increased T cell-mediated killing (A). Anti-PD-1 therapy did not significantly improve overall survival in the EGFR^{L858R} GEMM model. However, combination treatment of anti-IL-6 with anti-PD-1 treatment significantly increased overall survival (B).

Conclusion

IL-6 is upregulated in EGFR-mutant NSCLC tumors with acquired resistance to EGFR-TKIs and impairs anti-tumor immunity through suppression of T and NK cell function.

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