Abstract

Epidermal growth factor receptor *(EGFR)* is the most frequently mutated driver oncogene in non-smoking–related, non-small–cell lung cancer (NSCLC). *EGFR*-mutant NSCLC has a non-inflamed tumor microenvironment (TME), with low infiltration by $CD8^+$ T cells and, thus, immune checkpoint inhibitors, such as anti-programmed cell death-1 (anti–PD-1) have weak anti-tumor effects. Here, we showed that $CD8^+$ T-cell responses were induced by an EGFR-tyrosine kinase inhibitor (TKI) in syngeneic *Egfr*-mutant NSCLC tumors, which was further pronounced by sequential dual blockade of PD-1 and vascular endothelial growth factor receptor 2 (VEGFR2). However, simultaneous triple blockade had no such effect. PD-1/VEGFR2 dual blockade did not exert tumor-inhibitory effects without pre-treatment with the EGFR-TKI, suggesting that treatment schedule is crucial for efficacy of the dual blockade therapy. Pre-treatment with EGFR-TKI increased the $CD8^+$ T-cell/regulatory T-cell (Treg) ratio, while also increasing expression of immunosuppressive chemokines and chemokine receptors, as well as increasing the number of M2-like macrophages, in the TME. Discontinuing EGFR-TKI treatment reversed the transient increase of immunosuppressive factors in the TME. The subsequent PD-1/VEGFR2 inhibition maintained increased numbers of infiltrating $CD8^+$ T cells and $CD11c^+$ dendritic cells.

Depletion of $CD8^+$ T cells *in vivo* abolished tumor growth inhibition by EGFR-TKI alone and the sequential triple therapy, suggesting that EGFR inhibition is a prerequisite for the induction of $CD8^+$ T-cell responses. Our findings could aid in developing an alternative immunotherapy strategy in patients with cancers that have driver mutations and a non-inflamed TME.