Compensation between CSF1R⁺ macrophages and Foxp3⁺ Treg cells drives resistance to tumor immunotherapy

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Redundancy and compensation provide robustness to biological systems but may contribute to therapy resistance. Both tumor-associated macrophages (TAMs) and Foxp3⁺ regulatory T (Treg) cells promote tumor progression by limiting antitumor immunity. Here we show that genetic ablation of CSF1 in colorectal cancer cells reduces the influx of immunosuppressive CSF1R⁺ TAMs within tumors. This reduction in CSF1-dependent TAMs resulted in increased CD8⁺ T cell attack on tumors, but its effect on tumor growth was limited by a compensatory increase in Foxp3⁺ Treg cells. Similarly, disruption of Treg cell activity through their experimental ablation produced moderate effects on tumor growth and was associated with elevated numbers of CSF1R⁺ TAMs. Importantly, codepletion of CSF1R⁺ TAMs and Foxp3⁺ Treg cells resulted in an increased influx of CD8⁺ T cells, augmentation of their function, and a synergistic reduction in tumor growth. Further, inhibition of Treg cell activity either through systemic pharmacological blockade of PI3K

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Introduction

Tumors grow in immunocompetent hosts despite the ability of the adaptive immune system to rec-

that genetic inactivation of PI3K in mice protects against a wide range of solid tumors (27). This immunomodulatory effect was due to the inactivation of PI3K in CD4^{+}Foxp3^{+} Treg cells, unleashing CD8^{+} cytotoxic T lymphocytes which could then induce tumor regression (27). The PI3K inhibitor, idelalisib (Zydelig, Gilead) has proven highly effective for the treatment of chronic lymphocytic leukemia (26), and exerts its main effect by blocking the interactions between lymphocytic leukemia cells and stromal cells in their lymphoid niche. However, the extent to which PI3K inhibitors mayhic.429 4er

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Given the observation that CSF1R$^+$

these data indicate that CSF1R⁺ TAMs express PD-L1, secrete TGF-β_1, and are capable of limiting CD8⁺ T lymphocyte proliferation ex vivo, but other sources of immunosuppression may contribute to the failure of total tumor rejection with CSF1 ablation alone.

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Recent studies indicate that PI3K plays an important role in the maturation of Foxp3^{+} Treg cells and that this effect can supercede a smaller role for PI3K in CD8^{+} T cell function, such that tumors relying heavily on Treg -mediated suppression of CD8^{+} T cells for growth can be inhibited by deletion of PI3K (27). We investigated a potential role for PI3K in the MC38 model using mice with a Treg-specific deletion of PI3K .
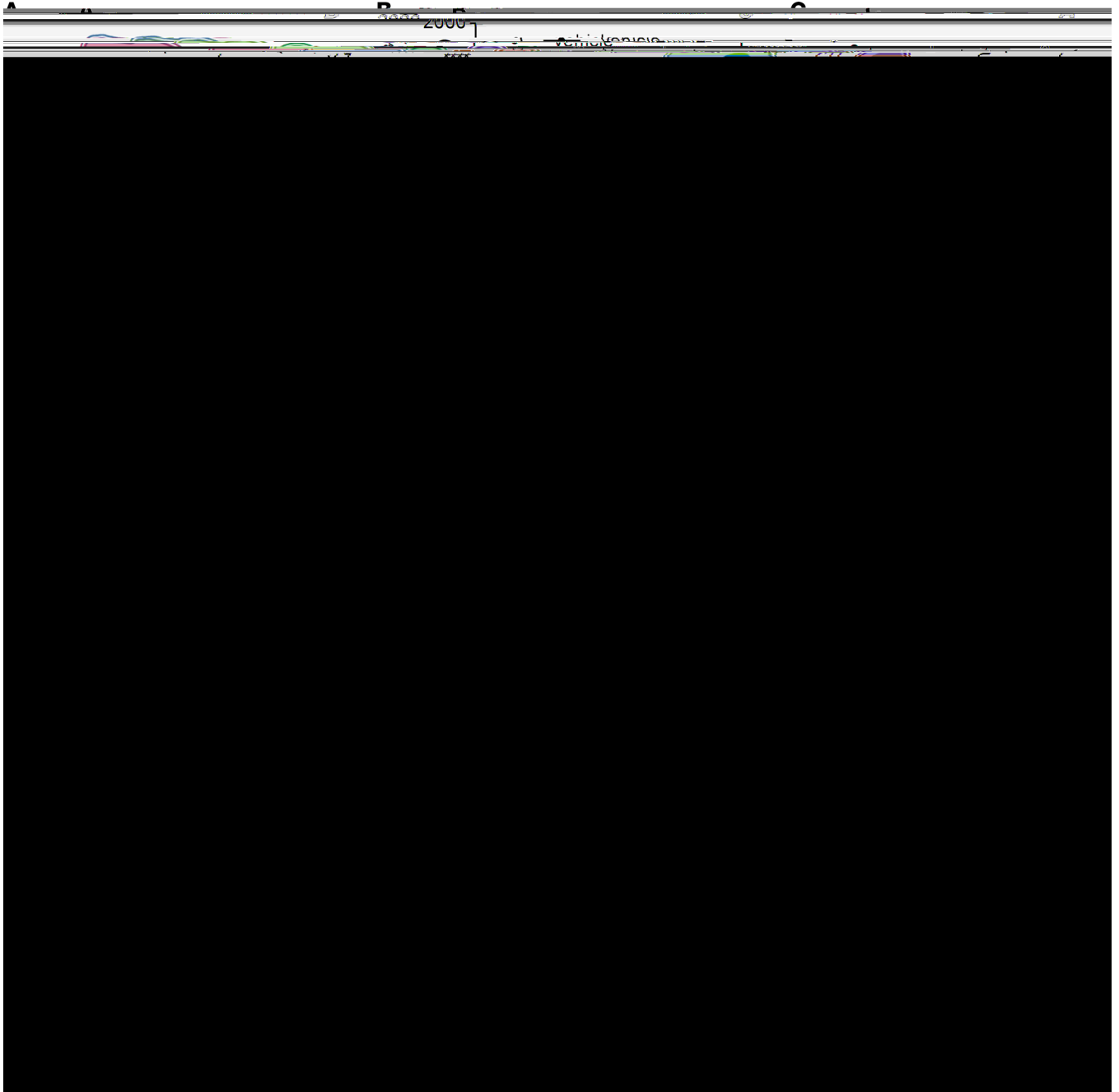
Figure 5. Combined CSF1R and PI3Kδ inhibition. (**A**) Graphical abstract. (

TAMs and Foxp3+ Treg cells might contribute to monotherapy resistance (Figure 5A). To this end, C57BL/6 mice were orally dosed with 40 mg/kg PLX3397 and/or 100 mg/kg idelalisib daily from day 7 after tumor implantation, when the tumors became palpable. Control mice received vehicle (0.5% w/v methylcellulose). Importantly, and consistent with compensatory immunosuppression driving therapy resistance to immune monotherapy, only tumors treated with the combination of PLX3397 and idelalisib showed a statistically significant reduction in tumor growth and primary tumor mass (Figure 5, B and C). By contrast, single-agent PLX3397 or idelalisib-treated tumors grew at the same rates compared with the

vehicle controls. As a confirmation of the depletion of TAMs by PLX3397, we detected a reduced proportion of CSF1R^{+} macrophages among intratumoral myeloid cells by flow cytometry (Figure 5, D and E). Similarly, flow cytometry confirmed the depletion of Treg cells by idelalisib as a reduced proportion of CD4^{+} Foxp3^{+} Treg cells among intratumoral lymphocytes (Figure 5, F and G). Consistent with a synergistic reversal of immunosuppression, we detected an increase in CSF1R^{+} macrophages in idelalisib-treated tumors (Figure 5, D and E), an increase in Foxp3^{+} Treg cells in PLX3397-treated tumors (Figure 5, F and G), and a significant increase in the proportion of CD8^{+} T lymphocytes among CD45^{+} cells in the MC38 tumors treated with the combination of PLX3397 and idelalisib (Figure 5, H and I). Further, we detected similar synergistic effects when combining CSF1 blockade with PI3K inhibition in the B16-F10 tumor model (Supplemental Figure 3, A and B). Collectively, these findings provide evidence of compensatory immunosuppression between CSF1R^{+} macrophages and PI3K -driven Foxp3^{+} Treg cells and provide a rationale for combinatorial therapy using CSF1- and PI3K -targeted approaches.

Discussion

TAMs and Treg cells are critical components of the tumor microenvironment, and contribute to every aspect of tumor growth and progression (5, 21, 30). Here, we ollectiVol 88 5.8 Tw -40.289(4 (R1.1900 Tc 0 Tw 5.101:

tumor implantation. Tumor growth was monitored as described above, and processing of tissues at the

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