Sunitinib, like other relatively unselective TKIs, affects not only malignant and endothelial cells but also immune cells. Indeed, it has been shown that sunitinib decreases the number of regulatory T cells (Tregs) in a mouse model of renal cancer as well as in metastatic renal cancer patients. Tregs accumulate in tumor-bearing mice and in the peripheral blood of cancer patients and negatively regulate antitumor T-cell responses. Moreover, although this remains a matter of debate, the accumulation of Tregs is generally associated with poor clinical outcomes. In a relevant mouse model of CRC (based on murine CT26 cells), we confirmed that sunitinib can inhibit the accumulation of Tregs, both in the spleen and within neoplastic lesions. Next, since sunitinib is a multi-kinase inhibitor that targets VEGFR, PDGFR, KIT and FLT3, we analyzed the impact of blocking the VEGFA/VEGFR signaling pathway in this model. The blockade of VEGFA with a specific antibody resulted in a decrease in Tregs similar to that achieved with sunitinib. In addition, the administration of masitinib (a TKI that targets KIT and PDGFR, but not VEGFR) had no effects on the accumulation of Tregs in CRC-bearing mice. These findings suggest that blocking the VEGFA/VEGFR signaling pathway is sufficient to inhibit Treg accumulation in a mouse model of CRC. In line with these preclinical data, we observed that the combination of bevacizumab with standard chemotherapeutic agents, but not chemotherapy alone, decreased the proportion of circulating Tregs in metastatic CRC patients.

Although we found that blocking VEGFA/VEGFR-transduced signals counteracts the induction of Tregs by malignant cells, the underlying mechanisms remained unclear. The accumulation of Tregs in the course of tumor progression is essentially attributed to the conversion of conventional T cells into Tregs or to the proliferation of pre-existing Tregs. It has previously been shown that sunitinib can prevent the conversion of CD4+FOXP3+ T cells into CD4+FOXP3+ T lymphocytes in vitro and in vivo by inhibiting the phosphorylation of signal transducer and activator of transcription 3 (STAT3) in melanoma-bearing mice. VEGFA also inhibits the maturation of dendritic cells and induce the expansion of myeloid-derived suppressor cells (MDSCs). Immature dendritic cells as
well as MDSCs can preferentially induce the development of Tregs. Since the administration of sunitinib had previously been shown to decrease the amount of MDSCs and as bevacizumab was known to restore dendritic cell maturation, we reasoned that both these mechanisms could be involved in the modulation of Treg abundance by VEGFA/VEGFR-targeting therapies. However, the direct effect of VEGFA on Tregs had never been studied before.

Since VEGFA is a mitogenic factor for endothelial cells and some types of cancer cells, we investigated the impact of VEGFA on Treg proliferation. Circulating VEGFA levels are increased in both tumor-bearing mice and metastatic CRC patients as compared with tumor-free animals and healthy volunteers, respectively. We observed that the proliferation of Tregs is accelerated in tumor-bearing mice and in metastatic CRC patients, a phenomenon that—in both settings—could be limited by the administration of anti-VEGFA antibodies. Of note, this effect was restricted to CD44hi memory Tregs, suggesting that VEGFA-targeting agents could reduce the cycling of a specific subset of tumor antigen-specific Tregs. In vitro, VEGFA stimulated the proliferation of Tregs purified from tumor-bearing but not from tumor-free mice. This observation could be attributed to the restricted expression of VEGFR1 and VEGFR2 on Tregs obtained from mice bearing neoplastic lesions but not from tumor-naive animals. Of note, only anti-VEGFR2 antibodies turned out to decrease the proliferation of Tregs exposed to VEGFA in vitro and their accumulation in tumor-bearing mice. Taken together, our results highlight the ability of tumor-derived VEGFA to stimulate the proliferation of VEGFR2+ Tregs, and that this phenomenon is efficiently inhibited by VEGFA/VEGFR-targeted therapies in preclinical models of CRC as well as in CRC patients.

Thus, VEGFA plays a key role in the establishment of an immunosuppressive tumor microenvironment, as it inhibits the maturation of dendritic cells, induces the expansion of MDSCs and stimulates the proliferation of Tregs (Fig. 1). The administration of recombinant VEGFA to tumor-free mice decreases the absolute and relative abundance of T cells and inhibits their functions. VEGFA also controls the infiltration of neoplastic lesions by T lymphocytes by modulating the expression of intercellular adhesion molecule 1 (ICAM1) and ICAM2 on endothelial cells. Thus, VEGFA can contribute to prevent the development of efficient antitumor immune responses by promoting local and systemic immunosuppression. VEGFA/VEGFR-targeting therapies may therefore revert such an immunosuppressive state and, in addition to their effects on the tumor stroma, positively modulate antitumor immunity. These agents offer some advantages since (1) they can simultaneously inhibit several immunosuppressive pathways; (2) they may restore the physiological proportion of Tregs and minimize the occurrence of autoimmune side effects, which are often associated with complete
Treg depletion; and (3) they do not deplete conventional T cells. The safety and antineoplastic activity of VEGFA/VEGFR-targeting agents combined with immunotherapy should now be evaluated in clinical settings.

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**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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