melanoma and the recent successes obtained in clinical trials using anti-PD1 and PD-L1 mAbs[2] demonstrate the potency of immunotherapy strategies. The clinical development of these immunomodulatory antibodies represents a paradigm shift in cancer therapy: for the first time the immune system is targeted rather than the tumor itself.

Although the immune system has been demonstrated to contribute to the activity of certain chemotherapies,[3] most are also affecting immune cells activated during the tumor cell destruction. Thus, the combination of tumor-targeted therapy with immunostimulation is an attractive approach as demonstrated by Yang and colleagues.[1] However, it is important to stress that targeted therapies may have previously undescribed effects that might alter immune cells.

Indeed, the immune effect of dasatinib reported here might operate via several mechanisms (see figure). First, by inducing targeted tumor cell death, dasatinib might lead to immunogenic tumor debris as described for other chemotherapies.[3] Second, the authors report here that dasatinib in vivo treatment results in selective decrease of regulatory T cells (Tregs), suggesting dasatinib is acting in part by reversing immuno-suppression. Indeed, in a recent study imatinib was also shown to act on another immuno-suppressive pathway, the IDO enzyme activity.[4] Because TKIs target multiple kinases, action on immune-related kinases might contribute to the observed immuno-modulatory effect of dasatinib. It has been reported that dasatinib inhibits T-cell expansion through blocking Lck,[5] a critical kinase in TCR signaling. The authors suggest that Tregs are more sensitive to dasatinib than effector T cells, suggesting a higher Lck dependence of Tregs. Importantly, different doses and schedules of drug administration might have opposite consequences on immunity, with strong CTL response with short-term dasatinib treatment, but immunosuppression with long-term treatment.[1] This supports the use of intermittent high-dose pulse dasatinib treatment.

Other immune-mediated activity of TKIs has been previously reported; in particular, imatinib has been reported to increase NK function likely through modulating NK/DC cross-talk.[4] In addition, dasatinib has been reported to increase NK reactivity in chronic myeloid leukemia patients[6] and in vitro NK cell expansion.

These observations argue for the need for comprehensive understanding of activities of targeted therapies on the immune system for optimal use in combination with immuno-stimulation.

Yang et al used agonistic anti-OX40 mAb to increase antitumor immunity, resulting in potent therapeutic synergy with dasatinib (see figure).[1] Recently, the combination of imatinib and anti–CTLA-4 was reported to induce tumor clearance in a spontaneous model of GIST.[4] Agonist anti-OX40 has been reported to increase immunity through both neutralization of Treg function and increased resistance of T effectors to suppression[7] and is currently under clinical development.[8] Although OX40 engagement leads to T-cell expansion, Treg numbers also increased in the present study. Thus, it will be critical to analyze the outcome of the antitumor immune response during in vivo decay of agonist anti-OX40 levels: would the neutralized Tregs take control again?

There is no doubt that the combination of targeted therapy and immunotherapy will be studied in clinical trials and will change clinical management. There is already an ongoing clinical trial in melanoma evaluating the combination vemurafenib (Braf inhibitor) plus anti–CTLA-4. Other immune strategies currently being studied include mAbs interfering with immune checkpoints (OX40, CD137, GITR) or endowed with Ab-dependent cell cytotoxicity capacities, vaccines (long peptides, poxviruses, synthetic mRNA), cytokines (IL-7), and vaccine adjuvants (TLR agonists).[9] Among the immune checkpoints, strategies directly targeting Tregs are also under development.[10] OX40 is one such target that neutralizes Treg function but not expansion, but will require careful monitoring during development.

In conclusion, the recent positive results of clinical trials with novel immuno-active drugs as well as the unexpected finding of a positive interaction between immunotherapy and targeted therapy should open a new era for the personalized immunotherapy of cancer.

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Angiogenic neutrophils: a novel subpopulation paradigm

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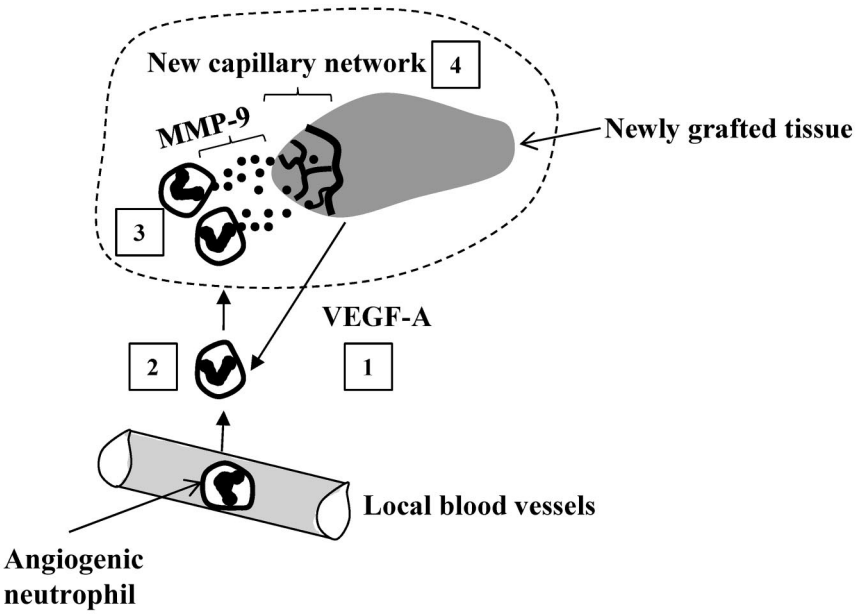
In this issue of *Blood*, Christoffersson et al provide data indicating the existence of a new neutrophil subset with angiogenic characteristics.[1]

Until recently, neutrophils were thought to be a homogeneous cell type that rapidly arrive at sites of infection or injury, eradicate infiltrating microbes, die, and are taken up by macrophages. However, recent reports indicate that there might be multiple neutrophil subpopulations, similar to monocytes, with not only pro-inflammatory but



Functioning of angiogenic neutrophils within the graft. The grafted tissues release VEGF-A that recruits a specific subset of angiogenic neutrophils from blood into the graft (1). The extravasated neutrophils (2) infiltrate the graft where they release MMP-9 (matrix metalloproteinase 9; (3) facilitating angiogenesis: development of a new capillary network (4) rescuing the graft from hypoxia.

also anti-inflammatory properties.[2-4] However, whether this is the same neutrophil at different stages of its life cycle, whether this is the same neutrophil just stimulated differently, or whether these are truly bona fide neutrophil subtypes remains debatable. If correct, however, multiple neutrophil subtypes could have significant implications for conditions ranging from chronic inflammation and cancer to organ transplant survival.

In the current study, Christoffersson and coworkers add additional evidence that multiple neutrophil subtypes exist, and propose a subset of neutrophils with pro-angiogenic properties, which are high in CXCR4, recruited to newly grafted tissues by VEGF-A, and release MMP-9 (matrix metalloproteinase 9) allowing for graft revascularization (MIP-2–recruited neutrophils had low levels of MMP-9 and CXCR4; see figure).[1] Although neutrophil subsets have been postulated to exist,[2-4] the prevailing criticism was that the neutrophil may be plastic, and as such could adapt to its environment. In vitro experiments suggested that neither VEGF-A nor MIP-2 could induce these phenotypic changes, suggesting 2 distinct populations before entry into tissues.[1] In addition to identifying pro-angiogenic neutrophils, this study also suggests new functions for VEGF-A (in neutrophil recruitment) and MMP-9 (in revascularization).

Neutrophils are the first immune cells to arrive at the inflammatory site. They leave blood vessels, passing barriers of endothelial cells, pericytes, and basement membrane, a complex and continuous structure composed of extracellular matrix (ECM) proteins. Digestion of ECM can be achieved by neutrophils through the release of proteases, including MMP-9. However, reports on the involvement of MMP-9 in extravasation are ambiguous. Some of the confusion may result from overlapping MMP substrate specificities and thus compensation. Alternatively, MMP-9 is dispensable for neutrophil extravasation in vivo, given that the cells actively choose ECM-poor regions of basement membrane for migration.[5] In this study, despite a 10-fold increase in MMP-9 content in angiogenic versus inflammatory neutrophils, both subtypes infiltrated tissue equally well, further supporting the view that MMP-9 is dispensable for extravasation. During infectious inflammation, neutrophils are the big kids at the playground, responsible for elimination of invading pathogens and debris clearance. During sterile inflammation (eg, caused by hypoxia), they infiltrate injured tissue and contribute to wound debris removal.[6] This latter function may be achieved by MMP-9 degrading intracellular matrix (ICM) proteins released from dead/damaged cells.[7] Finally, a cell type must then come in and help with revascularization and healing. Whether only inflammatory neutrophils enter infectious sites while both inflammatory and angiogenic neutrophils enter sterile injury to help with progression of inflammation and restitution remains unclear, but the current study may support such a scenario.

Christoffersson and colleagues report that neutrophil MMP-9 aids in the development of new vasculature in transplanted pancreatic islets. Mice genetically deprived of this enzyme have diminished revascularization during the first days and, although revascularization eventually occurs, it does so in a longer time frame and the vascular architecture of the new capillaries is significantly altered.[1] That revascularization occurs at all suggests potential compensatory action of other proteases, but it is clear that MMP-9 is responsible for proper angiogenesis.

The involvement of neutrophils in angiogenesis was reported previously in a corneal injury model.[8] This process also involved VEGF, but neutrophils engaged in this process were not phenotyped. In the current study Christoffersson and coworkers demonstrate that angiogenic neutrophils have preexisting features: high expression of CXCR4 and MMP-9.[1] While the latter is not surprising in light of MMP-9 involvement in vascularization, the importance of the chemokine receptor CXCR4 is interesting as its main ligand CXCL12 (SDF-1) was shown not to be involved in the recruitment of angiogenic neutrophils. In contrast, neutrophils were observed to infiltrate tissues after application of VEGF-A, and pancreatic islets from VEGF-deficient mice were unable to efficiently recruit neutrophils.[1] It is not known whether VEGF-A is able to directly recruit neutrophils.

It remains unexplained what the role of CXCR4 would be if not for migration. Previous studies have shown that CXCR4 is required for neutrophil release from the bone marrow.[9] Although this receptor is necessary for maximal neutrophil mobilization into blood, it is not essential for further neutrophil emigration to sites of inflammation. This might explain why, in the current model, CXCL12 was not necessary for neutrophil migration to the grafted tissue. On the other hand, the receptor is also important, although dispensable, for homing of aged, senescent neutrophils to the bone marrow and other organs.[9] If so, the expression of CXCR4 on the

angiogenic neutrophils might facilitate their clearance once they fulfill their function. This would require that the neutrophils leave the sterile injury, perhaps re-entering the vasculature, something proposed in zebra fish embryos.[10]

The current study by Christoffersson et al characterizes a new population of angiogenic neutrophils. Clinically, β cell replacement of the pancreatic islets is a promising therapeutic approach for a cure of type 1 diabetes, and from this point of view the role of neutrophils presented in this work is favorable; however, these same angiogenic neutrophils might also facilitate vascularization of tumors leading to the outgrowth of cancer tissues. It is this dual role of angiogenic neutrophils that opens exciting therapeutic possibilities. By regulating their recruitment to specific sites, it may be possible to either help support the revascularization of transplanted tissues, helping to ensure their survival, or to starve tumors of their blood supply, leading to hypoxia and tumor cell death.

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