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Previews

Redirecting tumor macrophage activity to fight cancer: Make room for the next era of anti-cancer drugs

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Functionally significant proteins expressed by tumor macrophages have emerged as promising anti-cancer targets. In this issue of *Cancer Cell*, Sun et al. identify two FDA-approved agents that together safely reprogram tumor macrophages into potent anti-tumor effectors, demonstrating the power of engaging *both* immune system arms to fight cancer.

The functional significance of mononuclear phagocytes in regulating key aspects of de-novo carcinogenesis and metastasis is now well accepted (Hanahan and Coussens 2012). Initial studies were based on colony stimulating factor-1 (CSF-1) regulation of monocyte and/or macrophage recruitment into carcinomas, which was investigated through the use of gain-of-function and loss-of-function approaches in orthotopic and spontaneous models of carcinogenesis. Development of highly selective (>1,000-fold) CSF-1 receptor (CSF-1R) small molecule inhibitors and blocking antibodies followed and thus affirmed that tumor-recruited monocytes and macrophages represented viable therapeutic targets in some tumor types (DeNardo and Ruffell 2019). Collectively, these studies have demonstrated that tissue-resident macrophage subsets, and recruited macrophage precursors (e.g., monocytes generated in spleen and bone marrow), respond to and functionally contribute to de-novo carcinogenesis by promoting extracellular matrix remodeling, angiogenesis, removing senescent and/or neoplastic cells, sustaining inflammation, and regulating anti-tumor adaptive immune responses. Since these proof-of-concept studies and initial human clinical trials were reported, much research has been devoted to improve understanding of the origins, plasticity, and effector functionality of tumor-associated macrophage subsets, and this has collectively led to more sculpted therapeutic approaches to neutralize their protumorigenic properties and/or enhance tumoricidal capabilities (DeNardo and Ruffell 2019, Weissleder and Pittet 2020). For example, therapeutics that target macrophage effector programs regulated by class IIa histone deacetylases, Brutons tyrosine kinase, phosphatidylinositol-3-Kinase γ, or CD40 (to name only a few) have emerged into the clinic with the goal of tipping the balance of tumor macrophage functionality toward T cell stimulation, thus leading to increased cytotoxic activity (DeNardo and Ruffell 2019). In addition, highly innovative myelomonocytic-based cellular therapies are now being investigated for delivery of immune-activating cytokines or chimeric antigen receptors (CARs) to tumor microenvironments that suppress primary and/or metastatic disease (De Palma, Mazzieri et al., 2008, Klichinsky, Ruella et al., 2020, Kaczanowska, Beury et al., 2021).

While the duality of macrophage functionality in tissues is recognized, an overarching question that has remained more-or-less unanswered has been which option, macrophage depletion or macrophage reprogramming strategies, would provide better efficacy (without toxicity), to what degree either approach would be efficacious, and whether the best approach would be used as monotherapy or require combination with cytotoxic drugs. In this issue of *Cancer Cell*, Sun and co-workers provide insight into these issues by investigating two FDA-approved drugs, monophosphoryl lipid A (MPLA) and interferon (IFN)γ, that could be used to reprogram macrophage effector function in cancer, and they demonstrate the power of dually leveraging agents that engage both the innate and adaptive arms of the immune system to fight malignancy (Sun, Kees et al., 2021).

MPLA is a toll-like receptor (TLR)4 agonist that is used as adjuvant in some vaccine formulations, and it displays reduced toxicities as compared to other TLR4 agonists such as lipopolysaccharide. IFNγ is a cytokine that is administered to treat chronic granulomatous disease and osteopetrosis, two diseases that involve mononuclear phagocytes. Although neither agent alone possesses potent anti-tumor activity, the two agents combined (MPLA/IFNγ) trigger macrophage-mediated killing of tumor cells, dependent on NOS2, with an effector-to-target ratio of three achieving >80% killing of patients' metastatic cancer cells. For reference, chimeric antigen receptor T (CAR-T) cells generally require an effector-to-target ratio between 20 and 50 to achieve similar levels of killing *in vitro* (Kiesgen, Messinger et al., 2021). Using *in vivo* immune-competent murine tumor models, Sun and colleagues demonstrate that the MPLA/IFNγ drug combination inhibits primary and metastatic mammary carcinoma progression,

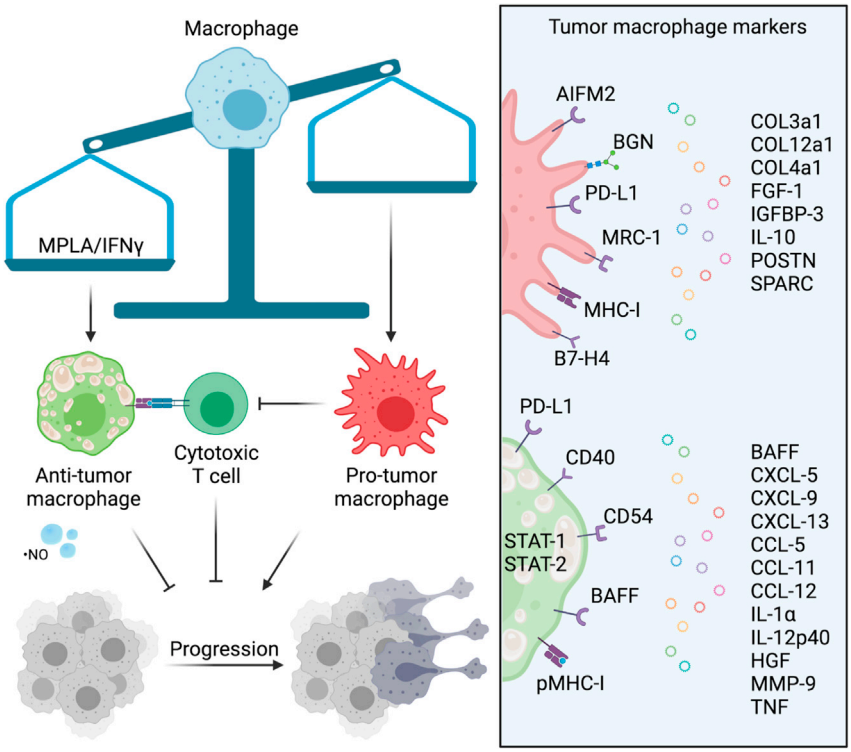

Figure 1. Reprogramming macrophages into anti-tumor effectors
Two FDA-approved agents, MPLA and IFNγ, rebalance tumor macrophage functionalities toward suppression of tumor progression and metastasis.

which is dependent on both macrophages and CD8$^+$ T cells, with superior efficacy as compared to therapies that blockade either CSF-1R or PD-1. Importantly, when combined with cisplatin-based chemotherapy in ovarian tumors, synergy was observed, resulting in increased survival of mice from 10% to 70% after 5 months. Although the response to TLR agonists may differ between mice and humans, the MPLA/IFNγ combination was well tolerated, as measured by liver toxicity and body weight after systemic injection. Notably, in addition to demonstrating efficacy of MPLA/IFNγ via systemic administration, the authors also reported potent anti-tumor activity when MPLA/IFNγ was administered either intratumorally or intraperitoneally. Together, these observations support the notion that a safe and effective strategy could likely be identified for translation of MPLA/IFNγ into human clinical studies.

Mechanistically, MPLA/IFNγ therapy induced local secretion of numerous chemokines that are functionally involved in leukocyte recruitment and effector activity (e.g., IL-12, TNFα, and CXCL9), as well as proteins that regulate antigen cross presentation (e.g., CD40, IL-12, BAFF). These responses correlated with increased density of CD45$^+$ cells within tumors, including CD4$^+$ T cells, inflammatory monocytes, and macrophages, which are associated with decreased presence of B cells. Macrophage transcriptional programs were significantly impacted, giving evidence of increased expression of type 1 IFN signaling, accompanied by decreased expression of genes which are more associated with angiogenesis and immune-suppression (Figure 1). Importantly, evaluation of MHC class I-restricted antigen-presentation revealed that macrophages (and dendritic cells) that are exposed to MPLA/IFNγ present tumor antigens at higher frequencies, and this is correlated with increased leukocyte infiltration and increased activation and induction of long-term CD8$^+$ T cell memory *in vivo*. For MPLA/IFNγ comparison with other therapeutics that are aimed at reprogramming macrophage functionality and also harbor anti-cancer activity (e.g., class IIa HDAC- and PI3Kγ-inhibitors, TMP195 and IPI-549 respectively), neither increased *Nos2* or *IL12b* expression or induced macrophage tumoricidal activity; this result indicates distinctive effector pathway involvement—which is not surprising, perhaps, when comparing agonistic versus antagonistic approaches. Thus, in this elegant study, Sun and colleagues have now identified a safe and efficacious approach not only for reversing macrophage-mediated immune suppression but also for triggering macrophage tumoricidal activity and long-lasting anti-tumor T cell memory that impacts both primary and metastatic disease.

A number of questions remain, however, around tumor-type specificity of the MPLA/IFNγ approach and in which contexts the addition of cytotoxic and/or checkpoint blockade therapy would be required to invoke the responses identified herein in human malignancies. We know that some chemotherapeutics promote immunogenic cell death and thereby provide additional "danger signals" that lower thresholds for effective anti-tumor immunity, but will these be synergistic with MPLA/IFNγ or instead result in adverse immune responses and limit efficacy?

There are also outstanding questions based on activation of long-term CD8$^+$ T cell memory responses following MPLA/IFNγ: To what degree are these clonal responses? Do they involve bystander T cells? Are abscopal effects and systemic adaptive immunity impacted, as recently reported by Sato and colleagues following administration of TLR9 agonists and PD-1 blockade (Sato-Kaneko, Yao et al., 2017), and if so, how are they impacted?

The mononuclear phagocyte system may also behave as a distributed "organ" in which individual "parenchymal cells" make contact with and share phagocytosed tumor material (Ruhland, Roberts et al., 2020). If so, activating one cell type, subset, or state (e.g., tumor macrophages) may unleash access to tumor-derived antigens for other mononuclear phagocytes, including dendritic cells; how will these responses be monitored, regulated, or tolerated? And, based on reported differences in TLR expression between mice and humans, and in particular TLR4 which may be absent or low in

human plasmacytoid dendritic cells, this begs the question as to whether TLR4 agonists will be as efficacious in humans, as observed herein by Sun and colleagues. And finally, a deeper understanding of lineage and plasticity is now more relevant than ever (Weissleder and Pittet 2020) as we move into the clinic with these (and other) myeloid-based reprograming agents and we discover new immune subtypes (or states) identified by single-cell RNA sequencing. Knowing the subsets or cellular states that have tumor-promoting versus tumor-suppressing roles will be critical not only for development of the next generation of targeted therapies but also for monitoring response and resistance mechanisms prospectively as therapies are disseminated to patients.

DECLARATION OF INTERESTS

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Restoring order at the cell cycle border: Co-targeting CDK4/6 and CDK2

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Overcoming resistance to CDK4/6 inhibitors is a major clinical challenge. In this issue of *Cancer Cell*, Freeman-Cook et al. study mechanisms of resistance to CDK4/6 inhibitors by employing a CRISPRa screen. They identify the cyclin E-CDK2 axis and Myc signaling as key pathways of resistance and develop PF-06873600, a selective CDK2/4/6 inhibitor.

The machinery of the cell cycle is highly conserved, and the borders between phases of the cell cycle are tightly regulated by checkpoints that are unique to each of the cell cycle phases. These checkpoints include the cyclin-dependent kinases (CDKs) whose activity depends on their association with their cyclin partners. The restriction point (R-point) at the end of the G1 phase is a key point for the cell's decision to proceed through the cell cycle. This point is dependent on mitogenic stimuli, mainly from receptor tyrosine kinases, that signal through cyclin D1-CDK4/6. Once the cell is committed to transition through the G1 phase, the cyclin D1-CDK4/6 complex phosphorylates the retinoblastoma protein (pRB). This results in the de-repression of pRb and the unleashing of the E2F transcription factors. E2F activates the cyclin E-CDK2 complex, which in turn phosphorylates pRB and enables progression to the S phase. Aberrant regulation of the cyclin D1-CDK4/6-pRb