

partly mediated by EZH2 (Figure 1B). In addition, other more tractable targets, such as TGF- β -FOXO signaling and PTGS1 (a key enzyme activating the Wnt/ β -catenin pathway), were also identified in the LIC signature shared by both SOX4 and C/EBP α leukemias (Figure 1B). TGF- β -FOXO signaling is required for the maintenance of LICs in chronic myeloid leukemia (Naka et al., 2010), whereas inhibition of β -catenin suppresses AML stem cells induced by MLL fusions (Wang et al., 2010; Yeung et al., 2010) (Figure 1B). Thus, future studies in delineating the molecular mechanisms and downstream targets mediating SOX4 oncogenic function will be instrumental for both understanding the biology of the

disease and designing better therapeutic strategies for C/EBP α leukemia.

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A New Twist on Radiation Oncology: Low-Dose Irradiation Elicits Immunostimulatory Macrophages that Unlock Barriers to Tumor Immunotherapy

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Tumor-infiltrating macrophages typically promote angiogenesis while suppressing antitumoral T cell responses. In this issue of *Cancer Cell*, Klug and colleagues report that clinically-feasible, low-dose irradiation redirects macrophage differentiation from a tumor-promoting/immunosuppressive state to one that enables cytotoxic T cells to infiltrate tumors and kill cancer cells, rendering immunotherapy successful in mice.

Macrophages are components of a stromal-cell network that orchestrates the angiogenic and immunosuppressive programming of neoplastic tissues (Hanahan and Coussens, 2012). In growing tumors, macrophages suppress T cells both by growth stimulation of aberrant and dysfunctional blood vessels, which can limit T cell extravasation, and via direct inhibitory effects on extravasated T cells. Macrophage depletion, or alternatively “reprogramming,” may present opportunities to restore T cell-mediated antitumor immunity and increase the efficacy of cancer immunotherapies (De Palma and

Lewis, 2013). In this issue of *Cancer Cell*, Klug et al. (2013) show that low-dose irradiation (LDI) of tumors or of isolated peritoneal macrophages followed by adoptive transfer promotes, in both cases, a differentiation switch that results in macrophages that attenuate angiogenesis-driven vascular abnormalities, facilitate T cell infiltration, and enhance the efficacy of immunotherapy in a genetically engineered mouse model (GEMM) of cancer and xenotransplanted human melanomas (Klug et al., 2013) (Figure 1).

In the RIP1-Tag5 GEMM of pancreatic islet carcinogenesis, LDI (2 Gy) enhanced

tumor infiltration by CD8⁺ T cells, the primary effectors of antitumor immune responses. Importantly, prior LDI also enhanced tumor infiltration and the efficacy of adoptively transferred, tumor-specific CD8⁺ T cells. The combination of prior LDI with T cell transfer dramatically extended the survival of tumor-bearing mice, while either treatment alone had equivocal effects. The extended survival is remarkable, because this GEMM develops multiple pancreatic tumors and dozens of angiogenic dysplasias. The antitumor efficacy of the combined treatment was associated with two important



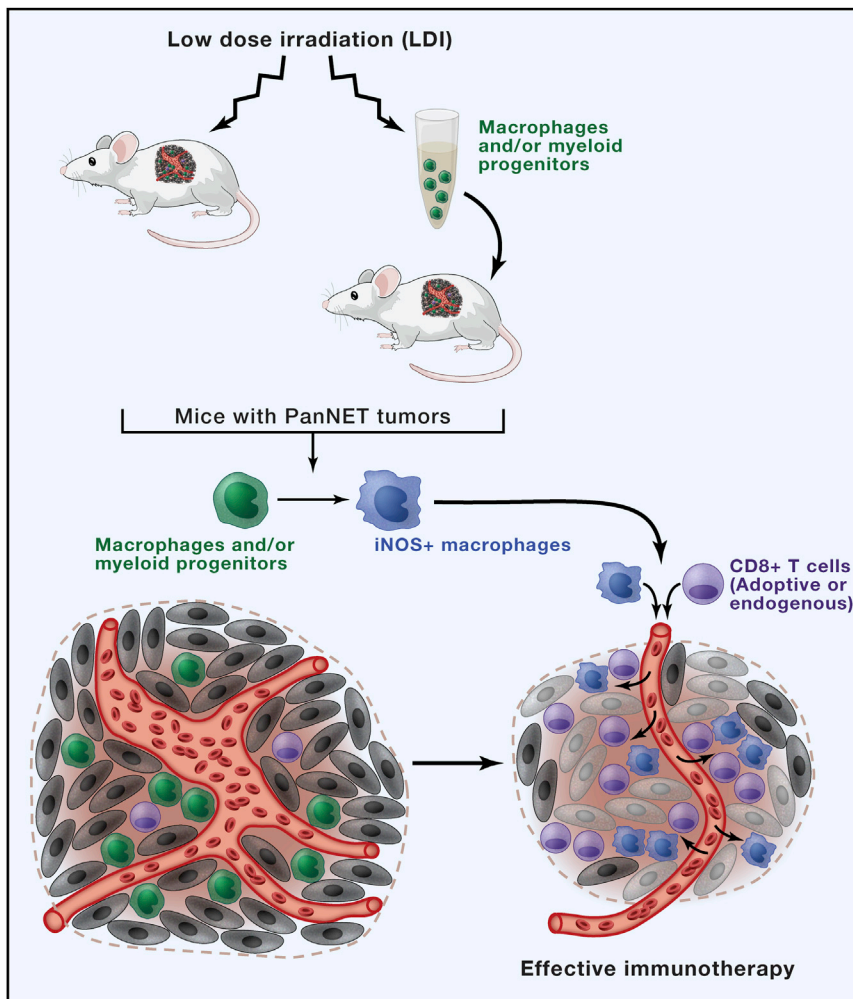


Figure 1. Low Dose Irradiation Programs/Reprograms Macrophages to Promote Antitumor Immunity

Irradiation of macrophages and/or myeloid progenitors in tumor-bearing mice, or ex vivo followed by adoptive transfer, elicits infiltration of tumors by iNOS-expressing macrophages, which normalize the tumor vasculature and increase the influx of CD8 T cells (either endogenous or following adoptive transfer). The net result is tumor shrinkage and remarkable survival benefit in a mouse model of multifocal pancreatic neuroendocrine cancer (PanNET), illustrating successful immunotherapy of late stage solid tumors involving a clinically feasible and likely combinable intervention.

histopathological changes in the tumors. First, LDI and T cell transfer converted the dysfunctional tumor blood vessels into a more “normalized” vascular network, concomitant with upregulated expression of the leukocyte (T cell) adhesion molecule VCAM1 on endothelial cells. Such changes likely facilitate the homing and extravasation of transferred (as well as endogenous) CD8⁺ T cells to the tumors. Second, LDI promoted tumor infiltration of a novel class of macrophages expressing higher levels of inducible nitric oxide synthetase (NOS2 or iNOS) compared to macrophages found in untreated tumors.

iNOS is an enzyme that converts L-arginine into nitrous oxide (NO), a free radical with diverse effects on cells. The use of a selective iNOS inhibitor prior to LDI and T cell transfer abrogated their antitumor effects in RIP1-Tag5 mice. Although this finding points to a key effector role for iNOS in promoting the immunostimulatory functions of macrophages, it is unclear how LDI induces iNOS upregulation in the macrophages or what its downstream effector functions might be, involving the macrophages themselves or other cells in the tumor microenvironment including infiltrating CD8⁺ T cells. The observation that macrophages upregulate iNOS

when treated by LDI ex vivo suggests a cell-intrinsic effect of LDI on macrophages or their precursors, resulting in induction of *Nos2* transcription. It is possible that a cellular stress response (perhaps independent of DNA damage) is triggered by LDI, thereby stimulating the expression/activity of nuclear-factor kappa-B (NF-κB) (Ahmed and Li, 2008) or the release of inflammatory cytokines that upregulate iNOS. Notably, the *Nos2* promoter contains consensus sequences for NF-κB.

It is conceivable that macrophage-derived NO and infiltrating CD8⁺ T cells—adoptively transferred or endogenous—together contributed to pruning and hence “normalizing” the tumor blood vessels, enabling further influx of iNOS⁺ cytotoxic macrophages and T cells into solid tumors. Notably, the abnormal tumor vasculature is typically not permissive for efficient T cell adhesion and transmigration. The molecular determinants of this barrier involve regulatory genes, endothelial cell receptors (e.g., endothelin B receptor, ET_BR), and proangiogenic growth factors, some of which may be directly or indirectly affected by NO produced by iNOS⁺ macrophages. Indeed, NO has been shown to induce endothelial cell anergy via ET_BR expressed on tumor endothelial cells (Motz and Coukos, 2011).

iNOS upregulation has been historically associated with immunosuppressive functions in tumor-infiltrating Gr1⁺ immature myeloid cells (iMCs). Indeed, accumulation of iMCs in tumors and their release of NO are known to suppress T cell function through several mechanisms, including the induction of T cell apoptosis and the nitration or nitrosylation of T cell receptors (Gabrilovich et al., 2012). In contradistinction, the current study reveals a provocative and unanticipated role for iNOS as an orchestrator of effective antitumor T cell responses. The authors note that, while LDI and T cell transfer increased iNOS⁺ macrophages, intratumoral Gr1⁺ iMCs were decreased. Conversely, pharmacological inhibition of iNOS decreased the intratumoral abundance of macrophages and T cells while increasing Gr1⁺ iMCs, suggesting a reciprocal inhibitory interplay between iNOS⁺ macrophages and (immunosuppressive) Gr1⁺ iMCs, as is the case for macrophages and Gr1⁺ cells in other tumor

models (Pahler et al., 2008). Thus, NO can play diverse roles in the tumor immune microenvironment, which may be context and cell-type dependent. The ostensibly dichotomous mechanisms of iNOS/NO in supporting versus suppressing CD8⁺ T cell activity in different tumor/immune microenvironments thus warrant further investigation; it remains unclear, for example, why NO is not suppressing T cells by the aforementioned mechanisms.

Seeking to further assess the functional importance of macrophages to the LDI phenotype, macrophages were depleted with clodronate liposomes prior to LDI and adoptive transfer of T cells. Macrophage depletion impaired T cell recruitment into the tumors and eliminated their antitumoral effects. Conversely, as noted above, the transfer of ex vivo irradiated macrophages into nonirradiated RIP1-Tag5 mice was sufficient to elicit vascular normalization and T cell recruitment and tumor control by the transferred CD8⁺ T cells. Thus, macrophage precursors (and perhaps mature macrophages) preconditioned by LDI can be programmed/reprogrammed into macrophages that modify the tumor microenvironment to unleash the cytotoxic functions of CD8⁺ T cells.

Klug et al. (2013) did not compare LDI with high-dose tumor irradiation (HDI; 10–25 Gy in single or fractionated doses), which is known to trigger a reparative response involving macrophages that facilitates tumor revascularization and regrowth. Interestingly, HDI of mouse tumors appears to program macrophages toward a “wound-healing” and protumoral phenotype, and macrophage depletion from HDI-treated tumors effectively limits post-therapy tumor relapse (Russell and Brown, 2013). An earlier study showed that HDI can also upregulate iNOS in macrophages and that HDI-irradiated iNOS⁺ macrophages enhanced tumor growth in mice (Tsai et al., 2007),

suggesting that a distinct mechanism is involved in the immunostimulatory, tumor-antagonizing phenotype of LDI-programmed macrophages.

Differential macrophage activation in tumors can elicit either pro- or antitumoral (immune) responses (De Palma and Lewis, 2013). The study by Klug et al. (2013) supports the emerging concept that macrophage programming/reprogramming—as opposed to a broad-brush macrophage depletion approach—may present an attractive means to improve the efficacy of anticancer therapies. This concept is supported by a number of preclinical and clinical studies highlighting the therapeutic benefits of targeting macrophages with immunomodulatory antibodies, such as anti-CD40 or anti-CD47, which are capable of reprogramming macrophages toward a tumoricidal and immunostimulatory phenotype (De Palma and Lewis, 2013; Tseng et al., 2013).

The potential clinical applicability of LDI-mediated programming of macrophages is supported here by a retrospective analysis of human pancreatic adenocarcinomas previously treated by LDI in a neoadjuvant setting. In these tumors, LDI significantly increased the proportion of iNOS⁺ macrophages and CD8⁺ T cells, and decreased the average size of the tumor blood vessels, possibly reflecting vascular normalization. Moreover, experiments employing an orthotopic xenotransplant mouse model of human melanoma in which tumor-bearing mice were treated with LDI and adoptive transfer of tumor-specific T cells largely recapitulated the findings in RIP1-Tag5 mice. Collectively, the results should incentivize discussion of clinical trials to further evaluate the potential of LDI, perhaps starting from dose-escalation studies that directly compare LDI with HDI in terms of effects on macrophages, the vasculature, and T cell infiltration and, in particular, to establish the optimal “low dose” to program macrophage dif-

ferentiation and enhance T cell infiltration while limiting toxicity. Importantly, LDI therapy should be readily deliverable in traditional radiation oncology facilities worldwide. As such, the likely tolerability and noninvasive modality of LDI make it an attractive candidate for combinations with novel immunotherapeutic agents, such as adoptive transfer of chimeric antigen receptor- or TCR-engineered T cells or treatment with immune checkpoint blockers (e.g., anti-PD1/PDL1 and/or anti-CTLA4), tumor vaccines, or immunogenic chemotherapy. If validated, LDI in such combinations could prove to be an important new interventional agent in the exciting frontier of tumor immunotherapy.

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