Myeloid-Derived Suppressor Cells and Radiotherapy

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ABSTRACT

Myeloid-derived suppressor cells (MDSC) are a heterogeneous population of pathologically activated, mostly immature, myeloid cells that exert robust immunosuppressive functions. MDSCs expand during oncogenesis and have been linked to accelerated disease progression and resistance to treatment in both preclinical tumor models and patients with cancer. Thus, MDSCs stand out as promising targets for the development of novel immunotherapeutic regimens with superior efficacy. Here, we summarize accumulating preclinical and clinical evidence indicating that MDSCs also hamper the efficacy of radiotherapy (RT), as we critically discuss the potential of MDSC-targeting strategies as tools to achieve superior immunotherapeutic tumor control by RT in the clinic.

Introduction

Myeloid-derived suppressor cells (MDSC) are a heterogeneous and highly plastic population of mostly immature myeloid cells (IMC) that expand systemically in the context of multiple pathologic conditions including cancer (1). In physiologic conditions, indeed, the IMCs that are released by the bone marrow (BM) as part of normal hematopoiesis migrate to peripheral organs and rapidly differentiate into granulocytes, macrophages or dendritic cells (DC; ref. 2). Conversely, in the context of indolent, chronic inflammation (as it occurs during cancer progression; ref. 3), BM-derived IMCs fail to differentiate and expand, both systemically and (at least in some malignancies) within the tumor microenvironment (TME; ref. 4). Moreover, tumor progression is frequently accompanied by at least some degree of extramedullary hematopoiesis, which also contributes to the expansion of the circulating pool of IMCs (5). Importantly, these IMCs represent a group of pathologically activated myeloid cells that mediate a plethora of immunosuppressive effects (4), ultimately underlying the widespread adoption of the term MDSCs (6).

Despite being developed as a cytostatic and cytotoxic agent, focal radiotherapy (RT)—at least when delivered according to specific fractionation regimens—mediates numerous immunostimulatory effects that actively contribute to tumor control (7). Consistent with this notion, accumulating evidence suggests that MDSCs actively counteract the therapeutic efficacy of RT, making them a promising target to develop novel combinatorial regimens that provide superior tumor control.

Here, we critically discuss preclinical and clinical data linking MDSC expansion to suppressed RT-driven immunostimulation, and we identify strategies that may potentially be harnessed to target MDSCs for enhancing tumor control by RT.

Myeloid-Derived Suppressor Cells

MDSCs were first reported and characterized for their immunosuppressive effects more than 40 years ago (8). It was only in the mid-2000s, however, that their morphologic, phenotypic, and functional heterogeneity began to emerge (9). Initially, two broad subpopulations of mouse MDSCs were identified: $CD11b^+Ly6C^+Ly6G^-$ cells originating from mononuclear cells, which are now dubbed monocytic (M-) MDSCs and $CD11b^+Ly6C^{low}Ly6G^+$ cells originating from low-density granulocytes, which are now referred to as granulocytic (G-) or polymorphonuclear (PMN-) MDSCs (9). In humans, M-MDSCs and G-MDSCs are commonly identified as $CD33^{high}CD11b^+$HLA-$DR^+CD14^+CD15^-$ and $CD33^{mid}CD11b^+$HLA-$DR^-CD14^-CD15^+$ cells, respectively (10). Alternatively, human MDSCs can be classified as G- based on a $CD11b^+CD14^-CD15^+$ or $CD11b^+CD14^-CD66b^+$ phenotype, or as M- based on a $CD11b^+CD14^+$HLA-$DR^{-/lo}CD15^-$ phenotype (11). Moreover, an early-stage (E-) population of MDSCs comprising even more immature progenitors of both the M- and G-type can be identified as $CD33^+$HLA-DR^- and Lin^- (meaning these cells do not express any mature lineage marker, including CD3, CD14, CD15, CD19, and CD56; ref. 11).

More recently, fibrocytic (F-) MDSCs have been reported as a population of MDSCs emerging from umbilical cord blood precursors and expressing not only common MDSCs markers such as CD33, CD11b, CD14, and CD15, but also CD163, S100A8, IL1B, FN1, TLR4, and ICAM1 (12, 13). Conversely, F-MDSCs do not express pure fibrocyte markers including ACTA2, CCR7, and COL6A1 (12, 13). So far, F-MDSCs have mostly been found to mediate beneficial effects in nonmalignant disorders including type I diabetes (12), wound healing (14), and chronic obstructive pulmonary disorders (15), with the notable exception of an F-MDSC population with indoleamine 2,3-dioxygenase 1 (IDO1)-dependent immunosuppressive activity that has been identified in subjects with metastatic pediatric sarcomas (16). Finally, so-called eosinophilic (Eo-) MDSCs have been reported to emerge in the context of *Staphylococcus aureus* infection in mice as an immature cell population expressing MDSC markers as well as eosinophil markers such as SIGLECF and low IL5RA levels (17). Whether a human MDSC population resembling mouse Eo-MDSCs exists and what pathophysiologic functions it potentially plays remain to be clarified.

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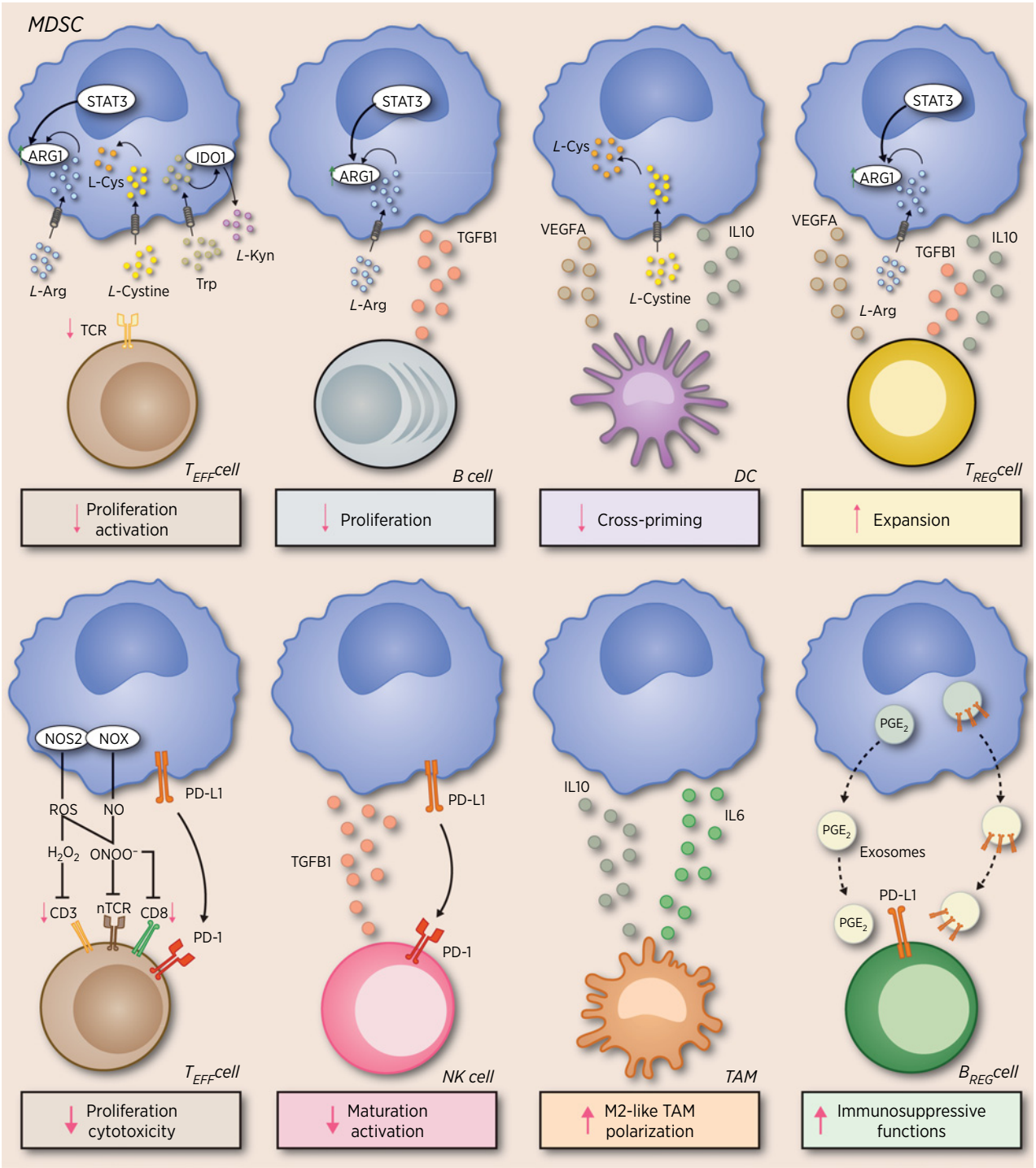

Figure 1.

Main mechanisms of MDSC-dependent immunosuppression. Myeloid-derived suppressor cells (MDSC) exert multipronged immunosuppressive effects, mostly, but not exclusively, as they condition their microenvironment from both the metabolic and immunologic standpoints. In general, such alterations suppress the activity of immune effector cells including effector T (T_{EFF}) cells, B lymphocytes, natural killer (NK) cells and dendritic cells (DC), as they expand or improve the functions of immunosuppressive cells including regulatory T (T_{REG}) cells, regulatory B (B_{REG}) cells, and M2-like tumor-associated macrophages (TAMs). Abbreviations: ARG1, arginase 1; H_2O_2, hydrogen peroxide; IDO1, indoleamine 2,3-dioxygenase 1; IL, interleukin; NO, nitric oxide; NOS2, nitric oxide synthase 2; NOX, NADPH oxidase; nTCR, nitrosylated TCR; $ONOO^-$, peroxynitrite anion; PD-1 (official name: PDCD1), programmed cell death 1; PD-L1 (official name: CD274); PGE_2, prostaglandin E_2; ROS, reactive oxygen species; STAT3, signal transducer and activator of transcription 3; TGFB1, transforming growth factor beta 1; VEGFA, vascular endothelial growth factor A.

Both M- and G-MDSCs exert immunosuppressive functions largely (but not exclusively) by altering the microenvironment of immune effector cells (**Fig. 1**; ref. 1). Specifically, MDSCs can secrete multiple cytokines with immunosuppressive activity such as interleukin 4 (IL4), IL10, transforming growth factor beta 1 (TGFB1), colony stimulating factor 2 (CSF2, best known as GM-CSF), vascular endothelial growth factor A (VEGFA), as well as other bioactive factors that potently inhibit anticancer immunity, like prostaglandin E_2 (PGE_2) and *L*-kynurenine (1). Moreover, MDSCs actively compete with immune effector cells, most notably $CD8^+$ cytotoxic T lymphocytes (CTL), for critical nutrients that are generally limited in the TME. These nutrients include not only glucose and fatty acids, but also amino acids like tryptophan, arginine, cysteine and cystine, a least in part as a consequence of IDO1 and arginase 1 (ARG1) overexpression (18). Finally, MDSCs (especially G-MDSCs) produce abundant amounts of reactive oxygen species (ROS) and reactive nitrogen species including nitric oxide (NO), which also contribute to establish an immunosuppressive microenvironment (19). That said, it is important to note that the specific immunosuppressive mechanisms used by MDSCs in different settings generally reflect their functional plasticity in response to microenvironmental cues (1).

Such a diversified armamentarium of immunosuppressive tools confers MDSCs with the ability to:

(i) block the recruitment, proliferation, differentiation, and/or activation of CTLs as a consequence of nitrosylation-dependent chemokine inactivation (20), limited tryptophan, arginine, cysteine, and cystine availability (18, 21, 22), CD3, CD8, and TCR nitration or nitrosylation (23, 24), as well as NO-dependent DNA damage (25);

(ii) inhibit the maturation and activation of natural killer (NK) cells by triggering TGFB1 signaling (26, 27);

(iii) suppress DC-dependent CTL cross-priming via cystine depletion plus active IL10- and VEGFA-dependent mechanisms that result in quenched IL12 and IL23 secretion by DCs (18, 28–30);

(iv) inhibit antitumor B-cell responses by an ARG1- and TGFB1-dependent mechanism (31, 32);

(v) promote the expansion of $CD4^+CD25^+FOXP3^+$ regulatory T (T_{REG}) cells by reducing arginine availability coupled to IL10, VEGFA, and TGFB1 secretion (28, 33–36);

(vi) stimulate the immunosuppressive function of regulatory B (B_{REG}) cells via exosomal PGE_2 (37); and

(vii) engage in metabolic and IL10-dependent cross talks with tumor-associated macrophages (TAMs) to receive trophic signals and promote the expansion of immunosuppressive M2-like TAMs (38, 39).

Moreover, at least some MDSCs express coinhibitory ligands such as CD274 (best known as PD-L1) on their surface, hence inhibiting effector T (T_{EFF}) cells and NK cells upon binding to the coinhibitory receptor programmed cell death 1 (PDCD1, best known as PD-1; refs. 40, 41), or upon transferring PD-L1 to other immunosuppressive cells such as B_{REG} cells via exosomes (42).

Of note, some MDSC-derived mediators that actively promote immunosuppression such as PGE_2, GM-CSF, and VEGFA can also be produced by (at least some) malignant cells, ultimately acting in an autocrine/paracrine manner to support the recruitment, expansion, and/or immunosuppressive activity of MDSCs themselves (43–46). Neoplastic cells (and notably the cancer stem cell compartment) can secrete various other factors that recruit MDSCs to the TME and support their immunosuppressive activity, including small metabolites

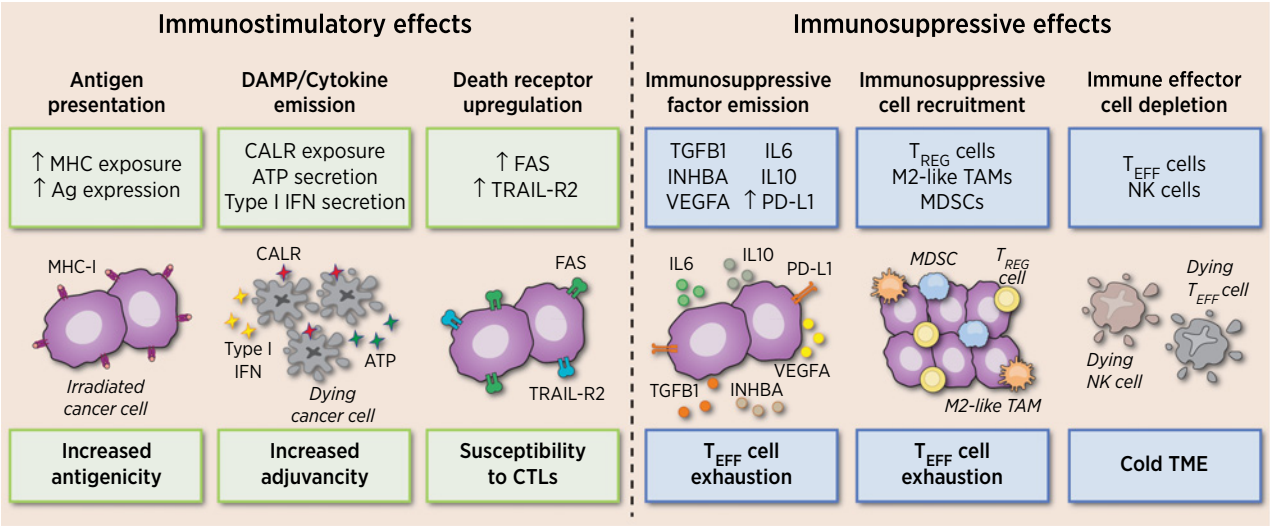

Figure 2.

Immunomodulatory properties of RT. Depending on multiple parameters, including (but not limited to) dose and fractionation schedule, radiotherapy (RT) can mediate robust immunostimulatory effects as well as a pronounced immunosuppressive activity. On the one hand, upon interacting with cancer cells, RT can promote the expression of otherwise silenced potentially immunogenic antigens (Ag), the exposure of MHC class I molecules (MHC-I) and death receptors on the cell surface, as well as the release of immunostimulatory cytokines and damage-associated molecular patterns (DAMP), ultimately boosting the antigenicity, adjuvanticity, and immune susceptibility of malignant cells. On the other hand, RT can stimulate the expression or secretion of immunosuppressive factors by malignant cells, favor the recruitment of immunosuppressive cell populations to the tumor microenvironment (TME), and mediate cytotoxic effects on immune effector cells, ultimately establishing permissive conditions for immunoevasion. CALR, calreticulin; FAS, Fas cell-surface death receptor; IFN, interferon; IL, interleukin; INHBA, inhibin subunit beta A; MDSC, myeloid-derived suppressor cell; NK, natural killer; PD-L1 (official name: CD274); TAM, tumor-associated macrophage; T_{EFF}, effector T; TGFB1, transforming growth factor beta 1; TRAIL-R2 (official name: TNFRSF10B), TNF receptor superfamily member 10b; T_{REG}, regulatory T; VEGFA, vascular endothelial growth factor A.

like lactate (47). In turn, MDSCs can release stemness-preserving signals, ultimately delineating a vicious cycle whereby cancer stem cells and MDSCs support each other in the context of accrued tumor progression (48).

Importantly, many of these processes are sensitive to RT and have been mechanistically linked to RT-driven MDSC recruitment or expansion (see below). Moreover, RT is a potent inducer of hypoxia and consequent hypoxia-inducible factor 1 subunit alpha (HIF1α) activation in cancer cells (49). Although the precise impact of RT-driven hypoxia on MDSCs has not been directly investigated, HIF1α signaling in malignant cells has been consistently associated with MDSC accumulation in the TME and enhanced immunosuppression (40, 50). Along similar lines, ROS are important regulators of MDSC functions (51), but how ROS generation downstream of RT influences MDSC-dependent immunosuppression remains to be specifically assessed.

Immunomodulatory Effects of RT

Focal RT has been used for the clinical management of various malignancies for over a century, reflecting considerable efficacy in multiple oncological indications as well as an extraordinary safety profile (52). The therapeutic activity of RT has classically been attributed to its ability to inflict direct and ROS-mediated damage to macromolecules, notably DNA and lipids (53). Besides the fact that modern technological platforms enable the delivery of high doses of RT to specific target volumes with extraordinary precision (hence maximally sparing healthy tissues), normal cells are more proficient than their malignant counterparts at repairing such macromolecular damage (54), offering a relatively large therapeutic window for clinical applications. That said, accumulating preclinical and clinical data challenge the traditional notion whereby RT purely operates as a cytostatic/cytotoxic agent as they highlight various RT-driven immunomodulatory pathways that ultimately influence therapeutic outcome (**Fig. 2**; refs. 7, 55).

On the one hand, RT, at least when delivered according to specific fractionation schedules (56, 57), can elicit robust immunostimulatory effects. For instance, RT can increase the antigenicity of malignant cells (and hence their visibility to the host immune system) by favoring the exposure of MHC class I molecules on the cell surface (58) as well as the transcriptional derepression of otherwise silenced genes encoding for mutated MHC class I and II epitopes (59). Moreover, hypofractionated RT is particularly effective at increasing the adjuvanticity of cancer cells, i.e., at promoting the emission of immunostimulatory signals that support the recruitment and activation of antigen-presenting cells, ultimately enabling the cross-priming of tumor-targeting CTLs (60). These signals encompass immunogenic cell death (ICD)-associated damage-associated molecular patterns (DAMP), such as the surface-exposed calreticulin (CALR) and secreted ATP (61, 62), as well as proinflammatory cytokines such as type I interferon (IFN; refs. 63, 64). Finally, RT can promote the upregulation of death receptors such as Fas cell-surface death receptor (FAS) and tumor necrosis factor (TNF) receptor superfamily member 10b (TNFRSF10B, best known as TRAIL-R2 or DR5) on the surface of cancer cells, de facto increasing their susceptibility to lysis by activated $CD8^+$ CTLs (59, 65). Abundant mechanistic data from preclinical tumor models and observational findings from clinical studies implicate most (if not all) these pathways in the therapeutic effects of RT.

On the other hand, RT can also elicit a plethora of immunosuppressive pathways. Thus, irradiated cancer cells can release immunosuppressive cytokines, such as TGFB1 (66), homodimeric inhibin subunit beta A (INHBA; ref. 67), IL6 (68), and IL10 (69), and express increased levels of PD-L1 on their surface (70, 71), ultimately inhibiting the activity of various immune effector cells potentially present in the TME. Moreover, RT can efficiently recruit and/or expand immunosuppressive cell populations including T_{REG} cells (67, 72), M2-like TAMs (73, 74), and MDSCs (see below). Finally, RT can limit tumor-targeting immune responses by directly killing immune effector cells and causing at least some degree of vascular disruption coupled to local fibrosis and hypoxia, which altogether can largely compromise T_{EFF} cell recruitment to the TME and activation (75–77). The relevance of these immunosuppressive pathways for the therapeutic effects of RT is substantiated by abundant mechanistic data in preclinical tumor models and emerging observational findings from clinical studies.

Importantly, the balance between RT-driven immunostimulation and immunosuppression, which ultimately dictates the efficacy of RT, at least in preclinical settings, is influenced by a variety of physical and biological parameters, including (but not limited to)

(i) RT dose, dose rate, fractionation, and administration schedule (in the context of combinatorial regimen; refs. 56, 57, 74, 78);
(ii) physical RT nature (i.e., which subatomic particles are used for irradiation) and linear energy transfer (i.e., the energy that an ionizing particle transfers to the material traversed per unit of distance; refs. 79–81);
(iii) tumor type, stage, anatomical localization, and baseline infiltration by immune cells (82, 83);
(iv) genetic, epigenetic, and immunologic intratumoral heterogeneity (84);
(v) local oxygenation (85); and
(vi) systemic microbial configuration (86).

These observations may provide an interpretative framework for apparently discrepant results emerging from studies investigating RT-dependent immunomodulation in both preclinical and clinical settings.

MDSCs and RT in Preclinical Tumor Models

A growing preclinical literature illustrates the ability of RT to alter the abundance of circulating or tumor-infiltrating MDSCs in a therapeutically relevant manner in mouse models of cancer (**Table 1**).

Mouse TRAMP-C1 prostate tumors (including the hormone-resistant TRAMP-HR variant) established subcutaneously in immunocompetent syngeneic hosts drive a robust systemic expansion of M- and G-MDSCs upon exposure to single RT doses > 15 Gy (87–89). Such an expansion culminates with the intratumoral accumulation of MDSCs expressing immunosuppressive factors (i.e., ARG1 and IDO1) as well as matrix metallopeptidase 9 (MMP9), an extracellular enzyme that favors disease dissemination, especially in central, necrotic tumor areas (88). In this setting, tumor infiltration by MDSCs limits the efficacy of RT, as demonstrated by depletion experiments (88). Moreover, the ability of RT to recruit MDSCs to the tumor bed mechanistically involves IL6, which has also been linked to resistance to hormonotherapy (87), and can be prevented, at least partially, by UVB irradiation or vitamin D_3 supplementation, correlating with (i) suppressed IL6 signaling, (ii) restored $CD3^+$ T-cell infiltration, and (iii) increased therapeutic efficacy (87, 88). A similar systemic expansion and intratumoral accumulation of MDSCs has also been documented in immunocompetent mice bearing mouse DVL3, RM-1,

Table 1. RT and MDSCs in syngeneic mouse models of cancer.

Type of cancer	Model	*In vivo* setting	RT schedule	Effect on MDSCs after RT	Ref.
Breast cancer	4T1 cells	Subcutaneous BALB/c	12 Gy × 2	- MDSC depletion upon RT, cyclophosphamide, PD-1 blockade and/or VISTA blockade	(103)
Breast cancer	4T1 cells	Subcutaneous BALB/c	8 Gy × 3	- G-MDSC expansion in the spleen and TME - Decreased MDSC infiltration and FASL expression upon RT, PI3K inhibition, and PD-1 blockade	(104)
Breast cancer	TS/A cells	Subcutaneous BALB/cAnN	8 Gy × 3	- G-MDSC recruitment upon RT, LTX-315, and CTLA4 blockade	(72)
Breast cancer	TUBO cells	Subcutaneous BALB/c	12 Gy × 1	- Limited effect on MDSC recruitment after 3 days - Depletion after 10 days with or without PD-L1 blockade	(111)
CRC	CT26 cells	Subcutaneous BALB/c	30 Gy × 1 3 Gy × 10	- Transient MDSC recruitment after 3 days and decreased MDSC infiltration after 14 days - MDSC recruitment after 14 days upon depletion of $CD8^+$ and $CD4^+$ T cells	(110)
CRC	CT26 cells	Subcutaneous BALB/c	2 Gy × 18 8 Gy × 3 16.4 Gy × 1	- MDSC recruitment after 14 days with 2 Gy × 18	(112)
CRC	MC38 cells	Subcutaneous C57BL/6	20 Gy × 1	- M-MDSC recruitment with RT alone - MDSC depletion upon RT plus IFNAR1 or cGAMP and CCR2 blockade or *Sting* knockout - Increased tumor control upon RT and CCR2 blockade or *Ccr2* knockout	(102)
CRC	MC38 cells	Subcutaneous C57BL/6	20 Gy × 1	- Increased RT efficiency and tumor control after MDSC depletion	(111)
Glioma	CT2A cells	Intercranial C57BL/6	3 Gy × 1.33 4 Gy × 1	- M-MDSC recruitment with fractionated schedule	(74)
Glioma	CT2A cells	Intercranial C57BL/6	2 Gy × 4	- Limited effect on MDSC recruitment - Depletion upon RT and injection of magnetic nanoparticles	(107)
Glioma	CT2A cells	Intercranial C57BL/6	4 Gy × 1	- Decreased M-MDSC infiltration	(108)
HCC	BNL-P2 cells	Orthotopic BALB/c	10 Gy × 1	- Limited effect on MDSC recruitment - Depletion upon RT and IL12 administration - Increased tumor control upon RT and IL12 administration	(106)
HCC	H22 cells Hepa1-6 cells	Subcutaneous C57BL/6 ICR	2.5 Gy × 16 4 Gy × 10 6 Gy × 6 + 4 Gy × 1 8 Gy × 5	- MDSC depletion in the tumor and peripheral blood - Decreased plasma levels of MDSC-related cytokines	(95)
Lung cancer	LL/2 cells	Subcutaneous C57BL/6	4 Gy × 9 11.5 Gy × 2	- MDSC recruitment (lower in AHFRT vs. CFRT) - MDSC depletion in the spleen and peripheral blood 13 days after AHFRT - PD-L1 overexpression (lower in AHFRT vs. CFRT)	(105)
Lung cancer	LLC cells	Subcutaneous C57BL/6	20 Gy × 1	- Increased RT efficiency and tumor control after M-MDSC depletion upon RT, CGAMP and CCR2 blockade	(102)
Lung cancer	LLC cells	Subcutaneous C57BL/6	20 Gy × 1	- G-MDSC expansion in the tumor and peripheral blood with increased ARG1 activity and PD-L1 expression - Depletion upon RT and PDE5 inhibition - Increased tumor control upon RT and PDE5 inhibition	(98)
Lung cancer	LLC cells	Subcutaneous C57BL/6	10 Gy × 1	- MDSC recruitment - Depletion upon RT and LXR agonist - Increased tumor control upon RT and LXR agonist	(99)
Lung cancer	LLC cells	Subcutaneous C57BL/6	2 Gy × 3	- Decreased MDSC infiltration after RT and PD-L1 blockade - Increased tumor control upon RT and PD-L1 blockade	(100)
Lung cancer	LLC cells	Subcutaneous C57BL/6	10 Gy × 4	- PD-L1 overexpression on MDSCs - Decreased MDSC infiltration after RT and PD-L1 blockade with or without VEGF blockade	(101)

(Continued on the following page)

Table 1. RT and MDSCs in syngeneic mouse models of cancer. (Cont'd)

Type of cancer	Model	*In vivo* setting	RT schedule	Effect on MDSCs after RT	Ref.
Lung cancer	LLC cells	Subcutaneous C57BL/6	6 Gy × 3 12 Gy × 3	- $IDO1^+$ MDSC recruitment in a dose-dependent manner - Depletion upon RT plus IDO1 blockade	(97)
Lung cancer	LLC-OVA cells	Subcutaneous C57BL/6	30 Gy × 1	- Decreased MDSC infiltration with or without SMAC mimetic - Partial restoration of MDSC infiltration upon RT and SMAC mimetic and IFNF blockade, $CD8^+$ T-cell or TNF depletion	(69)
Melanoma	B16 cells MelanA cells S91 cells	Subcutaneous BALB/c	5 Gy × 1	- MDSC depletion in the bone marrow, peripheral blood, spleen, and tumor (higher in CIRT compared with XR) - Decreased JAK2 and STAT3 expression in MDSCs (higher in CIRT compared with XR)	(109)
Melanoma	B16F10 cells	Subcutaneous C57BL/6	4 Gy × 9 11.5 Gy × 2	- MDSC recruitment (lower in AHFRT vs. CFRT) - MDSC depletion in the spleen and peripheral blood 13 days after AHFRT - PD-L1 overexpression (lower in AHFRT vs. CFRT)	(105)
PDAC	FC1242 cells PK5L1940 cells	Subcutaneous C57BL/6	8 Gy × 1	- MDSC recruitment and STAT3 activation in G-MDSCS - Decreased MDSC infiltration and STAT3 phosphorylation after RT and STAT3 blockade	(94)
PDAC	KC	Endogenous C57BL/6	6 Gy × 3	- Tumor cell–derived lactate induced MDSC activation - Upregulation of *S100a8*, *S100a9*, *Arg1*, *Nos2*, *Vegf*, and *Mmps* in MDSCs	(47)
PDAC	Panc-02 cells	Orthotopic C57BL/6	6 Gy × 3	- Tumor cell–derived lactate induced MDSCs activation - Upregulation of *S100a8*, *S100a9*, *Arg1*, *Nos2*, *Vegf*, and *Mmps* in MDSCs - Increased tumor control by RT upon *Ldha* knockdown in tumor cells or *Hif1a* deletion in myeloid cells	(47)
Prostate cancer	DVL3 cells	Subcutaneous C57BL/6J	2 Gy × 5	- Upregulation of an MDSC gene signature	(90)
Prostate cancer	RM-1 cells RM-9 cells Myc-CaP cells	Subcutaneous C57BL/6	3 Gy × 5	- MDSC expansion in tumor, peripheral blood, spleen, lymph node and lung - Depletion in tumor and spleen upon RT and CSF1R blockade	(92)
Prostate cancer	RM-1 cells Myc-CaP cells	Subcutaneous C57BL/6 FVB/NCrlBR	8 Gy × 1 8 Gy × 2	- Rapid MDSC recruitment after 2 days, transient decrease after 8 days, and final increase after 14 days - T_{REG} cell recruitment upon RT and MDSC depletion	(91)
Prostate cancer	TRAMP-C1 cells	Intramuscular C57BL/6J	8 Gy × 1 25 Gy × 1	- MDSC expansion in tumor, peripheral blood and spleen at high RT dose - G-MDSC aggregation in central necrotic and avascular hypoxic regions of irradiated tumors - Increased RT efficiency and tumor control after G-MDSC depletion	(88)
Prostate cancer	TRAMP-C1 cells TRAM-HR cells	Orthotopic subcutaneous C57BL/6J	15 Gy × 1	- MDSC recruitment (more so in hormone resistant vs. hormone-sensitive model) - Depletion upon RT and IL6 blockage, UVB irradiation or androgen deprivation therapy	(68, 89) (87)

Abbreviations: AHRT, ablative hypofractionated radiotherapy; CFRT, conventional fractionated radiotherapy; CIRT, carbon ion radiotherapy; CRC, colorectal carcinoma; G-, granulocytic; M-, monocytic; MDSC, monocyte-derived suppressor cell; NSCLC, non–small cell lung carcinoma; RT, radiotherapy; T_{REG}, regulatory T; XR, X-ray.

RM-9, and Myc-Cap prostate tumors upon irradiation with 2 RT fractions of 8 Gy each (DVL3 and RM-1), 5 RT fractions of 3 Gy each (Myc-Cap, RM-1, and RM-9) and a single RT fraction of 8 Gy (Myc-Cap; refs. 90–92). In RM-1 tumors treated with 2 RT fractions of 8 Gy each, early M- and G-MDSC infiltration is followed by an influx of functional T cells, and MDSC depletion offers no therapeutic benefits because of compensatory T_{REG} cell expansion (91). Conversely, RM-1 tumors responding to 5 RT fractions of 3 Gy each appear to recruit an MDSC population enriched in M-MDSCs, coupled to the intratumoral expansion of M2-like TAMs (92). In this latter setting, pharmacologic inhibition of colony stimulating factor 1 receptor (CSF1R), which is abundantly expressed by M-MDSCs (less so G-MDSCs) and M2-like

TAMs (93), limits MDSC infiltration, downregulates the expression of ARG1 and MMP9 and ultimately improves the therapeutic efficacy of RT (92).

Mouse FC1242 and PK5L1940 pancreatic ductal adenocarcinomas (PDAC) established subcutaneously in immunocompetent syngeneic hosts recruit MDSCs (especially, but not exclusively G-MDSCs) upon irradiation with a single fraction of 8 Gy. This correlates with increased signal transducer and activator of transcription (STAT3) phosphorylation, which is critical for MDSC-dependent immunosuppression, and accrued Ki67 positivity, which reflects MDSC proliferation (94). Consistent with a mechanistic involvement, STAT3 inhibition with an antisense oligonucleotide blunts MDSC recruitment to irradiated PDACs, coupled to limited tumor infiltration by T_{REG} cells, restored T_{EFF} cell recruitment, and increased therapeutic efficacy (94). Similar findings have been documented with mouse Panc-02 PDACs as well as with endogenous PDAC models exposed to 3 RT fraction of 6 Gy each (47). In this setting, irradiation also promoted the secretion of lactate by cancer cells, which was ultimately found to promote the recruitment and immunosuppressive functions of MDSCs via a STAT3-dependent pathway involving mechanistic target of rapamycin (MTOR) and HIF1α (47). Accordingly, both the depletion of MDSCs with a Ly6G-targeting antibody and deletion of *Hif1a* in myeloid cells improved the therapeutic effects of RT (47). Apparently at odds with these findings, conditioned media from mouse Hepa1-6 and H22 hepatocellular carcinoma (HCC) cells exposed to single RT doses of 2.5, 4, 6, or 8 Gy block MDSC differentiation and proliferation via suppressed IL6 secretion and consequent STAT3 inhibition in MDSC precursors (95). Consistent with this notion, a total dose of 40 Gy delivered in 2.5, 4, 6, or 8 Gy per fraction to Hepa1-6 and H22 HCCs growing in immunocompetent syngeneic mice appears to mediate considerable therapeutic activity coupled to both systemic and intratumoral MDSC depletion (95). Whether lactate secretion upon irradiation with a single RT dose of 6 Gy is a prerogative of PDAC cells remains to be elucidated. Alternatively, the differential response of PDACs and HCCs to RT with respect to MDSCs may reflect pathways elicited by other components of the TME, such as cancer-associated fibroblasts (CAF). CAFs, which are abundant in PDAC lesions, can respond to RT by producing high amounts of MDSC chemoattractants like C–X–C motif chemokine ligand 1 (CXCL1; ref. 96).

Mouse Lewis lung carcinoma (LLC) tumors established in immunocompetent syngeneic hosts and exposed to 3 RT fraction of 6 or 12 Gy each recruit a population of MDSCs that expresses CD11b and IDO1, intermediate levels of Ly6G, but no CD106 and CD11c, as well as T_{REG} cells (97). In this scenario, coadministration of a pharmacologic IDO1 inhibitor improves the efficacy of RT (delivered as 3 fractions of 12 Gy each) but fails to suppress MDSC recruitment (97). Conversely, blocking ARG1 or activating nuclear receptor subfamily 1 group H member 2 (NR1H2, best known as LXR) in the context of a single RT dose of 20 or 10 Gy, respectively, partially restores LLC infiltration by $CD8^+$ CTLs as it extends the therapeutic benefits of RT (98, 99). Similar findings have been obtained by blocking PD-L1 in the context of 3 RT fractions of 2 Gy each or 4 RT fractions of 10 Gy each (100, 101). Moreover, neutralizing C–C motif chemokine ligand 2 (CCL2), blocking C–C motif chemokine receptor 2 (CCR2), or deleting *Ccr2* efficiently prevents the RT-dependent infiltration of LLC tumors (and mouse MC38 colorectal carcinomas) by MDSCs (102). Ultimately, this enables superior therapeutic responses to RT plus a stimulator of interferon response cGAMP interactor (STING1) agonist (102). These findings, which mechanistically reflect CCL2 secretion downstream of STING1-dependent type I IFN signaling, nicely exemplify the complexity of RT-dependent immunomodulation and suggest that CCL2/CCR2 inhibition may represent a promising strategy to improve the efficacy of RT by offsetting MDSC expansion.

In preclinical models of breast cancer, including models of hormone receptor $(HR)^+$ breast cancer (TS/A) and triple-negative breast cancer (4T1), RT schedules associated with good local disease control but suboptimal immunostimulation (3 fractions of 8 Gy each for TS/A and 4T1, and 2 fractions of 12 Gy for 4T1) appear to have limited effects on tumor infiltration by M- and G-MDSCs (72, 103, 104). In the TS/A model, improved immunostimulation by RT, as achieved by the coadministration of an oncolytic peptide and a cytotoxic T lymphocyte-associated protein 4 (CTLA4)-targeting immune-checkpoint inhibitor (ICI), results in the compensatory expansion of intratumoral MDSCs (72). Conversely, in the 4T1 model, improved overall survival (in the context of virtually unaffected local disease control), as achieved by combining RT with various combinatorial regimens involving a PI3K inhibitor, a V-set immunoregulatory receptor (best known as VISTA), a programmed cell death 1 (PDCD1, best known as PD-1) inhibitor, and/or low-dose cyclophosphamide, is associated with profound MDSC depletion, at least in part reflecting abundant VISTA expression by tumor-infiltrating MDSCs (103, 104). Along these lines, mouse LL/2 lung carcinomas and B16F10 melanomas respond better to a total RT dose of 23 Gy delivered in 2 fractions 8 days apart from each other than they do to a total dose of 36 Gy delivered in 9 daily fractions (but only so in immunocompetent hosts), correlating with superior MDSC depletion, ARG1 and kinase insert domain receptor (the main VEGFA receptor) expression by MDSCs, limited VEGFA secretion by cancer cells, restored CTL infiltration as well as activation of a systemic immune responses that can delay the growth of the same tumors implanted contralaterally shortly after irradiation (105).

A single RT dose of 10 Gy mediates poor therapeutic effects against large BNL-P2 HCCs established subcutaneously or orthotopically in immunocompetent syngeneic mice as it fails to deplete intratumoral MDSCs (106). Conversely, RT plus intratumoral administration of a viral vector encoding IL12 enables superior disease control coupled to MDSC depletion and restored tumor infiltration by active CTLs and NK cells (106). Similar findings correlating the therapeutic effects of RT with M-MDSC (and M2-like TAM) depletion have been obtained in orthotopic models of glioma (CT-2A spheroids) treated with 4 RT fractions of 2 Gy each (107) as well as single RT doses of 2, 4, or 8 Gy (74, 108). In this latter setting, 3 RT fractions of 1.33 Gy was less efficient than an equivalent biologically effective dose (BED) of 4 Gy in a single fraction, and this correlated with increased intratumoral MDSC and M2-like TAM abundance (74). These findings provide additional support to the influence of fractionation on the immunobiological effects of RT. Of note, at least in preclinical models of melanoma (B16, MelanA, and S91), the ability of a single RT dose of 5 Gy to deplete MDSCs is considerably ameliorated (correlating with improved CTL activation and disease control) when carbon ions are used instead of X-rays, at least in part reflecting superior STAT3 inhibition (109). These data highlight the important of physical parameters other than dose and fraction on the effects of RT.

Finally, it is interesting to note that in mouse models of breast cancer (TUBO), colorectal carcinoma (CT26 and MC38), and lung carcinoma (LLC) exposed to single RT doses of 12 Gy (TUBO and MC38) or 30 Gy (CT26, MC38, and LLC), intratumoral G- and M-MDSC depletion linked to (at least partial) therapeutic efficacy mechanistically depends on the activation of a tumor-targeting adaptive immune response involving DC-dependent cross-presentation (110), $CD4^+$ T cell–dependent help (110), as well as TNF and IFNγ secretion by $CD8^+$ CTLs, ultimately resulting in apoptotic MDSC death (69, 110, 111). In

the CT26 model, 3 RT doses of 8 Gy as well as 18 RT fractions of 2 Gy each mediate superior therapeutic activity than a single RT fraction of 16.4 Gy, but whereas hypofractionation enables the recruitment of $CD8^+$ CTLs to the tumor bed, conventional fractionation causes pronounced intratumoral MDSC expansion (112). In most of these settings, improved tumor control can be achieved by adding immunostimulatory agents including ICIs targeting PD-1, PD-L1, or T-cell immunoreceptor with Ig and ITIM domains (TIGIT; refs. 69, 111, 112). Conversely, delivering 10 RT fractions of 3 Gy each after the initial dose of 30 Gy compromises therapeutic efficacy in the CT26 model, and this correlates with recovered MDSC infiltration and CTL depletion (110). Whether alternative fractionation schedules can be successfully used as maintenance regimens in this setting remains to be investigated.

Taken together, these observations highlight the complex relationship between RT and numerical as well as functional MDSC alterations in preclinical tumor models as they provide important mechanistic insights toward the development of therapeutic approaches harnessing RT and MDSC-targeting strategies. Specifically, it seems that the ability of RT to influence systemic MDSC expansion, recruitment, and immunosuppressive activity varies considerably with total dose and fractionation schedule, with hypofractionated regimens standing out as superior approaches for MDSC depletion (at least when total dose is clinically relevant). That said, only a few studies have comparatively assessed different fractionation schedules of the same BED (74, 105, 112), highlighting the need for additional work to deconvolute the impact of RT dose and fractionation on MDSCs.

MDSCs and RT in Cancer Patients

A few clinical studies have investigated the impact of RT on the abundance of circulating or tumor-infiltrating MDSCs in patients with cancer (**Table 2**).

No differences in circulating M- and G-MDSCs were detected in 7 patients with non–small cell lung carcinoma (NSCLC) 3 days, 1 month, 3 months, and 6 months after stereotactic body radiotherapy (SBRT) with 8 fractions of 7.5 Gy each or 4 fractions of 12.5 Gy each (113). In a distinct cohort of 18 subjects with NSCLC, the circulating levels of 10 specific MDSC subsets (114) at baseline did not differ from those of 8 healthy donors, and they were not altered by carboplatin–pemetrexed ($n = 7$) or carboplatin–vinorelbine ($n = 5$) chemotherapy (115). However, patients receiving a 5-week cycle of concurrent chemoradiotherapy (24 daily RT fractions of 2.75 Gy each) preceded

Table 2. RT and MDSCs in patients with cancer.

Type of cancer	No of patients	RT schedule[a]	Note	Effect on MDSCs after RT	Ref.
Cervical cancer	10	2 Gy × 23 7 Gy × 3–4	Combined with platinum-based chemotherapy and BT	- M-MDSC expansion in 9 of 10 patients	(122)
HNSCC	3	70 Gy	Combined with platinum-based chemotherapy	- MDSC expansion in 1 of 3 patients	(116)
HNSCC	15	2–2.2 Gy × 30–25	Combined with cisplatin and/or cetuximab	- G-MDSC expansion, correlating with IL6 levels and arginase activity - PD-L1 overexpression on MDSCs	(121)
HNSCC	20	70 Gy	With or without platinum-based chemotherapy	- MDSC expansion in 14 of 20 patients	(120)
HNSCC	43	<2 Gy × 30	No combination therapy	- MDSC depletion - Higher levels of MDSCs correlating with decreased overall survival	(125)
LARC	13	5 Gy × 5	SCRT	- MDSC depletion after 5 weeks - Lower levels of M-MDSCs corresponding to poor responders to SCRT	(124)
Melanoma	1	9.5 Gy × 3	No combination therapy	- Decrease in MDSCs	(126)
NSCLC	6	2.75 Gy × 24	Combined with cisplatin	- MDSC expansion types 4 and 7	(115)
NSCLC	7	7.5 Gy × 8 12.5 Gy × 4	SRBT	- G-MDSC expansion after 72 hours - Progressive decrease of MDSCs after 6 months	(113)
NSCLC	14	60–66 Gy	Combined with platinum-based chemotherapy	- MDSC expansion in 5 of 14 patients - MDSC depletion in 5 of 14 patients	(116)
OSCC	45	60–66 Gy	No combination therapy	- MDSC expansion - PD-L1 overexpression on MDSCs	(118)
OSCC	248	60–66 Gy	Adjuvant RT after surgery	- PD-L1 overexpression on MDSCs, correlating with high IL6 levels	(119)
PDAC	7	9 Gy × 3 10 Gy × 3 11 Gy × 3	SRBT	- MDSC expansion	(94)
Pharyngeal cancer	75	>66 Gy	Combined with chemotherapy	- PD-L1 overexpression on MDSCs, correlating with high IL6 levels	(119)
SCLC	3	4 Gy	Combined with platinum-based chemotherapy	- MDSC expansion in 2 of 3 patients	(116)

Abbreviations: BT, brachytherapy; G-, granulocytic; HNSCC, head and neck squamous cell carcinoma; LARC, locally advanced rectal cancer; M-, monocytic; MDSC, monocyte-derived suppressor cell; NSCLC, non–small cell lung carcinoma; OSCC, oral squamous cell carcinoma; PDAC, pancreatic ductal adenocarcinoma; RT, radiotherapy; SCRT, short-course radiotherapy; SCLC, small cell lung carcinoma.
[a]Total dose unless otherwise specified.

by low-dose cisplatin ($n = 6$) did exhibit a slight increase in circulating $CD14^+HLA\text{-}DR^{low}$ and $CD14^+CD33^+HLA\text{-}DR^{low}$ MDSCs that was paralleled by a decrease in blood-borne lymphocytes (115). Similar findings have been documented in a cohort of 20 patients with small cell lung carcinoma (SCLC), NSCLC, or head and neck squamous cell carcinoma (HNSCC) treated with total RT doses of 45, 60 to 66, or 70 Gy, respectively, in the context of cisplatin-based chemotherapy (116). In this setting, >20% elevations in circulating MDSCs were detected in 8 of 20 patients. This correlated with an increase in blood-borne T_{REG} cells and was associated with suppressed T-cell responses against the tumor-associated antigen telomerase reverse transcriptase (TERT; ref. 116). Of note, the negative correlation between circulating MDSCs and TERT-targeting immunity also appears to hold true and have negative prognostic value in other cancer types (e.g., anal squamous cell carcinoma) and irrespective of RT (117).

An expansion in circulating $CD11b^+HLA\text{-}DR^-CD33^+CD14^+$ MDSCs coupled to PD-L1 upregulation on the MDSC surface has also been documented in a cohort of 45 patients with oral squamous cell carcinoma (OSCC) 2 weeks after adjuvant RT at a total dose of 60 to 66 Gy, especially when OSCC cells expressed stem cell markers that have been linked with MDSC expansion in preclinical cancer models (46, 118). Similar results have been obtained in a partially overlapping cohort of 96 patients with HNSCC (encompassing OSCC and pharyngeal cancer) who received adjuvant RT at a total dose greater than or equal to 66 Gy for definite RT, especially in subjects with high circulating levels of IL6 (119); and in an independent cohort of 20 subjects with HNSCC who received a 7-week course of curative-intent RT (median dose 70 Gy) with or without chemotherapy (120). Like subjects with NSCLC, patients with (most often HPV^+) HNSCC receiving a total RT dose of 66 to 70 Gy (in 2–2.2 Gy/fraction RT over 7 weeks in the context of cisplatin- or cetuximab-based chemotherapy) exhibited a progressive accumulation of circulating $CD14^-CD15^+CD33^+$ G-MDSCs (but not M-MDSCs), correlating with increased blood-borne IL6 levels, increased ARG1 activity in the circulation, STAT3 activation in the $CD33^+$ compartment (but not in M- or G-MDSCs), as well as accrued PD-L1 expression on both M- and G-MDSCs (121). Interestingly, most of these changes largely resolved 5 weeks after RT interruption (121). Similarly, 9 of 10 patients with cervical carcinoma experienced a considerable expansion of circulating $CD3^-CD19^-CD1a^-HLA\text{-}DR^-CD14^+CD15^-$ M-MDSCs coupled to PD-1 upregulation on $CD4^+$ T cells and impaired T-cell reactivity upon receiving 23 RT fractions of 2 Gy each, but these alterations resolved 6 to 9 weeks after RT cessation (122), suggesting a transient nature for RT-driven MDSC elevation (at least in these oncological settings). An increase in circulating MDSCs with active STAT3 has also been documented in 7 patients with PDAC receiving RT in 3 fractions of 9, 10, or 11 Gy as part of disease management (94). In this context, however, blood-borne IL6 levels remained unchanged, while both VEGFA and TGFB1 increased after treatment, suggesting an IL6-independent mechanism of expansion (94).

In a cohort of 62 subjects with locally advanced rectal cancer (LARC) receiving long-course neoadjuvant RT (a total dose of 40–45 Gy delivered to the pelvis in 25 to 28 fractions over 5 weeks, with/or without a boost of 5.4 Gy to the primary tumor) followed by radical surgery, tumor-infiltrating MDSCs did not exhibit consistent numerical alterations post-RT across patients and were not statistically associated with progression-free survival or overall survival (OS; ref. 123). However, patients with low MDSCs at baseline had a higher chance to exhibit good disease control at surgical resection (123). Conversely, in a distinct cohort of 13 patients with LARC receiving short-course neoadjuvant RT (a total dose of 25 Gy delivered to the pelvis in 5 daily fractions over 1 week) patients with poor disease control at surgical resection experienced a major decrease in circulating $HLA\text{-}DR^-CD11b^+CD15^-CD14^+CD33^+$ M-MDSCs and (less so) $Lin^-HLA\text{-}DR^-CD11b^+CD15^+CD14^-CD33^+$ G-MDSCs 5 weeks from RT initiation, correlating with an increase in PD-1-expressing T_{REG} cells (124). Also in this setting, MDSC depletion driven by RT rapidly subsided after therapy (124). Along similar lines, circulating MDSCs were decreased in 43 patients with HCC receiving a total RT dose <60 Gy in conventional 2-Gy fractions over 4 to 6 weeks, and posttreatment MDSC abundance negatively correlated with OS in both univariate and multivariate Cox regression analyses (125). Moreover, palliative RT with a total dose of 28.5 Gy delivered in 3 fractions caused disease regression not only to the irradiated paraspinal lesion but also to nonirradiated lymphadenopathy and splenic lesions in a patient with melanoma progressing on CTLA4 blockage, which was paralleled by a trend for transient circulating MDSC depletion (126).

Altogether, these findings lend further support to the notion that the immunomodulatory effects of RT on MDSC abundance and function exhibit a high degree of context dependency, which considerably complicates the development of MDSC-targeting strategies to improve RT efficacy in the clinic (see below).

Conclusions and Outlook

The abundant literature discussed herein lend strong support to the notion that both the abundance and immunosuppressive activity of MDSCs can be altered by focal RT, which ultimately affects therapeutic outcome. Specifically, the suboptimal depletion and the expansion of MDSCs after RT have been linked with incomplete therapeutic responses in both preclinical tumor models and patients with cancer. Thus, MDSCs stand out as promising targets to ameliorate the efficacy of RT, as demonstrated (at least in preclinical settings) via a variety of pharmacologic interventions or genetic approaches. Of note, many pharmacologic agents that can be harnessed to directly or indirectly target MDSC-dependent immunosuppression are clinically available or currently under clinical development, although none of them has been conceived to specifically interfere with MDSCs.

Clinically approved agents that (at least based on preclinical data) target MDSCs or their functions include, but are not limited to, the IL6 inhibitor tocilizumab, which is currently licensed for use in patients with moderately to severely active rheumatoid arthritis (127), the MTOR inhibitor everolimus, which is commonly used to prevent transplant rejection as well as for the management of multiple neoplasms (128), various anticancer tyrosine kinase inhibitors that (specifically or alongside other kinases) block CSF1R (e.g., pexidartinib, dasatinib, and sunitinib; ref. 129), multiple chemotherapeutics that directly kill MDSCs (e.g., cyclophosphamide; ref. 130), the phosphodiesterase 5 (PDE5) inhibitor tadalafil (131), as well as a variety of ICIs targeting PD-1 (i.e., nivolumab and pembrolizumab) and PD-L1 (i.e., avelumab, atezolizumab, and durvalumab; ref. 132). MDSC-targeting drugs that are still under clinical development encompass IDO1 inhibitors (133) and CCR2 inhibitors (134), as well as VISTA- and TIGIT-targeting ICIs (132). Despite such an ample therapeutic armamentarium, however, none of these agents is used in combination with RT as part of routine clinical protocols for patients with cancer, and only a few are being/have been formally evaluated for their capacity to

ameliorate the efficacy of RT in clinical trials (55, 135), generally with rather deceiving results (136). As a notable exception, durvalumab has been shown to cooperate efficiently with SBRT as a neoadjuvant approach for patients with potentially resectable early-stage NSCLC (71). However, whether such a positive outcome involves MDSC depletion remains to be elucidated. Of note, several interventional clinical trials currently recruiting participants are assessing the therapeutic potential of RT in combination with one (or more) agent(s) that (at least based on preclinical data) can deplete or inhibit MDSCs (source www.clinicaltrials.gov). Most of these studies, however, involve FDA-approved (rather than investigational) agents and are not designed to ascertain the potential role of MDSC inhibition. As an exception, the rationale for testing tadalafil together with chemoradiation in patients with stage III–IV astrocytoma reflects the ability of this agent to inhibit MDSCs (NCT04757662). In addition, researchers conducting three clinical trials testing SBRT in patients with advanced malignancies are planning to monitor patients for circulating immune cell subsets including MDSCs (NCT04073745, NCT03348748, and NCT04068649).

In summary, although MDSCs stand out as promising targets to extend the clinical benefits of RT, additional research is needed for translating such a promise into a clinical reality. At least in part, this reflects the extraordinary plasticity of MDSCs, their multipronged immunosuppressive activity, and the existence of mechanisms that drive compensatory myelopoiesis upon MDSC depletion (4). Moreover, currently available preclinical data fail to identify a common denominator for the interaction between RT and MDSCs, which considerably complicates the development of therapeutic strategies that can be rapidly translated to clinical testing. Although RT dose and fractionation have been shown to influence the impact of RT on MDSCs by a few comparative studies (74, 105, 112), various other parameters remain to be comparatively analyzed in this respect. Such potential confounders include, but are not limited to:

(i) model and anatomic localization (most studies discussed herein relied on mouse cancer cell lines established subcutaneously in immunocompetent syngeneic hosts, which generally do not recapitulate the immunobiology of human malignancies as closely as orthotopically implanted or endogenous tumors; ref. 137);

(ii) time (in the preclinical literature that we summarized, MDSCs were most often quantified 8 to 14 days after RT initiation, with little attention given to early time points);

(iii) RT-related parameters beyond dose and fractionation (many studies fail to provide detailed information on particle type, dose rate, and linear energy transfer, which may all influence the biological effects of RT); and

(iv) MDSC identification (besides the use of surface markers that are not necessarily identical across different studies, the actual ability of MDSCs expanding or contracting after RT to mediate immunosuppressive functions has rarely been tested with *ex vivo* functional assays).

Despite these and other incognita, we surmise that obtaining additional insights into MDSC-dependent immunosuppression in adequate preclinical tumor models that consider the aforementioned points, developing strategies that target nonredundant MDSC functions, and selecting patients with prominent MDSC expansion/activity may ultimately unlock the full therapeutic potential of MDSC-targeting strategies as partners for RT in clinical cancer management.

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