INTERNATIONAL JOURNAL OF
RADIATION ONCOLOGY • BIOLOGY • PHYSICS
www.redjournal.org

CRITICAL REVIEW

The Effect of Radiation Treatment of Solid Tumors on Neutrophil Infiltration and Function: A Systematic Review

Léon Raymakers, MD,[*,†] Thijs J. Demmers, MSc,[*] Gert J. Meijer, PhD,[†] I. Quintus Molenaar, MD, PhD,[‡] Hjalmar C. van Santvoort, MD, PhD,[‡] Martijn P.W. Intven, MD, PhD,[†] Jeanette H.W. Leusen, PhD,[*] Patricia A. Olofsen, PhD,[*] and Lois A. Daamen, MD, PhD[†,‡]

[*]*Center for Translational Immunology, University Medical Center Utrecht, Utrecht, The Netherlands;* [†]*Department of Radiation Oncology, University Medical Center Utrecht, UMC Utrecht Cancer Center, Utrecht, The Netherlands; and* [‡]*Department of Surgery, Regional Academic Cancer Center Utrecht, UMC Utrecht Cancer Center and St. Antonius Hospital Nieuwegein, Utrecht University, Utrecht, The Netherlands*

Received Apr 17, 2024; Accepted for publication Jul 2, 2024

Radiation therapy (RT) initiates a local and systemic immune response which can induce antitumor immunity and improve immunotherapy efficacy. Neutrophils are among the first immune cells that infiltrate tumors after RT and are suggested to be essential for the initial antitumor immune response. However, neutrophils in tumors are associated with poor outcomes and RT-induced neutrophil infiltration could also change the composition of the tumor microenvironment (TME) in favor of tumor progression. To improve RT efficacy for patients with cancer it is important to understand the interplay between RT and neutrophils. Here, we review the literature on how RT affects the infiltration and function of neutrophils in the TME of solid tumors, using both patients studies and preclinical murine in vivo models. In general, it was found that neutrophil levels increase and reach maximal levels in the first days after RT and can remain elevated up to 3 weeks. Most studies report an immunosuppressive role of neutrophils in the TME after RT, caused by upregulated expression of neutrophil indoleamine 2,3-dioxygenase 1 and arginase 1, as well as neutrophil extracellular trap formation. RT was also associated with increased reactive oxygen species production by neutrophils, which can both improve and inhibit antitumor immunity. In addition, multiple murine models showed improved RT efficacy when depleting neutrophils, suggesting that neutrophils have a protumor phenotype after RT. We conclude that the role of neutrophils should not be overlooked when developing RT strategies and requires further investigation in specific tumor types. In addition, neutrophils can possibly be exploited to enhance RT efficacy by combining RT with neutrophil-targeting therapies.

Corresponding author: Lois A. Daamen, MD, PhD; E-mail: l.a.daamen-3@umcutrecht.nl, l.raijmakers@umcutrecht.nl

Authors Olofsen and Daamen made equal contributions to this study.

Disclosures: J.H.W.L. is a scientific founder of and holds stock in TigaTx and has received consulting fees from the company. M.P.W.I. has received honoraria for lectures from Elekta. P.A.O. was funded by a grant of the Dutch Cancer Society. This work was funded by a Health Holland, grant number TKI2122.

Data Sharing Statement: Research data are stored in an institutional repository and will be shared on request to the corresponding author.

Acknowledgments—The illustrations were created with BioRender.com, last accessed on the 17th of April 2024.

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijrobp.2024.07.2141.

https://doi.org/10.1016/j.ijrobp.2024.07.2141

Introduction

Radiation therapy (RT) is an important treatment modality for multiple types of cancer. Irradiation induces DNA damage which can result in tumor cell death via senescence, apoptosis or necrosis. Besides the direct cytotoxic effects, RT initiates a local and systemic immune response. These immune responses can have both antitumor and protumor effects through various mechanisms.[1-3]

First, radiation induced tissue damage activates and attracts immune cells to the tumor.[1-4] The first responders to tissue damage are neutrophils. Neutrophils are already present in the tumor microenvironment (TME) of most solid tumors and are often described as tumor associated neutrophils, which can exert an antitumor (N1) or protumor (N2) effect.[5-10] Multiple murine cancer models have shown that RT reduces tumor growth and increases overall survival and some studies suggest that neutrophils are essential for RT efficacy.[10-18] However, other studies show that neutrophil depletion improves response to RT, suggesting a protumor function of neutrophils.[10,11] In line with this, high levels of circulating neutrophils and high density of neutrophils in the TME are associated with poor outcomes in patients with cancer.[5,9,12,19-22]

Second, RT selectively kills radiation sensitive immune cells in the tumor, changing the immune cell composition of the TME, possibly in favor of tumor progression.[1,23-26] In addition, radiation can enhance the production of immunosuppressive cytokines and chemokines, upregulation of immune checkpoints and recruitment of immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs).[1,4,5,11,26-30]

MDSCs are a heterogeneous group of immature suppressive myeloid cells that expand under pathologic conditions such as chronic inflammation and cancer. MDSCs can be subdivided into polymorphonuclear MDSCs (PMN-MDSCs) and monocytic MDSCs.[5,31] PMN-MDSCs share similarities with neutrophils. However, whether they are distinct from protumorigenic tumor associated neutrophils and immature neutrophil fractions found in the blood (ie, also referred to as low density neutrophils) remains a topic of debate, especially because their terminology is used interchangeably.[5,32,33] Such immunosuppressive myeloid cells are also found in mice, were protumorigenic neutrophils are characterized by high expression of Siglec-F.[34,35] Within the TME, PMN-MDSCs exert immunosuppressive functions such as inhibition of $CD4^+$ and $CD8^+$ T cells, inducing Tregs and inhibition of NK cells.[5,36,37] RT can enhance PMN-MDSC recruitment, resulting in a more immunosuppressive TME which contributes to therapy resistance.[29,38-41] Consequently, apart from intrinsic radiation resistance of tumor cells, changes in the immune microenvironment may contribute to tumor regrowth and progression after RT.

To improve the antitumor effect of RT in patients with cancer, it is important to understand the interplay between RT and neutrophils. We therefore performed a systematic review of the literature to assess how RT affects the infiltration and function of neutrophils in the TME of solid tumors.

Methods

A literature search was performed in PubMed and EMBASE on May 24, 2024 using a combination of the terms "neutrophils" or "myeloid-derived suppressor cells" and "radiotherapy," including synonyms (Appendix E1). Identified records were imported into Rayyan for article selection.[42] Duplicates were detected and removed. Titles, abstracts and subsequent full-text articles were screened. Meta-analysis or reviews, case reports, case series, conference articles, or articles without available full text were excluded. Inclusion criteria for articles were the mentioning of neutrophils or MDSCs in a cancer or tumor model in combination with external beam RT as monotherapy.

Studies investigating combination therapies were included if separate results of RT as monotherapy were available. Because we were interested in the direct effect of RT on neutrophils, we excluded association or prediction studies. For example, studies looking at neutrophils or neutrophil-to-lymphocyte ratios as predictive markers for disease outcome. We focused our search on the most frequently used form of RT; photon external beam RT, and excluded studies using brachytherapy or particle RT, for example, with protons, because these types of RT can have different biologic effects.

Articles with murine models were included if Ly-6G was used as a specific neutrophil marker. Another frequently used "neutrophil" marker, Gr-1 (staining both Ly-6G and Ly-6C), can also be found on subsets of monocytes, macrophages, dendritic cells, and lymphocytes.[43-47] Therefore, if studies were used describing cell populations stained with Gr-1 or other nonspecific neutrophil markers, we refer to them as myeloid cells. When reviewing RT-induced neutrophil infiltration in the tumor, data from murine tumor models were only included if neutrophil specific markers, such as Ly-6G, were used. To determine alterations in neutrophil levels in these models, neutrophil levels were compared with tumors of nonirradiated mice at the same time point. Articles were divided into articles which reported on direct effects of RT on neutrophils and articles presenting data on indirect effects, such as cytokines release (Fig. 1).

Results

A total of 3025 unique records were found from which 48 articles were included. Thirty-six articles presented direct effects of RT on neutrophils (Table 1)[10-13,17,18,26,37,48-75] and 12 articles provided information on indirect effects (Fig. 1). Relevant outcomes were obtained from in vitro cell experiments in 8 studies, from in vivo murine models in 44 studies and from patient data in 10 studies. Some studies reported on both in vivo and in vitro or in vivo and patient data (Table 1).[10-13,17,18,26,37,48-75]

Included studies presented data on how RT affects neutrophil infiltration in the tumor (n = 27), the systemic

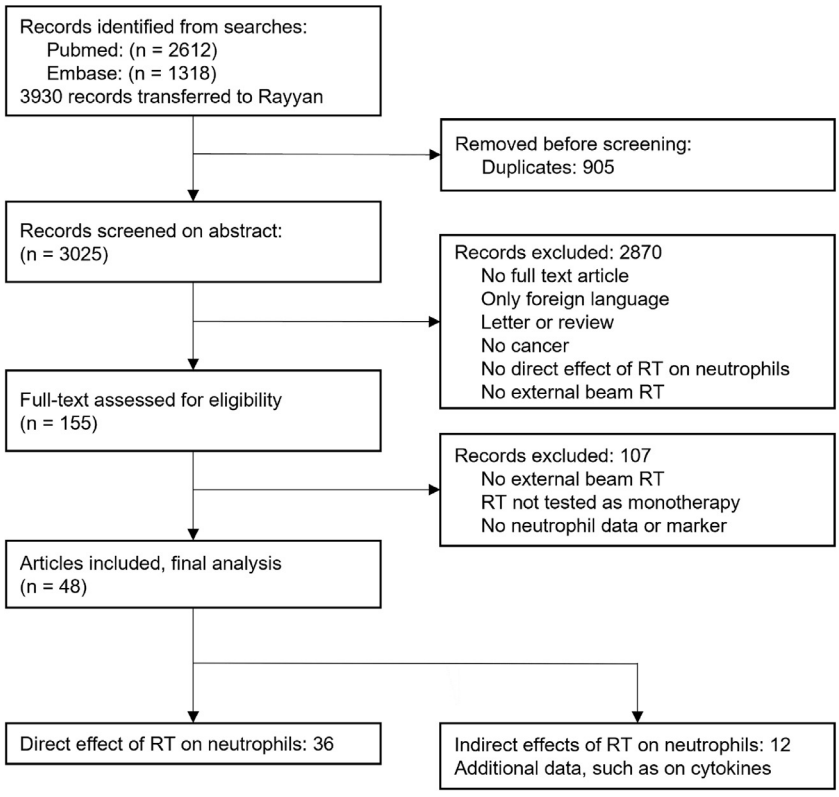

Fig. 1. Flowchart of article inclusion. RT, radiation therapy.

response of neutrophils (n = 11), cytokine release (n = 22), and the neutrophil phenotype and function (n = 26) (Table 1).[10-13,17,18,26,37,48-75] Most frequently used murine tumor models were C57BL/6 and BALB/c mice (n = 25 and n = 11, respectively). Six articles that used Gr-1 as a neutrophil or MDSCs marker instead of Ly-6G were included because they provided additional data on RT fractionation strategies, cytokine release and immunosuppressive effects of myeloid cells.[16,38,48,49,76,77] Two studies did use Ly-6G as neutrophil marker for flow cytometry staining, but used anti-Gr-1 for neutrophil depletion, since efficient long-term neutrophil depletion with Ly-6G antibodies was not possible until recently.[17,26,43,78] Different terms were used in the articles to describe neutrophils such as "tumor associated neutrophil" and "PMN-MDSCs." To simplify the results we adhered to the term neutrophil.

Radiation induced neutrophil infiltration in the tumor

Murine tumor models

Neutrophil infiltration was studied in murine tumor models (n = 25) of varying cancer types, using different RT regimens and methods for neutrophil assessment (Table 2).[10-13,17,18,26,37,48-75] Neutrophil infiltration increased in most tumor models following RT and specifically accumulated in RT-induced necrotic regions of the tumor, as shown in a murine prostate cancer model.[50] Although both irradiated and nonirradiated tumors have necrotic regions, neutrophil aggregation only occurred in irradiated tumors.[50]

Most studies used syngeneic mouse models, and subcutaneous, intradermal or intramuscular injected tumor cells (n = 20). In these murine models, it was found that neutrophils were not present at the time of injection but could infiltrate during tumor development. Radiation was delivered in one fraction in 11/25 studies and fractionated RT was used in 17/25 studies, including 3 studies testing both. RT fractionation differed considerably with 3 × 8 gray (Gy) being the most frequently used scheme (6/17 studies).[51-56] Changes in neutrophils were measured in absolute numbers as count per gram of tissue, number of events per field in immunofluorescence staining, immunohistochemistry (IHC) staining, or imaging mass cytometry, and in relative numbers as percentage of all live cells, $CD45^+$ leukocytes or $CD11b^+$ myeloid cells using flow cytometry. Different methods of measurement could result in varying outcomes, and interpretation of results should therefore be put into perspective. For example, one study reported an increase in neutrophils relative to the percentage of $CD45^+$ leukocytes, but not an increase in the number of events as measured with IHC staining.[57]

Dynamics of neutrophil infiltration

Neutrophils appeared to be the main responding immune cells in vivo after RT, especially when compared with T cell infiltration.[26,53] In various murine tumor models, neutrophil

Table 1 Included studies with direct effects of radiation therapy on neutrophils (n = 36)

			Studied material					RT		
					Mouse model					
Author	Cancer type	Protumor or antitumor role of neutrophils	Cells in vitro	Patient	Strain	Tumor cell line	Genetically engineered	Dose (Gy)	Fraction	Effect of RT on PMN infiltration or function
Wisdom et al[10]	Soft tissue sarcoma Cervix	Pro		+			KrasLSL-G12D/+, Trp53flox/flox (−/+MRP8cre, −/+ R26lsl-dtx+)	20 ~80*	1 × 5 + 1 × 15 25 × 2 + 30*	PMN in tumor PMN in blood Function
Zhang et al[11]	Lung	Pro			C57BL/6	LLC		20	1 × 20	PMN in tumor Function
Shinde-Jadhav et al[12]	Bladder	Pro		+	C57BL/6	MB49		2 10	1 × 2 2 × 5 1 × 10	PMN in tumor PMN in blood Function: NETs
Takeshima et al[13]	Prostate Breast Thymoma	Anti			C57BL/6 BALB/c C57BL/6	RM-9 4T1 EG7		15 15 1.3	1 × 15 1 × 15 1 × 1.3	PMN in tumor Function
Mao et al[17]	Head and neck SCC	Pro		+	C3H/HeN C57BL/6	SCC7 4MOSC2		27	3 × 9	PMN in tumor Function
Zhang et al[18]	Colon/colorectal Prostate	Anti	+		C57BL/6	MC38 RM-9		15	1 × 15	PMN in tumor PMN in blood
Lin et al[26]	Prostate	Pro			C57BL/6	RM-1 Myc-Cap		16 8	2 × 8 1 × 8	PMN in tumor Function
Yin et al[37]	Esophageal squamous cell carcinoma	Pro			C57BL/6		*4NQO induced*	3 9 10	1 × 3 3 × 3 1 × 10	PMN in tumor
Ji et al[48]	Colon/colorectal	Both		+	C57BL/6	MC38		12	1 × 12	Function: SIRPα
Li et al[49]	Lung	Pro			C57BL/6	LLC		18 36	3 × 6 3 × 12	Function: IDO1
Fu et al[50]	Prostate	Pro			C57BL/6	TRAMP-C1		8 25	1 × 8 1 × 25	PMN in tumor PMN in blood Function
Han et al[51]	Breast	Pro	+		BALB/c HuNSG	4T1 PDX		24	3 × 8	PMN in tumor
Rodriguez-Ruiz et al[52]	Breast Colon Melanoma	Pro			BALB/c C57BL/6	4T1 MC38 B16OVA		24	3 × 8	PMN in tumor

(*Continued*)

Table 1 (*Continued*)

Author	Cancer type	Protumor or antitumor role of neutrophils	Studied material					RT		Effect of RT on PMN infiltration or function
			Cells in vitro	Patient	Mouse model			Dose (Gy)	Fraction	
					Strain	Tumor cell line	Genetically engineered			
Liu et al[53]	Lung	Anti	+		C57BL/6	LLC		24	3 × 8	PMN in tumor
Kim et al[54]	Breast	Pro			BALB/c	4T1		24	3 × 8	PMN in tumor
Boustani et al[55]	Colon/colorectal	Pro			BALB/c C57BL/6	CT26 MC38		24 36	3 × 8 18 × 2	PMN in tumor
Yazdimamaghani et al[56]	Breast	Anti			BALB/c	T11-AP		24	3 × 8	PMN in tumor
Boivin et al[57]	Lung	Both			C57BL/6		KrasLSL-G12D/+, Trp53flox/flox (KP mice)	11.7	1 × 11.7	PMN in tumor Function
Zhang et al[58]	Colon/colorectal Breast	Pro	+	+	C57BL/6 BALB/c	CT26 MC38 4T1 3T3		25	5 × 5	PMN in tumor PMN in blood
Napolitano et al[59]	Rectal	-		+				25	5 × 5	PMN in tumor PMN in blood
Nagtegaal et al[60]	Rectal	-		+				25	5 × 5	PMN in tumor
Lee et al[61]	Pancreas	Pro			C57BL/6	UN-KC-6141		10 25 40	1 × 10 1 × 25 4 × 10	PMN in blood
Oweida et al[62]	Pancreas	Pro	+	+	C57BL/6	FC1242 PK5L1940		8	1 × 8	PMN in tumor PMN in blood Function
Chen et al[63]	Liver	Pro			C57BL/6	Hepa1-6 H22		40	5 × 8 6 × 6 + 1 × 4 10 × 4 16 × 2.5	PMN in blood Function: ROS
Lennon et al[64]	Pancreas	Both			C57BL/6 nu/nu	FC1242 PDX		5 12	1 × 5 1 × 12	PMN in tumor PMN in blood
Navarro-Martín et al[65]	Lung	Pro		+				50 60	4 × 12.5 8 × 7.5	PMN in blood
Reijmen et al[66]	Lung	Pro			C57BL/6	LLC		12.8	4 × 3.2	PMN in tumor
Yamamoto et al[67]	Bladder	Pro			C57BL/6	MB49 MB49R		20	2 × 10 4 × 5	PMN in tumor + abscopal tumor

(*Continued*)

Table 1 (*Continued*)

			Studied material					RT		
					Mouse model					
Author	**Cancer type**	**Protumor or antitumor role of neutrophils**	**Cells in vitro**	**Patient**	**Strain**	**Tumor cell line**	**Genetically engineered**	**Dose (Gy)**	**Fraction**	**Effect of RT on PMN infiltration or function**
Leonard et al[68]	Breast Colon/colorectal Rectal	Pro	+	+	BALB/c	4T1 CT26 HCT116		4 8 12	1 × 4 1 × 8 1 × 12	Function: ARG1
Haidenberger et al[69]	-	-	+					2 6 12 18	1 × all doses 3 × 2 6 × 2 9 × 2	Function: ROS
Bian et al[70]	Colon/colorectal Pancreas	Both			C57BL/6	MC38 Pan02 KPC	SIRPα −/−	4 8 15	1 × 4 1 × 8 1 × 15	Function: ROS + SIRPα
Teijeira et al[71]	Breast Colon/colorectal	Pro	+		BALB/c	4T1		0.25 0.5 1 5 10	1 × all doses	Function: NETs
Kim et al[72]	Breast Colon/colorectal Fibrosarcoma	Pro			BALB/c BALB/c-nude C3H/HeJ	4T1 CT26 FsaII		20 40	2 × 10 2 × 20	PMN in tumor
Reichardt et al[73]	Breast	Pro			BALB/c	TS/A		16	2 × 8	PMN in tumor
Ali et al[74]	Glioblastoma	Both			C57BL/6	005 GBM	MPO ko	10	1 × 10	PMN in tumor
Chen et al[75]	Melanoma	Pro			C57BL/6	B-16 B-16R		30	2 × 15	PMN in tumor

Abbreviations: ARG1 = arginase 1; Gy = gray; IDO1 = indoleamine 2,3-dioxygenase 1; MPO = myeloperoxidase; PMN = neutrophil; NET = neutrophil extracellular trap; RT = radiation therapy; ROS = reactive oxygen species; SIRPα = signal regulatory protein alpha; SCC = squamous cell carcinoma.

* RT dose for patients is not mentioned, for a standard chemoradiotherapy regimen for cervix carcinoma this should be a total cumulative dose of about 80 Gy, consisting of 45 to 50 Gy delivered by stereotactic body radiation therapy (25 × 1.8-2 Gy in 5 weeks) and an additional dose via brachytherapy.

Table 2 Neutrophil (PMN) infiltration

Author	Murine tumor model	Route of administration, location	Cell line/*GEMM*	RT		Time points of measurement after RT	PMN decrease/ increase −/+	Method of measurement
				Dose (Gy)	Fractionation			
Han et al[51]	Breast	s.c. hind leg R	4T1	24	3 × 8	17d*	+ +	IF % CD11b
Kim et al[54]	Breast	s.c. hind leg R	4T1	24	3 × 8	17d	=/−	% CD11b
Reichardt et al[73]	Breast	s.c. flank	TS/A	16	2 × 8	7d	−	IMC
Yazdimamaghani et al[56]	Breast	s.c. mammary fat pad	T11-AP	24	3 × 8	24d	+	% of all cells
Zhang et al[58]	Breast Colon	s.c. hind leg R	4T1 CT26 MC38	25	5 × 5	2d, 9d, 16d	+, +, ++ =/+, ++, =/+ ++, ++, +	% CD45
Zhang et al[18]	Colon Prostate	s.c. hind leg R	MC38 RM-9	15	1 × 15	24h/1d	+ +	% of live cells % of all events
Takeshima et al[13]	Breast Prostate Thymoma	i.d. hind leg R	4T1 RM-9 EG7	15 15 1.3	1 × 15 1 × 15 1 × 1.3	6h, 12h, 24h, 48h, 96h	−, +, ++, =, = −, =, ++, +, = −, +, ++, =, =	% CD11b
Boustani et al[55]	Colon	s.c. flank R	CT26	24 36	3 × 8 18 × 2	7d	− +	% of total cells
Rodriguez-Ruiz et al[52]	Colon	s.c. flank R	MC38	24	3 × 8	2d	−	Count/gram Total count
Shinde-Jadhav et al[12]	Bladder Normal bladder	s.c. flank R	MB49	2 10 10	1 × 2 1 × 10 2 × 5 1 × 10	3d, 7d	=, = +++,++ +, + =†, =†	IF
Yamamoto et al[67]	Bladder	s.c. dorsal Abscopal dorsal‡ caudal	MB49 MB49R	20	2 × 10 2 × 10 4 × 5	1d, 8d	++, + ++, + ++, +	% CD11b‡
Ali et al[74]	Glioblastoma	Intracranially	005 GBM	10	1 × 10	7d	+	% CD45
Boivin et al[57]	Lung	lung infection, lentivirus induced	*KrasLSL-G12D/+, Trp53flox/flox (KP mice)*	11.7	1 × 11.7	1.5d	= +	IHC % CD45
Reijmen et al[66]	Lung	i.v.	LLC	12.8	4 × 3.2	5d	+	% CD45
Liu et al[53]	Lung	s.c. flank R	LLC	24	3 × 8	12h, 24h, 48h, 72h, 84h, 96h	−−, +++, ++, +, +, =	% of all cells

(*Continued*)

Table 2 (*Continued*)

Author	Murine tumor model	Route of administration, location	Cell line/*GEMM*	RT		Time points of measurement after RT	PMN decrease/ increase −/+	Method of measurement
				Dose (Gy)	Fractionation			
Zhang et al[11]	Lung	s.c. dorsal	LLC	20	1 × 20	21d 3d, 7d, 14d, 21d	+ =/+, =, =, ++	IHC % CD11b
Chen et al[75]	Melanoma	i.d. inguinal	B-16 B-16R	30	2 × 15	14d	− =	Count/gram
Lennon et al[64]	Pancreas	s.c. flank	FC1242 PDX (PANC272)	5 12	1 × 5 1 × 12	7d	=/+ −−	IHC
Oweida et al[62]	Pancreas	s.c. flank R	FC1242	8	1 × 8	4d	+	% of all cells
Fu et al[50]	Prostate	s.c. hind leg R	TRAMP-C1	8 25	1 × 8 1 × 25	2d, 7d[†] 2d, 14d	+, = ++, ++	IF
Lin et al[26]	Prostate	s.c. thigh L	Myc-Cap RM-1	8 16	1 × 8 2 × 8	2d, 10d, 20d 2d, 8d, 14d	++, −, − =, −, ?	% CD45
Kim et al[72]	Fibrosarcoma	s.c. hind leg	FsaII	40	2 × 20	10d, 36d	=, =	Count/gram % CD11b
Wisdom et al[10]	Soft tissue sarcoma		*KrasLSL-G12D/+, Trp53flox/flox (KP mice)*	20	1 × 5 + 1 × 15	7d	=/−	% CD45
Mao et al[17]	HNSCC	s.c. inguinal R	SSC7	27	3 × 9	4d	=	% CD45
Yin et al[37]	ESCC	Oral, 4NQO induced	4NQO induced	3 9 10	1 × 3 3 × 3 1 × 10	4d, 7d	+, + =, =/+ =/+, =	% CD45

+, increase; −, decrease; =, no difference; =/+ or =/−, trend of increase or decrease but nonsignificant; ?, unknown due to absence of control;

ESCC = esophageal SCC; GEMM = genetically engineered mouse model; Gy = gray; HNSCC = head and neck squamous cell carcinoma; i.d. = intradermal; i.v. = intravenous; IF = immunofluorescence; IHC = immunohistochemistry; IMC = imaging mass cytometry; L = left; PDX = patient derived xenograft; PMN = neutrophil; R = right; RT = radiation therapy; s.c. = subcutaneous.

[*] Days after RT not clearly stated.

[†] No PMN infiltration was measured.

[‡] Abscopal tumors were measured.

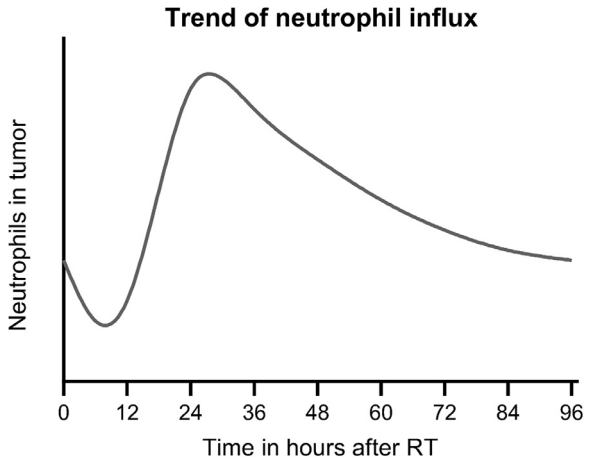

Fig. 2. Trend of neutrophil levels after radiation therapy. Neutrophil levels decreased directly after dose delivery. After initiation of the immune response, neutrophils are the first cells to infiltrate the tumor and showed an elevation that peaked after 24 hours. Over time neutrophil levels decreased back to baseline.[13,53]

dynamics were measured in the first few hours to days after RT.[13,26,53] It was shown that intratumoral neutrophils declined 6 to 12 hours after RT, suggesting radiosensitivity of tumor resident neutrophils. This was followed by neutrophil infiltration which peaked after 24 hours.[13,53] In line with these results, most studies measured the highest neutrophil levels 24 to 72 hours after RT,[11,50,51,58,79] after which neutrophil levels slowly declined, reaching baseline levels after 48 to 96 hours (Table 2, Fig. 2).[10-13,17,18,26,37,48-75] RT dose might delay and extent the duration of neutrophil influx, since studies using relatively higher cumulative doses of 20, 24, and 25 Gy measured elevated levels at later time points and up to 3 weeks after RT.[11,50,51,58] In addition, different RT fractionation strategies can influence neutrophil levels.[12,55] RT delivery in more than one fraction seems to delay neutrophil influx when comparing different studies using similar models (Table 2).[10-13,17,18,26,37,48-75] RT with 10 Gy showed lower neutrophil infiltration when fractionated in 2 doses compared with 1 dose.[12] Dynamics of neutrophil infiltration tend to follow a similar pattern in different tumor models, although the exact timing of this trend seemed to differ based on tumor biology or RT treatment regimen.[11-13,37,50,51,53,55,58]

Patient tumors

Only 4 studies assessed neutrophil infiltration in patient samples after RT. In patients with muscle invasive bladder cancer, neutrophil infiltration was measured before and after RT. Neutrophils were significantly increased in 6 out of 9 patients 1 month after RT.[12] In line with this, 2 independent studies on patients with rectal cancer showed increased infiltration of $CD11b^+$ myeloid cells, which contain neutrophils.[58,59] Specific markers to differentiate neutrophils from these myeloid cells were lacking, raising the question whether neutrophils were among the myeloid cells that infiltrated the tumor.[58,59] On the contrary, 1 study with 1304 patients with rectal cancer found lower levels of neutrophils in resected tumors after RT when compared with resection alone.[60] This could be the result of the timing of resection, and subsequent measurement of neutrophil numbers, since tumors were resected within 5 days after patients received fractionated RT (5 × 5 Gy). In murine tumor models a similar treatment regimen resulted in a delayed onset of neutrophil infiltration.[58,60]

Radiation induced systemic neutrophil response

Murine tumor models

In addition to local tumor infiltration, RT can induce a systemic immune response. In patients treated with RT, high neutrophil-to-lymphocyte ratios and high neutrophil levels in tumor and peripheral blood are associated with poorer outcomes.[9,12,21,22] Animal studies showed that RT can increase neutrophils in peripheral blood.[50,61,62] Conflicting results were found, since other studies detected a decrease of neutrophils in murine tumor models after RT,[18,58,63] or no alterations at all.[64] Although not always the case, increased blood neutrophil levels were more often reported after a single dose of RT of 8 to 25 Gy (Table 1).[10-13,17,18,26,37,48-75] Two studies showing a decrease delivered RT of respectively 25 and 40 Gy in multiple fractions, ranging from 5 up to 16 dose deliveries,[58,63] although a decrease with a single dose of radiation was also found in 1 study (Table 1).[10-13,17,18,26,37,48-75]

Patient studies

In patients with lung cancer, an increase in neutrophils was observed 72 hours after RT, followed by a decrease at later time points, albeit not significant.[65] In roughly 50% of the patients with cervical cancer, neutrophil levels were elevated after chemoradiotherapy and higher neutrophils levels were associated with more local and distant disease recurrence.[10] On the contrary, in patients with rectal cancer a decrease in neutrophils in the blood was found.[59] In these patients, $CD11b^+/CD15^+$ neutrophils in the blood decreased, although this coincided with an increased infiltration of $CD11b^+$ myeloid cells in the tumor. Interestingly, lower levels of $CD11b^+/CD15^+$ neutrophils were found in the blood in patients with poor response to RT, while simultaneously more myeloid cell infiltration in the tumor was observed.[59]

Compared with murine tumor models, treatment regimens for patients with cancer consisted of higher doses between 25 and 80 Gy delivered in 5 to 25 fractions (Table 1).[10-13,17,18,26,37,48-75] RT treatment regimen might influence systemic neutrophil levels. A decrease in circulating neutrophils could be the result of migration of these cells into the tumor, which could result in a more immunosuppressive TME.[59] However, alterations in neutrophil levels in the circulation did not always correspond to intratumoral infiltration.

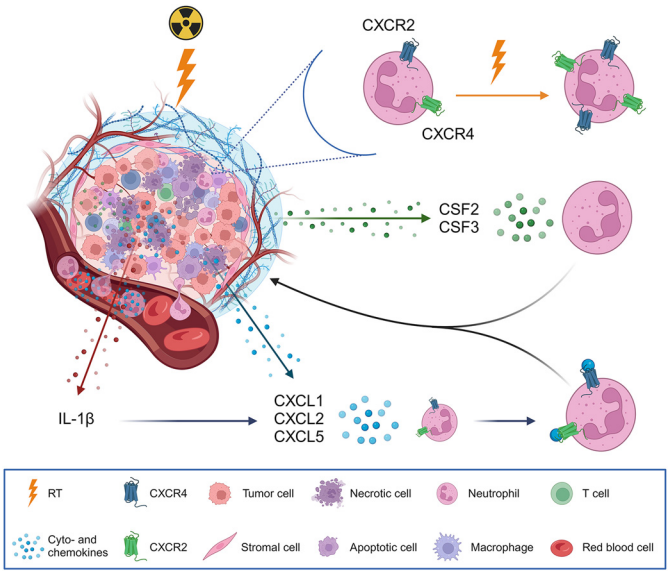

Fig. 3. Radiation therapy (RT) induced tissue damage causes cytokine release. RT induces the release of various cytokines such as interleukin-1 beta (IL-1β), C-X-C motif ligand 1 (CXCL1), CXCL2, CXCL5, colony-stimulation factor 2 (CSF2), and CSF3. IL-1β further induces the release of CXCLs. CSF2, CSF3, and CXCLs attract neutrophils to the tumor. Radiation upregulates C-X-C motif chemokine receptor 2 (CXCR2) and CXCR4 on neutrophils which bind to CXCLs, resulting in enhanced neutrophil recruitment.

Radiation induced cytokine and chemokine release by the tumor

Cytokines in murine tumor models following RT

Several studies indicated that RT-induced cytokine and chemokine release from the tumor is responsible for neutrophil recruitment and could also influence their phenotype and function.[80] Despite some slight variation between murine tumor models, increased expression of colony-stimulation factor 2 (CSF2, also known as GM-CSF), CSF3 (also known as G-CSF), chemokine C-C motif ligand 2 (CCL2), CCL5, C-X-C motif ligand 1 (CXCL1), CXCL2, CXCL5, interleukin-1 beta (IL-1β), and IL-6 was observed in multiple murine tumor models at different time points.[13,18,53,50,58,77,79,81-85]

RT-induced IL-1β increased neutrophil recruitment to the tumor via upregulation of CXCLs.[18] Inhibition or reduction of CSF3, CCL5, CXCL1, and CXCL2 reduced intratumoral neutrophil levels, showing that these cytokines are involved in neutrophil recruitment (Fig. 3).[53,81] Receptors for these cytokines such as C-C chemokine receptor type 1 (CCR1), CCR2, CCR5, C-X-C motif chemokine receptor 1 (CXCR1), CXCR2 and CXCR4 are present on myeloid cells in the tumor.[30,57,58,79] In particular, CXCR2 and CXCR4 are highly expressed on neutrophils and are shown to be upregulated by RT in multiple murine tumor models.[18,57] Blocking CXCR2 reduced neutrophil infiltration in vivo, showing that CXCR2 is one of the chemokine receptors responsible for neutrophil migration into the tumor after RT (Fig. 3).[14,18,79]

Cytokines which were less frequently measured or found elevated are CXCL3, CXCL7, CXCL12, CCL1, CCL3, IL-17α, interferon gamma, and vascular endothelial growth factor.[11,13,16,18,50,58,64,66,76,82-84,86]

Cytokines in circulation following RT

RT can also induce systemic alterations in cytokine and chemokine levels which can drive myelopoiesis and attract neutrophils. In murine bladder, breast, lung, prostate, and squamous cell tumor models cytokines were measured in blood after RT. CCL2, CSF2, IL-6, IFN-β, and interferon gamma were elevated in multiple tumor models,[37,50,54,66,67,76] although CSF2 was not elevated in another study.[67] CSF3, CCL5, CXCL1, CXCL2, IL-1β, IL-10, tumor necrosis factor alpha, and vascular endothelial growth factor were only increased in the blood of 1 tumor model.[50,51,58,66,67] In the blood of patients with pancreatic cancer, cytokines and chemokines were measured before, during and after RT treatment. Increased expression of CCL2, CCL4, transforming growth factor beta, and vascular endothelial growth factor was detected after, but not during RT.[62]

Effect of radiation on neutrophil phenotype and function

Phenotype of RT-induced neutrophils in mice

RT was found to influence neutrophil phenotype and function in the TME of mice, which can enhance both antitumor and protumor responses.[87] It was shown that RT can decrease the proportion of more protumorigenic Siglec-F^{high} neutrophils, which is compensated by the recruitment of newly produced more antitumorigenic Siglec-F^{low} neutrophils.[57] In murine tumor models RT was combined with neutrophil depletion to test whether neutrophils have an antitumor or protumor role after RT, but results were not consistent. Some studies showed improved RT efficacy with neutrophil depletion or inhibition of neutrophil effector

function,[10,11,62] while others showed no effect,[26,57,64] or decreased RT efficacy.[13,17,18] These differences could be due to technical difficulties since specific, efficient, long-term neutrophil depletion was deemed to be impossible until recently.[43,78]

Function of RT-induced neutrophils

One of the main mechanisms of immunosuppression by neutrophils is T cell inhibition. Neutrophils and myeloid cells can inhibit antitumor T cell proliferation and function via multiple pathways and all these mechanisms can play a role in case of increased neutrophil infiltration. Here, we will only discuss pathways altered by RT.[88]

As mentioned before, neutrophils aggregate in the necrotic region after RT (Fig. 4). Interestingly, these neutrophils were found to have higher indoleamine 2,3-dioxygenase 1 (IDO1) and arginase 1 (ARG1) expression than other neutrophils in the TME.[50] IDO1 and ARG1 are enzymes that respectively deplete tryptophan and L-arginine from the environment, which are essential amino acids for $CD8^+$ T cell proliferation and effector functions. Consequently, neutrophils recruited by RT can inhibit the T cell antitumor response via IDO1 and ARG1 release (Fig. 5).

IDO1

Besides T cell inhibition, IDO1 suppresses NK cells, stimulates Tregs, and induces angiogenesis.[36,88,89] In a murine lung tumor model, higher doses of RT increased IDO1 expression on $Gr\text{-}1^+$ myeloid cells, although neutrophil specific markers were not included. Furthermore, addition of an IDO1 inhibitor to RT was more effective in suppressing tumor outgrowth than RT alone, suggesting an immunosuppressive role for IDO1.[49] IDO1 inhibitors are currently tested in clinical trials and could have synergistic effects with RT.[49,89]

ARG1

Depletion of L-arginine from the environment by ARG1 causes proliferative arrest and downregulation of the CD3-dzeta-chain of the T cell receptor complex in T cells, which causes impaired T cell activation and proliferation.[88] In contrast to healthy donors, almost all circulating neutrophils were found to express ARG1 in patients with rectal cancer.[68] This shows that rectal cancer can already induce immunosuppressive functions in neutrophils and different phenotypes of neutrophils could be recruited in patients with cancer after RT.

In a murine prostate cancer model, intratumoral ARG1 expression as well as ARG1 activity were significantly increased after RT. Consequently, a lower percentage of $CD8^+$ T cells expressed the interferon gamma receptor, which reduced the number of activated $CD8^+$ T cells in the tumor.[11,90] In contrast to these results, data obtained from a murine pancreatic model showed that interferon gamma receptor expression by $CD4^+$ as well as $CD8^+$ T cells was increased after RT.[62]

Interestingly, targeting or depleting neutrophils increased the $CD8^+$ T cell activation state after RT and improved RT response in both studies.[11,62] When compared with RT alone, combination therapy with ARG1 inhibitors delayed tumor growth, increased the percentage of $CD8^+$ T cells and decreased the percentage of neutrophils in the tumor.[11] These results confirm that neutrophils increase ARG1 expression upon RT, resulting in $CD8^+$ T cell inhibition (Fig. 5). This makes ARG1 inhibition an interesting intervention in combination with RT.

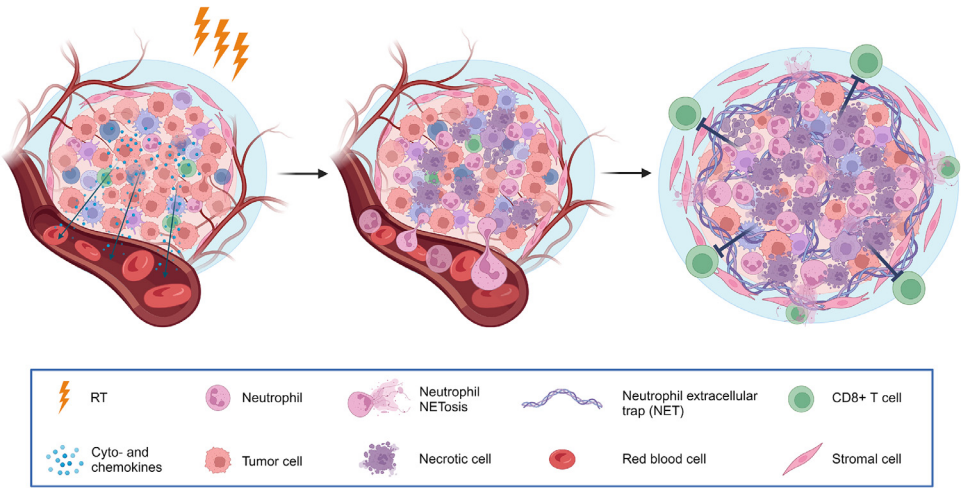

Fig. 4. Effects of radiation therapy (RT) on neutrophils in cancer. RT-induced cell death initiates an immune response. Neutrophil production is upregulated and neutrophils are attracted to the tumor. In the tumor, they accumulate in the necrotic area where they can inhibit the antitumor immune response via arginase 1 and indoleamine 2,3-dioxygenase 1 or can kill tumor cells via the release of reactive oxygen species. RT-induced neutrophil NETosis is activated via various pathways and can inhibit the T cell antitumor immunity. The neutrophil extracellular traps create a physical barrier between the tumor and the surrounding stroma that blocks $CD8^+$ T cells from entering.

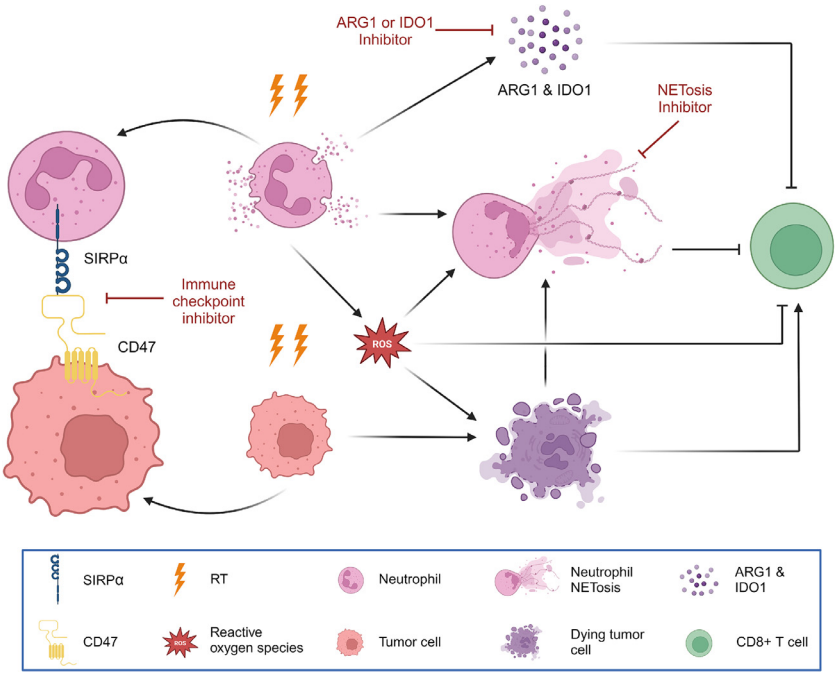

Fig. 5. Effect of radiation therapy (RT) on neutrophil immune response and potential targets for combination neutrophil-targeting therapies. RT recruits neutrophils into the tumor and increases the secretion of the molecules arginase 1 (ARG1) and indoleamine 2,3-dioxygenase 1 (IDO1) which inhibit $CD8^+$ T cells. Upon RT neutrophils release reactive oxygen species (ROS) which can kill tumor cell, but also inhibit $CD8^+$ T cells. NETosis by neutrophils is induced via various pathways: directly by RT, via ROS or via dying tumor cells. RT upregulates signal regulatory protein alpha (SIRPα) on myeloid cells and CD47 on tumor cells which inhibits antitumor immunity. Inhibition of potential therapeutic targets is depicted with red arrows.

Reactive oxygen species

Neutrophils are activated by danger signals and respond via the production and release of reactive oxygen species (ROS).[91,92] Radiation can influence the ability of neutrophils to release ROS after activation in in vitro experiments. Single doses of 6, 12, and 18 Gy were able to increase ROS production, whereas fractionated RT reduced ROS release compared with the unirradiated control.[69]

In murine thymoma, breast, prostate, and pancreatic cancer models, RT increased ROS production by neutrophils in irradiated tumors when compared with unirradiated tumors.[13,70] RT-induced neutrophils had an antitumor role in these models and inhibition of ROS production reduced the antitumor effect of neutrophils.[13,77]

Administration of CSF3 in combination with RT further increased ROS production by RT-induced neutrophils, resulting in more oxidative damage and apoptosis of tumor cells. As mentioned before, CSF3 is increased after RT and could play a role in ROS-mediated antitumor activity of neutrophils.[13,50,63] Interestingly, $CD8^+$ T cell depletion diminished the enhanced treatment response. These results suggest that neutrophils can also trigger a T cell antitumor response after RT via ROS-induced tumor cell death.[13]

In contrast to these results, some studies report that ROS production by neutrophils can suppress T cell proliferation and function.[88,93] RT-induced ROS production might have a dual role and antitumor or protumor effects are probably dependent on tumor type and characteristics (Fig. 5).

Neutrophil extracellular trap formation

One of the antimicrobial defense mechanisms of neutrophils is neutrophil extracellular trap (NET) formation. NETs are web-like structures that can be actively extruded by neutrophils to trap pathogens.[94] However, in cancer, NET formation by tumor infiltrating neutrophils can exert protumorigenic effects.[95]

In vitro, neutrophils isolated from blood of healthy donors and patients with cancer show NET formation after radiation with 0.25 to 10 Gy.[71] When irradiated neutrophils were added to colon carcinoma spheroids, NETs formed around the spheroids, which blocked T cells from interacting with tumor cells. In a killing assay, NETs protected against T and NK cell cytotoxicity, whereas NET degradation with DNAse treatment abrogated these effects.[71]

In an in vivo murine bladder cancer model NET formation was only seen in irradiated tumors. After RT, tumor cells released high mobility group box 1 which mediates NET formation by neutrophils (Fig. 5). Inhibiting NETosis delayed tumor growth and improved overall survival.[12] In accordance with in vitro experiments, IHC staining showed that NETs form a barrier between the tumor and stroma after RT and that $CD8^+$ T cell infiltration into the tumor region significantly increased when NETs were degraded. In nude $CD4^+$ and $CD8^+$ T cell lacking mice, NET degradation did not affect RT response, which suggests that RT-induced NET formation directly affects $CD8^+$ T cell antitumor response.[12] In a murine breast cancer model, more tumor

load was detected when intravenous tumor cell injection was preceded by injection of radiation induced NETs, suggesting that NETs can also enhance metastasis.[71]

Results from the murine bladder cancer model were in line with patient data. NET formation was seen in the TME of patients with muscle invasive bladder cancer (n = 104) after RT. Compared with RT responders, nonresponders had significantly more NETs in the TME. In addition, post-RT NET deposition was associated with worse overall survival.[12] In the nonresponding group, higher NET deposition correlated with significantly higher neutrophil infiltration. In the tumors $CD8^+$ T cells were surrounded by NETs but did not colocalize with them, showing that NETs prevent $CD8^+$ T cells from contacting tumor cells.[12]

These combined results indicate that RT induces NETosis by neutrophils, which is able to inhibit T cell antitumor response by physically blocking $CD8^+$ T cells from reaching tumor cells (Figs. 4 and 5).

Signal regulatory protein alpha-CD47 myeloid immune checkpoint

Tumor cells often overexpress immune checkpoints such as programmed cell death-ligand 1, which interacts with programmed cell death protein 1 on T cells to inhibit the T cell antitumor response.[87,96] Programmed cell death protein 1 and programmed cell death-ligand 1 are frequently upregulated after RT, which allows for combination therapy with immune checkpoint inhibitors in the clinic.[4,11,87,97]

Myeloid cells, including neutrophils, express signal regulatory protein alpha (SIRPα) which interacts with CD47 to inhibit myeloid cell effector functions such as phagocytosis. Similar to the programmed cell death protein 1 or programmed cell death-ligand 1 axis, RT was found to upregulate SIRPα on myeloid cells and CD47 on tumor cells in murine tumor models.[48,70] Specific markers to distinguish neutrophils from these myeloid cells were not used. Both SIRPα blockade and SIRPα knockout improved RT efficacy.[48,70] In addition, tumors of irradiated SIRPα knockout mice showed more neutrophil infiltration with higher levels of ROS, which was positively correlated with rapid tumor elimination.[70] These results suggest that therapies targeting neutrophil immune checkpoints can alter their role in the TME and combination with RT could be a promising therapeutic strategy (Fig. 5).

Discussion

RT-induced tissue damage triggers a local and systemic immune response. Neutrophils are the first responding immune cells and appear to infiltrate the tumor after RT. In most murine tumor models neutrophils infiltrate the tumor about 12 to 48 hours after RT. Neutrophil levels may decline after this initial peak but can be elevated up to 3 weeks after RT, although conflicting results were found. Specifically higher RT doses or more fractionated RT can delay the timing of neutrophil infiltration. Cytokines such as IL-1β, CSF3, and CXCLs are involved in RT-induced neutrophil chemotaxis to the tumor. Neutrophils can have an antitumor role in the TME after RT, specifically via increased ROS formation. However, in most cancers, neutrophils exert protumor effects via upregulation of ARG1 and IDO1 expression and NET formation which suppress the antitumor immune response. In addition, RT can upregulate the myeloid immune checkpoints SIRPα on neutrophils and CD47 on tumor cells which can inhibit the antitumor immune response.

Patient data on neutrophil infiltration are scarce, which might be explained by the complexity to obtain tumor material both before and after RT.[12,58-60] Consequently, researchers predominantly rely on in vivo murine models to study neutrophil infiltration. However, these models exhibit some limitations.

First, neutrophils in the tumor are often measured in relation to the amount of $CD45^+$ leukocytes or $CD11b^+$ myeloid cells. As radiation can kill or attract these or other immune cell populations, an increase in neutrophils does not always imply an absolute increase. For example, a study by Boivin et al[57] showed an increase in neutrophils relative to the number of $CD45^+$ cells with flow cytometry, but not an absolute increase in the number of events measured with IHC staining (Table 2).[10-13,17,18,26,37,48-75] In future experiments beads can be added to flow cytometry to quantify the number of cells and thus help to make a better estimation whether neutrophils numbers increase or not.

Second, most studies use a syngeneic tumor model, most often via subcutaneous injection of a cell line. For this reason, immune cells are not present in the tumor at the time of inoculation. In many unirradiated control groups, neutrophil infiltration increased with tumor development.[16,67] Therefore, we chose to present an increase in neutrophils relative to unirradiated control groups. Some studies use xenograft models, injecting human derived cells, requiring the use of immunocompromised mice.[64,72] These mice lack (functional) T and B cells, whereas sometimes NK cells are also affected, thereby preventing graft rejection. Absence of those immune cell subsets will influence the composition of the TME. In addition, mice are kept under pathogen-free conditions that can influence the activation state of immune cells. Syngeneic and xenograft tumor models are associated with rapid tumor growth, high tumor burden, and minimal matrix formation.[98] Genetically engineered mouse models undergo initial phases of tumor development, resulting in a TME that better represent that of patients. Differences in the TME will influence the immune response and can influence the phenotype of tumor infiltrating neutrophils.[98] There are also major differences between neutrophils in mice and in humans. Not only do mice have significantly fewer circulating neutrophils (10%-25% compared with 50%-70% of the white blood cell population in humans), mice lack specific genes important for neutrophil function, such as CXCL8. This is an important cytokine for neutrophil attraction and activation in humans.[98,99] Therefore, neutrophils could have different functions in mice compared with humans which makes translation of data from murine models almost impossible and emphasizing the need to confirm data obtained in mice in patients studies.[98] Novel

techniques such as imaging mass cytometry of tumor tissue after resection can help to improve translation by better visualization of the TME in murine models and patients.[73] In addition, imaging mass cytometry can improve understanding of the dynamics of the TME by providing information on infiltration, localization, interactions and functions of different cells in the TME.[73,100,101]

Third, the dose required to induce neutrophil infiltration differs between tumor models due to differences in radiosensitivity.[12,13] Whether higher doses result in increased or decreased infiltration of neutrophils remains unclear for now. In a prostate tumor model a higher irradiation dose resulted in increased neutrophil infiltration. However, the lower dose investigated in this study might have been insufficient since it only delayed tumor outgrowth by 1.5 days.[50]

Last, in most mice studies, lower irradiation doses were delivered, often in 1 fraction, which deviates from RT regimens delivered in clinical practice (Table 1).[10-13,17,18,26,37,48-75] In addition to differences in tumor biology, this impedes a direct translation of outcomes from mice studies to humans. A few studies compared different fractionation strategies, but data is limited.[12,37,67] A hypofractionated RT strategy may have beneficial effects regarding the TME, since it shows less myeloid cell infiltration.[16,55,61] However, a specific marker to distinguish neutrophils from other myeloid cells was only used in 1 study to show reduced neutrophil infiltration.[55]

As mentioned previously, the composition of the TME can also be altered by radiation induced apoptosis of tumor resident immune cells, which raises the question to what extend neutrophils are sensitive to radiation. Two murine tumor models suggest that neutrophils are among the radiosensitive immune cells, since intratumoral neutrophil levels showed a decline in the first 12 hours after RT.[13,53] In line with this, a decline in neutrophil numbers was observed in the patients with tumors of rectal cancer 5 days after RT.[60] However, this decline was only observed in a few studies. The lack of proliferation markers impedes proper assessment of radiosensitivity of neutrophils in tumor models and patients with cancer, as decreased neutrophil levels can be compensated by infiltration of new neutrophils.[79] As such, radiosensitivity of neutrophils is mainly studied in healthy donors or non-cancer models. The majority these studies suggest that neutrophils and macrophages are more resistant to radiation when compared with other immune cells subsets.[23-25,102] Nevertheless, it is important to note that radiation sensitivity of neutrophils is hard to study, since neutrophils go into apoptosis shortly after being cultured ex vivo. This challenges the reliability of outcomes, as only a small fraction of neutrophils remain.[23,24] Therefore, it remains difficult to provide conclusions on whether neutrophils are sensitive to radiation. In addition, radiosensitivity of neutrophils could differ between mice and men or between different neutrophil subsets and may depend on the sample from which they are obtained (blood vs healthy tissue vs tumor).

Neutrophil recruitment was shown to be mediated through local and systemic cytokine release after RT. Specifically IL-1β, CSF3, CCL5, CXCL1, and CXCL2 appeared to be involved in neutrophil recruitment.[13,18,50,53,58,79,81,83] Neutrophils highly expressed CXCR2, a receptor for CXCL1 and CXCL2, after RT and blocking this receptor or interfering with these cytokines reduced neutrophil infiltration.[18,57] Multiple other cytokines were measured, but the direct effects on neutrophil recruitment were not tested. Murine models were most often used for cytokine measurement. However, mice lack specific genes important for neutrophil function, such as the abovementioned CXCL8.[98] In addition, not every study measured the same set of cytokines. Besides neutrophil recruitment, cytokine release might also polarize neutrophils to a protumorigenic or antitumorigenic phenotype.[80] Since we did not primarily focus on the role of RT on cytokines release, other cytokines could be involved in neutrophil recruitment as well.

The way in which RT-induced neutrophil recruitment influences the immune response is dependent on the type of neutrophils that are recruited. RT can decrease the proportion of old Siglec-F^{high} protumorigenic neutrophils in mice, which is compensated by recruitment of young Siglec-F^{low} neutrophils that have a more antitumor phenotype.[57] Siglec-F^{high} neutrophils have been shown to exert tumor promoting functions and radioresistance is mediated by old Siglec-F^{high} neutrophils rather than young, newly produced, Siglec-F^{low} neutrophils.[15] This effect might be temporary and these young, recruited neutrophils could be converted to a protumor role. This could explain why most studies describe a protumor role of neutrophils in the TME,[13,17,18] and high neutrophil infiltration is associated with a poorer response to RT.[9,10,12,21,22]

Studies used neutrophil depletion in murine tumor models to determine the role of neutrophils in the TME. Neutrophil depletion could improve RT efficacy,[10,11] reduce RT efficacy,[13,17,18] or have no effect on RT efficacy.[57,64] The method of neutrophil depletion matters and can impact the immune response. Two studies used an anti-Gr-1 antibody for neutrophil depletion which is less specific since it can also decrease other immune cell subsets, but does result in efficient long-term depletion of Ly-6G and Ly-6C positive cells.[17,26,43,44] Depletion antibodies are often of rat origin which can result in reduced efficacy when mouse anti-rat antibodies are produced.[78] Furthermore, when testing the efficacy of neutrophil depletion, the depleting antibody can interfere with the detection antibody when similar epitopes are targeted. In addition, 1 study shows that neutrophils lose the Ly-6G antigen via internalization when treated with the rat Ly-6G (1A8) antibody.[57] Two studies used this rat Ly-6G antibody which is able to induce short-term neutrophil depletion, but fails to effectively deplete neutrophils for >2 days.[13,64] Long-term neutrophil depletion can be achieved by either masking rat Ly-6G antibodies with anti-rat antibodies, or by using a murinized version of the Ly-6G 1A8 antibody.[43,78] Finally, high neutrophil turnover and enhanced myelopoiesis due to neutrophil depletion could influence the immune response.[15,43,57,103]

RT can enhance antitumor response of neutrophils via upregulation of ROS release.[13,77] However, ROS release can also contribute to protumor effects.[88,93] RT can upregulate

ARG1 and IDO1 expression of neutrophils, which inhibits the T cell antitumor response.[50,88] RT can also upregulate NET formation by neutrophils which physically block immune cells from entering the tumor or getting access to tumor cells (Fig. 4).[12,71] The SIRPα-CD47 myeloid immune checkpoint is upregulated after RT and may inhibit antitumor responses and determine the faith of neutrophils to a protumor phenotype (Fig. 5).[48,70] Multiple other immunosuppressive properties of neutrophils were not found to be upregulated by RT, but could play a role as a consequence of increased neutrophil infiltration.[88]

Reduction of neutrophil infiltration and inhibition of immunosuppressive neutrophil effector functions can improve RT efficacy.[10-12,49,62] However, RT-induced neutrophil infiltration could also be exploited. Blocking myeloid immune checkpoints such as SIRPα-CD47 can enhance neutrophil antitumor immunity.[48,70] In addition, monoclonal antibodies of the IgA isotype directed against tumor antigens can activate both protumorigenic and antitumorigenic neutrophils to induce tumor cell killing.[104] Combination of IgA antibodies with RT could therefore be a promising therapeutic strategy.

To summarize: RT-induced tissue damage appears to recruit neutrophils to the tumor via cytokines released during the first few days after RT. Neutrophils play an important role in protumor and antitumor immune response and could contribute to therapy resistance. A dual role of neutrophils is suggested, but polarization in antitumor and protumor neutrophils is still not proven. The timing and extend of neutrophil infiltration is dependent on RT dose and fractionation, with higher irradiation doses and more fractionated RT showing a later onset of neutrophil infiltration. Data on tumor infiltrating neutrophils in patients is lacking due to logistical, ethical, and technical challenges. The role of neutrophils should be carefully considered when developing RT treatment strategies, since they can both enhance and reduce RT effects. Targeting neutrophils could overcome RT resistance and RT could improve therapies targeting neutrophils. In this review we showed that different methods are available to synergize with RT, such as inhibitors for IDO1, ARG1, and NET formation, as well as blocking the myeloid immune checkpoint CD47-SIRPα. However, the timing of neutrophil-targeting therapies in combination with RT may play an important role in therapy efficacy since neutrophil infiltration, as well as neutrophil polarization, is dynamic.

References

1. Barker HE, Paget JT, Khan AA, Harrington KJ. The tumour microenvironment after radiotherapy: Mechanisms of resistance and recurrence. *Nat Rev Cancer* 2015;15:409-425.
2. Krysko DV, Garg AD, Kaczmarek A, Krysko O, Agostinis P, Vandenabeele P. Immunogenic cell death and DAMPs in cancer therapy. *Nat Rev Cancer* 2012;12:860-875.
3. Kaur P, Asea A. Radiation-induced effects and the immune system in cancer. *Front Oncol* 2012;2(2):191.
4. Ko EC, Raben D, Formenti SC. The integration of radiotherapy with immunotherapy for the treatment of non-small cell lung cancer. *Clin Cancer Res* 2018;24:5792-5806.
5. Raskov H, Orhan A, Gaggar S, Gögenur I. Neutrophils and polymorphonuclear myeloid-derived suppressor cells: An emerging battleground in cancer therapy. *Oncogenesis* 2022;11:22.
6. Giese MA, Hind LE, Huttenlocher A. Neutrophil plasticity in the tumor microenvironment. *Blood* 2019;133:2159-2167.
7. Kuang DM, Zhao Q, Wu Y, et al. Peritumoral neutrophils link inflammatory response to disease progression by fostering angiogenesis in hepatocellular carcinoma. *J Hepatol* 2011;54:948-955.
8. Powell DR, Huttenlocher A. Neutrophils in the tumor microenvironment. *Trends Immunol* 2016;37:41-52.
9. Schernberg A, Blanchard P, Chargari C, Deutsch E. Neutrophils, a candidate biomarker and target for radiation therapy? *Acta Oncol* 2017;56:1522-1530.
10. Wisdom AJ, Hong CS, Lin AJ, et al. Neutrophils promote tumor resistance to radiation therapy. *Proc Natl Acad Sci U S A* 2019;116:18584-18589.
11. Zhang Md J, Zhang Md L, Yang Md Y, et al. Polymorphonuclear-MDSCs facilitate tumor regrowth after radiation by suppressing $CD8^+$ T cells. *Int J Radiat Oncol Biol Phys* 2021;109:1533-1546.
12. Shinde-Jadhav S, Mansure JJ, Rayes RF, et al. Role of neutrophil extracellular traps in radiation resistance of invasive bladder cancer. *Nat Commun* 2021;12:2776.
13. Takeshima T, Pop LM, Laine A, Iyengar P, Vitetta ES, Hannan R. Key role for neutrophils in radiation-induced antitumor immune responses: Potentiation with G-CSF. *Proc Natl Acad Sci U S A* 2016;113:11300-11305.
14. Liu X, Lan T, Mo F, Yang J, Wei Y, Wei X. Antitumor and radiosensitization effects of a CXCR2 inhibitor in nasopharyngeal carcinoma. *Front Cell Dev Biol* 2021;9(9) 689613.
15. Ancey PB, Contat C, Boivin G, et al. GLUT1 expression in tumor-associated neutrophils promotes lung cancer growth and resistance to radiotherapy. *Cancer Res* 2021;81:2345-2357.
16. Lan J, Li R, Yin LM, et al. Targeting myeloid-derived suppressor cells and programmed death ligand 1 confers therapeutic advantage of ablative hypofractionated radiation therapy compared with conventional fractionated radiation therapy. *Int J Radiat Oncol Biol Phys* 2018;101:74-87.
17. Mao L, Zhou JJ, Xiao Y, et al. Immunogenic hypofractionated radiotherapy sensitising head and neck squamous cell carcinoma to anti-PD-L1 therapy in MDSC-dependent manner. *Br J Cancer* 2023;128:2126-2139.
18. Zhang F, Mulvaney O, Salcedo E, et al. Radiation-induced innate neutrophil response in tumor is mediated by the CXCLs/CXCR2 axis. *Cancers (Basel)* 2023;15:5686.
19. Gentles AJ, Newman AM, Liu CL, et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. *Nat Med* 2015;21:938-945.
20. Shen M, Hu P, Donskov F, Wang G, Liu Q, Du J. Tumor-associated neutrophils as a new prognostic factor in cancer: A systematic review and meta-analysis. *PLoS One* 2014;9:e98259.
21. Bahig H, Taussky D, Delouya G, et al. Neutrophil count is associated with survival in localized prostate cancer. *BMC Cancer* 2015;15:594.
22. Choi N, Kim JH, Chie EK, Gim J, Kang HC. A meta-analysis of the impact of neutrophil-to-lymphocyte ratio on treatment outcomes after radiotherapy for solid tumors. *Med (Baltim)* 2019;98:e15369.
23. Falcke SE, Rühle PF, Deloch L, Fietkau R, Frey B, Gaipl US. Clinically relevant radiation exposure differentially impacts forms of cell death in human cells of the innate and adaptive immune system. *Int J Mol Sci* 2018;19:3574.
24. Heylmann D, Ponath V, Kindler T, Kaina B. Comparison of DNA repair and radiosensitivity of different blood cell populations. *Sci Rep* 2021;11:2478.
25. Heylmann D, Rödel F, Kindler T, Kaina B. Radiation sensitivity of human and murine peripheral blood lymphocytes, stem and progenitor cells. *Biochim Biophys Acta* 2014;1846:121-129.

26. Lin L, Kane N, Kobayashi N, et al. High-dose per fraction radiotherapy induces both antitumor immunity and immunosuppressive responses in prostate tumors. *Clin Cancer Res* 2021;27:1505-1515.
27. Ostrand-Rosenberg S, Horn LA, Ciavattone NG. Radiotherapy both promotes and inhibits myeloid-derived suppressor cell function: Novel strategies for preventing the tumor-protective effects of radiotherapy. *Front Oncol* 2019;9(9):215.
28. Chiang CS, Fu SY, Wang SC, et al. Irradiation promotes an m2 macrophage phenotype in tumor hypoxia. *Front Oncol* 2012;2:89.
29. Xu J, Escamilla J, Mok S, et al. CSF1R signaling blockade stanches tumor-infiltrating myeloid cells and improves the efficacy of radiotherapy in prostate cancer. *Cancer Res* 2013;73:2782-2794.
30. Liang H, Deng L, Hou Y, et al. Host STING-dependent MDSC mobilization drives extrinsic radiation resistance. *Nat Commun* 2017;8:1736.
31. Veglia F, Perego M, Gabrilovich D. Myeloid-derived suppressor cells coming of age. *Nat Immunol* 2018;19:108-119.
32. Bronte V, Brandau S, Chen SH, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat Commun* 2016;7:12150.
33. Ost M, Singh A, Peschel A, Mehling R, Rieber N, Hartl D. Myeloid-derived suppressor cells in bacterial infections. *Front Cell Infect Microbiol* 2016;6:37.
34. Gungabeesoon J, Gort-Freitas NA, Kiss M, et al. A neutrophil response linked to tumor control in immunotherapy. *Cell* 2023;186:1448-1464.e20.
35. Soukup K, Joyce JA. A long-distance relay-tionship between tumor and bone. *Immunity* 2018;48:13-16.
36. Jiménez-Cortegana C, Galassi C, Klapp V, Gabrilovich DI, Galluzzi L. Myeloid-derived suppressor cells and radiotherapy. *Cancer Immunol Res* 2022;10:545-557.
37. Yin X, Tian M, Zhang J, et al. MiR-26b-5p in small extracellular vesicles derived from dying tumor cells after irradiation enhances the metastasis promoting microenvironment in esophageal squamous cell carcinoma. *Cancer Lett* 2022;541 215746 541.
38. Chen MF, Hsieh CC, Chen WC, Lai CH. Role of interleukin-6 in the radiation response of liver tumors. *Int J Radiat Oncol Biol Phys* 2012;84:e621-e630.
39. Kozin SV, Kamoun WS, Huang Y, Dawson MR, Jain RK, Duda DG. Recruitment of myeloid but not endothelial precursor cells facilitates tumor regrowth after local irradiation. *Cancer Res* 2010;70:5679-5685.
40. Kioi M, Vogel H, Schultz G, Hoffman RM, Harsh GR, Brown JM. Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice. *J Clin Invest* 2010;120:694-705.
41. Chaturvedi P, Gilkes DM, Takano N, Semenza GL. Hypoxia-inducible factor-dependent signaling between triple-negative breast cancer cells and mesenchymal stem cells promotes macrophage recruitment. *Proc Natl Acad Sci U S A* 2014;111:E2120-E2129.
42. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. *Syst Rev* 2016;5:210.
43. Boivin G, Faget J, Ancey PB, et al. Durable and controlled depletion of neutrophils in mice. *Nat Commun* 2020;11:2762.
44. Quail DF, Amulic B, Aziz M, et al. Neutrophil phenotypes and functions in cancer: A consensus statement. *J Exp Med* 2022;219 e20220011.
45. Hestdal K, Ruscetti FW, Ihle JN, et al. Characterization and regulation of RB6-8C5 antigen expression on murine bone marrow cells. *J Immunol* 1991;147:22-28.
46. Fleming TJ, Fleming ML, Malek TR. Selective expression of Ly-6G on myeloid lineage cells in mouse bone marrow. RB6-8C5 mAb to granulocyte-differentiation antigen (Gr-1) detects members of the Ly-6 family. *J Immunol* 1993;151:2399-2408.
47. Daley JM, Thomay AA, Connolly MD, Reichner JS, Albina JE. Use of Ly6G-specific monoclonal antibody to deplete neutrophils in mice. *J Leukoc Biol* 2008;83:64-70.
48. Ji K, Zhang Y, Jiang S, et al. SIRPα blockade improves the antitumor immunity of radiotherapy in colorectal cancer. *Cell Death Discov* 2023;9:180.
49. Li A, Barsoumian HB, Schoenhals JE, et al. IDO1 inhibition overcomes radiation-induced "rebound immune suppression" by reducing numbers of IDO1-expressing myeloid-derived suppressor cells in the tumor microenvironment. *Int J Radiat Oncol Biol Phys* 2019;104:903-912.
50. Fu SY, Chen FH, Wang CC, Yu CF, Chiang CS, Hong JH. Role of myeloid-derived suppressor cells in high-dose-irradiated TRAMP-C1 tumors: A therapeutic target and an index for assessing tumor microenvironment. *Int J Radiat Oncol Biol Phys* 2021;109:1547-1558.
51. Han MG, Jang BS, Kang MH, Na D, Kim IA. PI3K$\gamma\delta$ inhibitor plus radiation enhances the antitumour immune effect of PD-1 blockade in syngenic murine breast cancer and humanised patient-derived xenograft model. *Eur J Cancer* 2021;157:450-463.
52. Rodriguez-Ruiz ME, Rodriguez I, Garasa S, et al. Abscopal effects of radiotherapy are enhanced by combined immunostimulatory mAbs and are dependent on CD8 T cells and crosspriming. *Cancer Res* 2016;76:5994-6005.
53. Liu Q, Hao Y, Du R, et al. Radiotherapy programs neutrophils to an antitumor phenotype by inducing mesenchymal-epithelial transition. *Transl Lung Cancer Res* 2021;10:1424-1443.
54. Kim S, Jeon SH, Han MG, Kang MH, Kim IA. BRD4 inhibition enhances the antitumor effects of radiation therapy in a murine breast cancer model. *Int J Mol Sci* 2023;24:13062.
55. Boustani J, Lecoester B, Baude J, et al. Targeting two radiation-induced immunosuppressive pathways to improve the efficacy of normofractionated radiation therapy in a preclinical colorectal cancer model. *Int J Radiat Biol* 2024;100:912-921.
56. Yazdimamaghani M, Kolupaev OV, Lim C, et al. Tumor microenvironment immunomodulation by nanoformulated tlr 7/8 agonist and pi3k delta inhibitor enhances therapeutic benefits of radiotherapy. Preprint. Posted online March 13, 2024. bioRxiv. doi:10.1101/2024.03.09.584084.
57. Boivin G, Ancey PB, Vuillefroy de Silly R, et al. Anti-Ly6G binding and trafficking mediate positive neutrophil selection to unleash the antitumor efficacy of radiation therapy. *Oncoimmunology* 2021;10 1876597.
58. Zhang B, Hu M, Ma Q, et al. Optimized CAR-T therapy based on spatiotemporal changes and chemotactic mechanisms of MDSCs induced by hypofractionated radiotherapy. *Mol Ther* 2023;31:2105-2119.
59. Napolitano M, D'Alterio C, Cardone E, et al. Peripheral myeloid-derived suppressor and T regulatory PD-1 positive cells predict response to neoadjuvant short-course radiotherapy in rectal cancer patients. *Oncotarget* 2015;6:8261-8270.
60. Nagtegaal ID, Marijnen CA, Kranenbarg EK, et al. Short-term preoperative radiotherapy interferes with the determination of pathological parameters in rectal cancer. *J Pathol* 2002;197:20-27.
61. Lee YH, Yu CF, Yang YC, Hong JH, Chiang CS. Ablative radiotherapy reprograms the tumor microenvironment of a pancreatic tumor in favoring the immune checkpoint blockade therapy. *Int J Mol Sci* 2021;22:2091.
62. Oweida AJ, Mueller AC, Piper M, et al. Response to radiotherapy in pancreatic ductal adenocarcinoma is enhanced by inhibition of myeloid-derived suppressor cells using STAT3 anti-sense oligonucleotide. *Cancer Immunol Immunother* 2021;70:989-1000.
63. Chen J, Wang Z, Ding Y, et al. Hypofractionated irradiation suppressed the off-target mouse hepatocarcinoma growth by inhibiting myeloid-derived suppressor cell-mediated immune suppression. *Front Oncol* 2020;10:4.
64. Lennon S, Oweida A, Milner D, et al. Pancreatic tumor microenvironment modulation by EphB4-ephrinB2 inhibition and radiation combination. *Clin Cancer Res* 2019;25:3352-3365.
65. Navarro-Martín A, Galiana IL, Berenguer Frances MA, et al. Preliminary study of the effect of stereotactic body radiotherapy (SBRT) on the immune system in lung cancer patients unfit for surgery: Immunophenotyping analysis. *Int J Mol Sci* 2018;19:3963.
66. Reijmen E, De Mey S, De Mey W, et al. Fractionated radiation severely reduces the number of $CD8^+$ T cells and mature antigen presenting cells within lung tumors. *Int J Radiat Oncol Biol Phys* 2021;111:272-283.

67. Yamamoto S, Kato M, Takeyama Y, et al. Irradiation plus myeloid-derived suppressor cell-targeted therapy for overcoming treatment resistance in immunologically cold urothelial carcinoma. *Br J Cancer* 2023;128:2197-2205.
68. Leonard W, Dufait I, Schwarze JK, et al. Myeloid-derived suppressor cells reveal radioprotective properties through arginase-induced l-arginine depletion. *Radiother Oncol* 2016;119:291-299.
69. Haidenberger A, Hengster P, Kunc M, et al. Influence of fractionated irradiation on neutrophilic granulocyte function. *Strahlenther Onkol* 2003;179:45-49.
70. Bian Z, Shi L, Kidder K, Zen K, Garnett-Benson C, Liu Y. Intratumoral SIRPα-deficient macrophages activate tumor antigen-specific cytotoxic T cells under radiotherapy. *Nat Commun* 2021;12:3229.
71. Teijeira A, Garasa S, Ochoa MC, et al. Low-dose ionizing γ-radiation elicits the extrusion of neutrophil extracellular traps. *Clin Cancer Res* 2024. https://doi.org/10.1158/1078-0432.CCR-23-3860.
72. Kim H, Lee E, Cho H, et al. Five-day spacing of two fractionated ablative radiotherapies enhances antitumor immunity. *Int J Radiat Oncol Biol Phys* 2024;118:498-511.
73. Reichardt CM, Muñoz-Becerra M, Rius Rigau A, et al. Neutrophils seeking new neighbors: Radiotherapy affects the cellular framework and the spatial organization in a murine breast cancer model. *Cancer Immunol Immunother* 2024;73:67.
74. Ali M, Fulci G, Grigalavicius M, et al. Myeloperoxidase exerts antitumor activity in glioma after radiotherapy. *Neoplasia* 2022;26 100779.
75. Chen HY, Xu L, Li LF, Liu XX, Gao JX, Bai YR. Inhibiting the $CD8^+$ T cell infiltration in the tumor microenvironment after radiotherapy is an important mechanism of radioresistance. *Sci Rep* 2018;8:11934.
76. Lee IJ, Lee EJ, Park H, et al. Altered biological potential and radioresponse of murine tumors in different microenvironments. *Cancer Res Treat* 2016;48:727-737.
77. Wu CT, Chen MF, Chen WC, Hsieh CC. The role of IL-6 in the radiation response of prostate cancer. *Radiat Oncol* 2013;8:159.
78. Olofsen PA, Stip MC, Jansen JHM, et al. Effective, long-term, neutrophil depletion using a murinized anti-Ly-6G 1A8 antibody. *Cells* 2022;11:3406.
79. Zhang Z, Yao Z, Zhang Z, et al. Local radiotherapy for murine breast cancer increases risk of metastasis by promoting the recruitment of M-MDSCs in lung. *Cancer Cell Int* 2023;23:107.
80. Tsioumpekou M, Krijgsman D, Leusen JHW, Olofsen PA. The role of cytokines in neutrophil development, tissue homing, function and plasticity in health and disease. *Cells* 2023;12:1981.
81. Chao T, Furth EE, Vonderheide RH. CXCR2-dependent accumulation of tumor-associated neutrophils regulates T-cell immunity in pancreatic ductal adenocarcinoma. *Cancer Immunol Res* 2016;4:968-982.
82. Guan L, Nambiar DK, Cao H, et al. NFE2L2 mutations enhance radioresistance in head and neck cancer by modulating intratumoral myeloid cells. *Cancer Res* 2023;83:861-874.
83. Krombach J, Hennel R, Brix N, et al. Priming antitumor immunity by radiotherapy: Dying tumor cell-derived DAMPs trigger endothelial cell activation and recruitment of myeloid cells. *Oncoimmunology* 2018;8 e1523097.
84. Trappetti V, Potez M, Fernandez-Palomo C, et al. Microbeam radiation therapy controls local growth of radioresistant melanoma and treats out-of-field locoregional metastasis. *Int J Radiat Oncol Biol Phys* 2022;114:478-493.
85. Yang X, Lu Y, Hang J, et al. Lactate-modulated immunosuppression of myeloid-derived suppressor cells contributes to the radioresistance of pancreatic cancer. *Cancer Immunol Res* 2020;8:1440-1451.
86. Cho WK, Shin SW, Kim SY, et al. Immunomodulatory effect of captopril and local irradiation on myeloid-derived suppressor cells. *Radiat Oncol J* 2016;34:223-229.
87. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: From T cell basic science to clinical practice. *Nat Rev Immunol* 2020;20:651-668.
88. Yang Y, Li C, Liu T, Dai X, Bazhin AV. Myeloid-derived suppressor cells in tumors: From mechanisms to antigen specificity and microenvironmental regulation. *Front Immunol* 2020;11:1371.
89. Liu M, Wang X, Wang L, et al. Targeting the IDO1 pathway in cancer: From bench to bedside. *J Hematol Oncol* 2018;11:100.
90. Whitmire JK, Tan JT, Whitton JL. Interferon-gamma acts directly on $CD8^+$ T cells to increase their abundance during virus infection. *J Exp Med* 2005;201:1053-1059.
91. Laforge M, Elbim C, Frère C, et al. Tissue damage from neutrophil-induced oxidative stress in COVID-19. *Nat Rev Immunol* 2020;20:515-516.
92. Nguyen GT, Green ER, Mecsas J. Neutrophils to the ROScue: Mechanisms of NADPH oxidase activation and bacterial resistance. *Front Cell Infect Microbiol* 2017;7:373.
93. Rashida Gnanaprakasam JN, Wu R, Wang R. Metabolic reprogramming in modulating T cell reactive oxygen species generation and antioxidant capacity. *Front Immunol* 2018;9:1075.
94. Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. *Science* 2004;303:1532-1535.
95. Demkow U. Neutrophil extracellular traps (NETs) in cancer invasion, evasion and metastasis. *Cancers (Basel)* 2021;13:4495.
96. Jiang Y, Chen M, Nie H, Yuan Y. PD-1 and PD-L1 in cancer immunotherapy: Clinical implications and future considerations. *Hum Vaccin Immunother* 2019;15:1111-1122.
97. Voronova V, Vislobokova A, Mutig K, et al. Combination of immune checkpoint inhibitors with radiation therapy in cancer: A hammer breaking the wall of resistance. *Front Oncol* 2022;12 1035884.
98. Eruslanov EB, Singhal S, Albelda SM. Mouse versus human neutrophils in cancer: A major knowledge gap. *Trends Cancer* 2017;3:149-160.
99. Mestas J, Hughes CC. Of mice and not men: Differences between mouse and human immunology. *J Immunol* 2004;172:2731-2738.
100. Hoch T, Schulz D, Eling N, Gómez JM, Levesque MP, Bodenmiller B. Multiplexed imaging mass cytometry of the chemokine milieus in melanoma characterizes features of the response to immunotherapy. *Sci Immunol* 2022;7:eabk1692.
101. Kuett L, Catena R, Özcan A, et al. Three-dimensional imaging mass cytometry for highly multiplexed molecular and cellular mapping of tissues and the tumor microenvironment. *Nat Cancer* 2022;3:122-133.
102. Ponath V, Heylmann D, Haak T, Woods K, Becker H, Kaina B. Compromised DNA repair and signalling in human granulocytes. *J Innate Immun* 2019;11:74-85.
103. Moses K, Klein JC, Männ L, Klingberg A, Gunzer M, Brandau S. Survival of residual neutrophils and accelerated myelopoiesis limit the efficacy of antibody-mediated depletion of Ly-6G+ cells in tumor-bearing mice. *J Leukoc Biol* 2016;99:811-823.
104. Brandsma AM, Bondza S, Evers M, et al. Potent Fc receptor signaling by IgA leads to superior killing of cancer cells by neutrophils compared to IgG. *Front Immunol* 2019;10:704.