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REVIEW

Immunotherapy as sensitizer for local radiotherapy

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ABSTRACT

The purpose of this report was to systematically review the radiation enhancement factor (REF) effects of immunotherapy on radiotherapy (RT) to the local tumor in comparison with other traditional radiation sensitizers such as cisplatin. PubMed and Medline databases were searched until February 2019. Reports with abscopal effect in the results were excluded. Graphs of the selected papers were digitized using Plot Digitizer (Sourceforge.net) in order to calculate the tumor growth delay (TGD) caused by immunotherapy. To enable comparison between different studies,the TGD were used to define the REF between RT versus the RT/immunotherapy combination. Thirty-two preclinical papers, and nine clinical series were selected. Different mouse models were exposed to RT doses ranging from 1 to 10 fractions of 1.8 to 20 Gray (Gy) per fraction. Endpoints were heterogeneous, ranging from regression to complete local response. No randomized clinical studies were identified. The median preclinical REF effect of different immunotherapy was varying from 1.7 to 9.1. There was no relationship observed either with subclasses of immunotherapy orRT doses. In the clinical studies, RT doses ranged from 1 to 37 fractions of 1.8 to 24 Gy per fraction. Most clinical trials used ipilimumab and interleukin-2. Local control rate in the clinical series ranged from 66% to 100%. A strong REF of immunotherapy (1.7 to 9.1) was observed, this being higher than traditionally sensitizers such as cisplatin (1.1). This result implies that for the same RT dose, a higher local control was achieved with a combination of immunotherapy and RT in preclinical settings. This study therefore supports the use of combined RT and immunotherapy to improve local tumor control in clinical settings without exacerbation of toxicities.

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Introduction

Radiotherapy (RT) is one of the three anticancer treatments, besides surgery and systemic therapies like chemotherapy, hormonal therapy, or immunotherapy. Several randomized trials and meta-analyses have shown that the addition of either cisplatin or 5-fluorouracil-based chemotherapy to RT significantly improves local control and survival over RT alone in several cancer subtypes such as esophagus, head and neck, lung, rectum, anal, cervix, and bladder cancer.[1–7] Although RT primarily damages the DNA of local cancer cells, it also changes the tumor microenvironment by generating local inflammatory reactions and enhancing tumor cell recognition by the host's immune system. These local processes can even be enhanced when triggering the immune system by immunotherapy.[8,9] RT-induced cancer cell damage exposes tumor-specific antigens to the immune system through a process called immunogenic cell death (ICD).[10] This process leads to improved priming and activation of cytotoxic T cells.[11] Furthermore, RT leads to the release of T-cell-attracting chemokines and the upregulation of surface receptors that makes tumor cells more vulnerable to T-cell-mediated cell killing. Such a combination may lead to increased effectiveness of *local* RT. Additionally, the RT + immunotherapy combination may even lead to an improved *systemic* effect, also known as the 'abscopal' effect (ab scopus: on a distant site) where the immune system starts to combat tumor deposits outside the radiation field more efficiently.[12] However, the abscopal effect is not within the scope of this review. The primary aim of this article is to systematically review the literature on the *local* effect of immunotherapy on RT in preclinical and clinical data. To this end, an estimation of the radiation enhancement factor (REF) for (the different forms of) immunotherapy was derived from the literature.

Materials and methods

A systematic review of the relevant literature search in the PubMed/Medline database was performed in February 2019 by BV. Search terms included 'radiotherapy' AND 'immunotherapy' AND 'local effect(s)'. Furthermore, an additional

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Table 1. The three levels of response according to their assumed clinical relevance and reliability of the study endpoints (Table 1).

Level of response	Study Endpoints	Clinical Releavance
1	**Local Tumor Control > 6 months**	**Sustained complete Response = Cure**
2	**Local tumor control < 6 months**	**Complete Response**
3	**Growth Delay**	**Partial Response**

search was performed using the terms 'radiotherapy' AND 'immunotherapy' AND 'local' NOT 'review' NOT 'abscopal' NOT 'metastatic'. Results were limited to manuscripts in the English language. Preclinical and clinical data were included. A manual review of filtered records was conducted for relevance by screening on their titles and abstracts alone. Articles were excluded if solely describing the (systematic) abscopal effect, or if other concurrent cytotoxic treatments (chemotherapy, hyperthermia) were also administered. Clinical case reports on single patients were excluded. Finally, the selected clinical and preclinical papers from prior knowledge of the authors were also screened for additional papers that met the selection criteria.

To assess the quality differences of the preclinical studies, we divided these into three levels of response according to their assumed clinical relevance and reliability of the study endpoints (Table 1). Level 1 represented the highest level of response with a complete remission of the local tumor over a long follow-up period of at least 6 months to exclude regrowth.[13] The 6 months threshold was chosen because in several experiments this level is taken as a cutoff, f.e. in a clinical trial, results would be reported as a percentage of complete responses. This level is denoted as *cure* and was scored as a percentage of test animals with a complete remission after a long time. Level 2 response represented a complete remission over a shorter follow-up period of less than 6 months. This level is defined as complete disappearance of the tumor after treatment, followed by regrowth within 6 months. Level 3 response represented growth delay as the reported endpoint, without achieving cure.

To obtain a quantitative number of the local RT sensitizing effect of immunotherapy for the Level 3 studies, all graphs in the selected papers were digitized using Plot Digitizer (v2.6.8, Oct 2015, downloaded from https://sourceforge.net). Tumor growth delayed (TGD) was obtained for every specific immunotherapy agent and was calculated as:

$$TGD = [T_{tv} \times 4] - [T_{cv} \times 4]$$

where $T_{tv} \times 4$ and $T_{cv} \times 4$ is the time to reach four fold tumor volume increase compared to treatment start, based on an exponential growth fit in treated tumors (tv) and in untreated control tumors (cv), respectively.

When $T_{tv} \times 4$ was not reached due to stable disease, i.e. tumor was not growing or tumor was cured (progression-free): the volume of the last day of follow-up was used.

These calculated TGD were used to obtain the radiation enhancement factor (REF) by this formula:

$$REF = TGD_{RT + IO}/TGD_{RT}$$

When no graphics of tumor volume were available for calculating REF, the specific ratios are used: when survival curves were available, the REF was calculated as:

$$REF = \text{Median Survival}_{RT + IO}/\text{Median Survival}_{RT}$$

Again, if the median survival was not reached, the last day of follow-up was used.

When percentages of responses were available, the REF was calculated as:

$$REF = \%\ DFS_{RT + IO}/\%\ DFS_{RT}$$

where DFS is the disease-free survival.

Beside the three levels of responses in preclinical studies, the clinical results are reported as a percentage of partial responses.

All forms of immunotherapy were divided into different subclasses according to their working mechanism: immune checkpoint inhibitors: anti-PD-(L)1; anti-CTLA4; cytokines: r-IL2; vaccines/dendritic cells; CPG/Toll-like receptor; and others.

A non-parametric Kruskal–Wallis test is performed with a Dunn's multiple comparisons test to obtain a significant differentiation of the subclasses of immunotherapy and in comparison of immunotherapy with cisplatin. A p-value <0.05 was considered statistically significant.

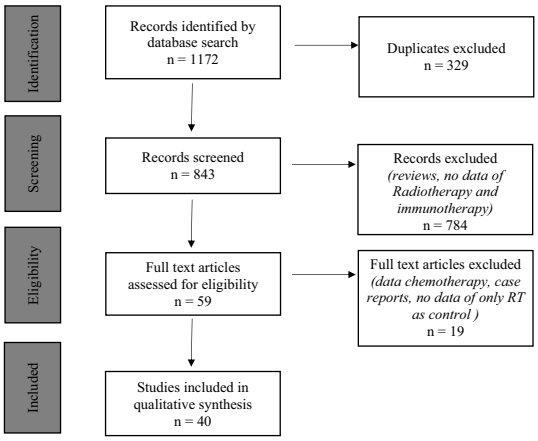

Figure 1. Flowchart of studies, which were identified by the literature search, screened excluded or included from analysis.

Results

We identified 1172 PubMed/Medline references (Figure 1). Thirty-seven preclinical papers were retrieved that directly reported local effects, which are summarized in Tables 2 and 3. All experiments were performed in mice except one report described experiments performed in rats, [17] All selected studies used RT in combination with immunotherapy to sensitize the local radiotherapy effect. Some reports also described the systemic effect of RT.

Seven different immune-competent mouse strains had been used: the C57BL/6 and Balb/c were most frequently presented. These mice had been mostly used because the tumor models were syngeneic with these genetic strains (See Table 2). Only one report used nude mice to investigate the role of T cells in

Table 2. Overview of level 2 preclinical studies according to the search criteria.

First Author	Year	Tumor type	Implantation site	Animal strain	Immune competent/ Syngeneic	Radiotherapy (Site – Total Dose [Gy]/ Fractions)	Immunotherapy	Observed Effect	Level of response	Suggested mediator
Plautz[14]	1996	fibrosarcomaMCA 205	i.c.	C57BL/6 J (B6) mice	+/+	Whole Body Irradiation – 5 Gy/1x	Adoptive transfer of SEC2-activated tumor-draining lymph node cells from MCA 205 subcutaneous tumor-bearing B6 mice	CR 100%	2	CD4 CD8 MHC-I MHC-II
Everse[15]	1997	SL2 lymphoma M8013 mammary carcinoma	s.c on one or both thighs	DBA/2JIco mice C57BL/ 6 JIcoU mice	+/+	Local tumor – 10 to 25 Gy/ 1-4 x	7000 IU/day rIL-2 20,000 IU/day rIL-2 daily p.t. 5 to 10 d	CR 100%	2	NA
J rgenliemk-Schulz[16]	1997	SL2 lymphoma M8013 mammary carcinoma	s.c. on one or both flanks	DBA/2JIco mice C57BL/ 6JIcoU mice	+/+	Local tumor – 10 to 25 Gy/ 1- 10x	7000 IU/day rIL-2 20,000 IU/day rIL-2 daily p.t. 5 to 10 d	CR 90%	2	CD8 CD4
Meng[17]	2005	9 L glioma	s.c. into the right flank or the right leg	Fisher rat	+/+	Flank – 30 Gy/ 10x	CpG oligodeoxynucleotide 28	CR: 66%	2	Toll-like Receptor 9
Mason[18]	2005	Fibrosarcoma - C3Hf	i.m. of the right hind leg	KamLaw mice	+/+	Leg – 10 to 90 Gy/10x	CpG oligodeoxynucleotide 1826	CR: 25 to 88%	2	Toll-like Receptor 9
Zegers[19]	2015	C51colon carcinoma, Lewis lung carcinoma, or 4T1 mammary carcinoma cells	s.c. Flank	C57Bl/6 + Balb/c mice	+/+	Flank – 10 Gy/ 1x	L19-IL2	C51: CR: 75% LLC: additive effect 4T1: no effect	2	an increased combination of NK and cytotoxic T cells 4T1: low/negative ED-B–expressing
Van den Heuvel[20]	2015	Lewis lung carcinoma	i.m. right quadriceps muscle	C57Bl/6 mice	+/+	Leg – 3.6 Gy/2x	NHS-IL2	CR 80 to 100%	2	Upregulated expression of effector T cells (CD3, CD4, CD8, CD25)
Schölch[21]	2015	pancreatic adenocarcinoma cell lines: Panc-1 a+ BxPC3 Colorectal carcinoma cell lines HT29, HCT-116, and CT26	s.c. in the right flank	BALB/c fg + C57Bl/ 6 mice	+/+	Flank – 10 Gy/ 5x	Toll-like receptor 7/8 agonists	CR: 50%	2	Upregulate antigen-presenting activity of dendritic cells + T cells
Connolly[22]	2016	Colon38, Glioma261, Line1	i.m. left leg	C57BL/6 + BALB/ cJ mice	+/+	Leg – 15 Gy / 1x	CCR2/CCR5 antagonist	CR: 40%	2	Increase of circulating + intratumoral inflammatory monocytes, chemokines; promote migration of myeloid cells, upregulation of CCL2 and CCL5 transcripts
Wu[23]	2018	BNL-P2 HCC cells	s.c. in the right flank	Balb/c mice	+/+	Flank – 10 Gy/ 1x	adenoviral vector +,IL 12	CR: 40%; PR: 50%	2	expression of MHC class II + CD40, CD86 on tumor-infiltrating dendritic cells
Zhuang[24]	2018	Lewis lung carcinoma -cells	s.c. in the right leg	C57BL/ 6mice	+/+	Leg – 8 Gy/1x	CpG (intratumoral), Anti-PD-1	CR: 100%	2	Toll-like Receptor 9, CD8 + T-cell infiltration + PD-L1 expression

i.c.: intracranial injection, s.c.: subcutaneous, i.m.: inta-musculair CR: Complete Response, PR: Partial Response, NA: not appropriated

Table 3. Overview of level 3 preclinical studies according to the search criteria.

First Author	Year	Tumor type	Implantation site	Animal	Immune competent / Syngeneic	Radiotherapy (Site – Total Dose [Gy] / Fractions)	Immunotherapy	Observed Effect	Level of response	Suggested mediator
Buchegger[25]	1995	Col 12 and LS174T	Transplants on the middle of their backs at 2 cm from the tail	nude mice	- / +	Back – 16 Gy/2x	3 monoclonal anti-CEA antibodies (mAb 35, CE25-B7, and B93)	Tumor growth delay	3	NA
Chiang[26]	2000	Fibrosarcoma – C3H/HeN	s.c. in the right thigh	C3H/HeJ mice	+ / +	right thigh – 25 to 35 Gy/1 x	IL-3 tumor vaccin	Tumor growth delay	3	increased intra tumoral levels of intercellular adhesion molecule-1, Mac-1, EB22/5.3, tumor necrosis factor
Lohr[27]	2000	4T1 mammary tumor cfC3H – B16 melanoma	s.c. in the right hind leg	BALB + C57BL/6 mice	+ / +	Leg – 18 to 33 Gy/3 x	Adenovirus, IL-12 – B7.1	Tumor growth delay	3	Upregulation T-cells and NK-cells
Teitz-Tennenbaum[28]	2003	D5melanoma or MCA 205 sarcoma	s.c. in the mid-right flank	C57BL/6 mice	+ / +	Flank – 42.5 Gy/5x	Dendritic cell	D5: Tumor inhibition 65.9% MCA 205: tumor inhibition	3	IFN- production by host-derived T cells
Huang[29]	2007	renal cell carcinoma	s.c. in the right axilla	C57Bl/6 mice	+ / +	Right Axilla – 35 Gy / 5x	Dendritic cell	Tumor growth delay	3	Down-regulation of Bcl-2, up-regulation of Bax, Expression of TNFα, IL-2, IL-4 m IFN-γ, IgG, IgM.
Meng[30]	2012	melanoma cell line B16SIY	s.c. in the right leg	C57Bl/6 mice	+ / +	Right Leg – 6 or 12 Gy / 1x	poly(ADP-ribose) polymerase inhibitor veliparib	Tumor growth delay	3	express immune stimulatory cytokines (CCL2, CCL5, CXCL9, CXCL10, and CXCL11) to activate cytotoxic T lymphocytes
Wang[31]	2012	T-26, a murine colon carcinoma cell line	s.c.in the left flank	BALB/c mice	+/+	Left flank – 8 Gy / 1x	Dendritic cell + Recombinant heat shock protein 70	Tumor growth delay	3	Expression of TNFα, IL-12, T-cell upregulation
Wei[32]	2013	murine D5 melanoma	s.c.in the right flank	C57BL/6 (B6) and B6.PL-Thy1a/CyJ (CD90.1) mice	+/+	bilateral flanks – 8.5 Gy / 5x	IL-2	Tumor growth delay	3	expression of the effector cytokines IFN-γ and TNF-α by donor and host CD4+ and CD8 + T cells
Dovedi[33]	2014	CT26 murine colon carcinoma cells	s.c. – not further specified	BALB/c and C57Bl/6 mice	+ / +	Local – 10 Gy / 5x	anti PD-L1	Tumor growth delay	3	NK cells, CD8+ T-cell upregulation
Lim[34]	2014	B16 melanoma	i.m. into the lower left thigh	C57BL/6J	+ / +	Leg – 15 Gy / 1x	Listeria monocytogenes-based cancer vaccine	Tumor growth delay	3	increase in intratumoral numbers of activated T cells, antigen-specific CD8 + t cells, natural killer cells + levels of effector molecules, such as interferon γ and granzyme B
Rekers[35]	2015	F9 terato-carcinoma cells	s.c.in the flank	129/SvHsd mice		Flank – 12 Gy / 1x	L19-IL2	Tumor growth delay	3	Extra Domain-B expression and infiltration of cytotoxic T cells.
Blanchard[36]	2015	B16-OVA melanoma	s.c. in the hind limb.	C57BL/6 mice	+ / +	Lower limb – 20 Gy/1x	vesicular stomatitis virus- tumor-associated antigen viral immunotherapy	Tumor growth delay	3	Upregulation T cells
Mondini[37]	2015	TC1/Luccells / HNSCC implantation model –	submucosal site of the right inner lip	C57BL/6 mice	+ / +	head and neck region – 2.6 –7.5 Gy / 1-4x	STxB-E7vaccine	Tumor growth delay	3	tumor-infiltrating, antigen-specific CD8 + T cells

(*Continued*)

Table 3. (Continued).

First Author	Year	Tumor type	Implantation site	Animal	Immune competent / Syngeneic	Radiotherapy (Site – Total Dose [Gy] / Fractions)	Immunotherapy	Observed Effect	Level of response	Suggested mediator
Sharabi[38]	2015	MC38-OVA cells; B16-OVA melanoma cells; 4T1HA breast carcinoma cell	s.c.in the right flank	C57BL/6, BALB/cJ, and MHC Class I knockout mices	+/+	Right flank – 10 to 20 Gy / 1x	Anti- PD-L1	Tumor growth delay	3	increased T-cell infiltration in tumor: CD8+, CD4+ CD25 + Foxp3 + T-regulatory cells
Monjazeb[39]	2016	B16 melanoma or 4T1 breast adenocarci-noma	into the flank	C57BL/6 or BALB/c mice	+ / +	Mammary fat pad / flank – 8 Gy/ 1x	CpG oligodeoxynucleotide, enzyme indolamine-2,3-dioxygenase blockade	Tumor growth delay	3	upregulation Toll-like Receptor 9, CD4+
Young[40]	2016	CT26 murine colorectal carcinoma	s.c.in the right hind limb	BALB/c and FVB mice	+/+	Limb – 20 Gy / 1x	OX40 (CD134) Anti-CTLA4	Tumor growth delay	3	Depletion of CD4+ or CD25
Zheng[41]	2016	Panc02 and MC57-SIY cells	s.c. on the back of the mice	C57BL/6 mice	+ / +	Local – 20 Gy/ 1x	Vaccination+ antiPD-L1	Tumor growth delay	3	CD8 + T cell infiltration; upregulation of CXCL10 and CCL5 chemokine
Oweida[42]	2017	LY2 + B4B8 squamous cell carcinoma	Submucosal via the buccal mucosa	BALB/c mice	+/+	Buccal + regional neck level – 10 Gy/1x	anti PD-L1	Tumor growth delay	3	upregulation of PD-L1 increased T-cell infiltration in tumor
Weiss[43]	2017	SMA glioma cell lines	intracranial (the right striatum)	C57BL/6 CD45.2 mice	+/+	Cranial – 4 Gy/ 1x	NKG2D-Based CAR T Cells	Tumor growth delay	3	high IFNg production and cytolytic activity in vitro
Choi[44]	2018	CT-26 colon carcinoma cells	s.c. into the right legs and left flanks	BALB/c mice	+/+	Leg – 15 Gy/ 1x –	Dendritic cell	Tumor growth delay	3	Maximum Dendritic cell sensitization and T-cell stimulation with IL-10, IL-12, and interferon (IFN)-γ production
Wang[45–50]	2019	Lewis lung carcinoma	s.c. into the left upper flank	C57Bl/6 mice	+ / +	Flank – 24 Gy/ 3x	α-PD-L1	Tumor growth delay	3	CD8 T-cell infiltration; PD-L1 expression

s.c.: subcutaneous, i.m.: inta-musculair, PR: Partial Response, NA: not appropriated

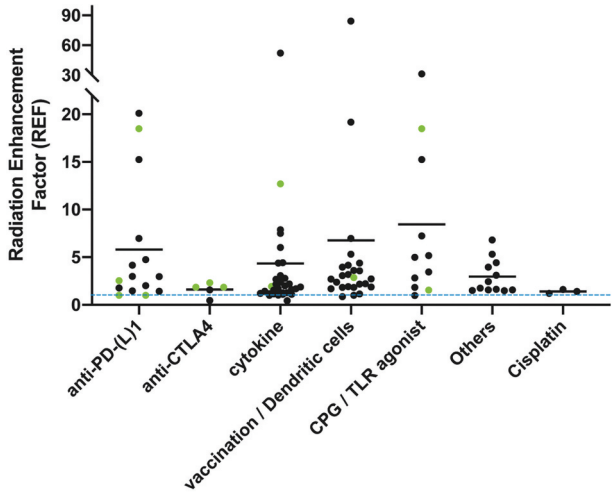

Figure 2. The radiotherapy sensitizing effect of Immunotherapy is compared between different studies: therefore tumor growth delay(TGD) was calculated and used to define the Radiation Enhancement factor (REF) between radiotherapy combined with immunotherapy and radiotherapy alone. The X axis displays the various classes of immunotherapies used in the studies: (1) anti-PD-(l)-1, (2) CTLA4, (3) cytokines: r-IL-2, (4) Vaccination / Dendritic cells, (5) CPG / Toll-like receptor, and (6) others. The Y-axis represents the value of the REF, from 0 to 90. The blue-dashed line is a REF value of 1, meaning RT + immunotherapy has the same effect as RT solely. Every dot represents a single calculated REF of one preclinical study. Several dots are calculated per study. A horizontal line represents the mean REF per immunotherapy. A green dot represents the calculated REF's based on survival curves or on response rates: these are based on the volume of the last day of follow-up because the tumor was progression free. Therefore these dots are a minimal representation of the REF because in reality it concerns a higher REF.

the association of RT and immunotherapy. Radiation doses varied from conventional schedules of 1.8 to 2 Gy per fraction to extreme hypofractionation, ranging from 1 to 10 fractions of 1.8 to 20 Gray (Gy) per fraction (Table 2). Responses varied from local regression to complete cure. Data were available from many different immunotherapy classes in regards to their working mechanisms, see Table 2.

Results from level 1 studies

No studies reported on level 1 outcome with a follow-up of longer than 6 months. Several studies observed a long follow-up, however, none longer than 180 days have been described.

Results from level 2 studies

Table 2 provides an overview of the 11 studies reporting Level 2 response. The preclinical reports describing complete responses in 100% of cases were using Staphylococcal enterotoxins (SEC2)-activated T lymphocytes, IL-2, CpG (intratumoral), anti-PD-1, and adenoviral vector + IL-12. The calculated REF's are represented in Figure 2. Thirty-four graphics are analyzed with median REF of 9.1, 1.7, 2.8, 7.3, and 3.1 for anti-PD-(L)1; cytokines: r-IL2; vaccines/dendritic cells; CPG/Toll-like receptor; and other immunotherapies, respectively. REF varied between 0.4 and 52.1.

Results from level 3 studies

Table 3 shows an overview of the 21 studies reporting level 3 response. REF varied between 0.4 and 84.3. These calculated REF's are represented in Figure 2. Sixty-five graphics are analyzed with median REF of 2.5, 1.9, 1.9, 2.7, 2.3, and 1.8 for anti-PD-(L)1; anti-CTLA4; cytokines: r-IL2; vaccines/dendritic cells; CPG/Toll-like receptor; and other immunotherapies, respectively.

All forms of immunotherapy were divided into different classes to obtain more differentiation of the subclasses. However, neither a relationship was observed between the type of immunotherapy, nor in the dose, nor the timing of RT. A significant difference was observed of the immunotherapy subclasses of vaccines/dendritic cells, and others versus cisplatin; p = .0484 and 0.0324, respectively.

Table 4. Overview of clinical studies according to the search criteria.

First Author	Year	Tumor: Histology (Origin)	*N*	Radiotherapy (Site – Dose [Gy]/ Fractions)	Immunotherapy	Local response	PFS after response
Brinkmann[51]	2005	RCC (Renal)	20	Bone/ Kidney – 45-50 Gy/ 25x	IL-2, IFN-a	15% CR, 15% PR, 45% SD, 25% PD	NA
Jacobs[52]	2005	Nasopharyngeal Carcinomas	10	70 Gy/35x	IL-2	Local control 77%	63% 5 y
Seung[53]	2012	RCC(renal) + Melanoma (skin)	12	60 Gy /3x	IL-2	LC: 100% M+	16 months
Barker[54]	2013	Melanoma	29	30 Gy / 5x	Ipilimumab	Local response: 77%	39 months
Abei[55]	2013	HCC (Hepato Cellular Carcinoma)	9	52.8– 87.6 Gy / 22-37x	In situ injection of "CalTUMP"(BCG extract + hydroxyapatite +microparticulated tuberculin)	Local response: 66%	6 months
Kiess[56]	2015	Melanoma (Skin)	46	Brain – 15-24 Gy/1x	Ipilimumab	1-y LC 87 to100%*	NA
Twyman-Saint Victor[57]	2015	Melanoma Skin)	22	12– 24 Gy/2-3X	Ipilimumab	5% CR; 28% PR; 41% SD; 18% PD – 8% NA	3.8 months
Nardin[58]	2018	Melanoma Skin)	74	Brain	Pembrolizumab	LC: 80% M+	4 months

Results from clinical studies

No randomized clinical studies were identified. Table 4 provides an overview of the clinical studies. Eight series of patients have been reported, from which melanoma and renal cell carcinoma were the most frequent tumor histology types. RT doses were widely dispersed, ranging from 1 to 37 fractions of 1.8 to 24 Gy per fraction. The two most commonly used immunotherapy agents were ipilimumab and IL-2, administered in 3 and 2 clinical reports, respectively. In four trials the immunotherapy has been prescribed during RT, whereas in two trials it was prescribed before, during, and after RT. In two other trials, the immunotherapy started several days after commencing RT. Local tumor control rates varied from 66% to 100%.

Discussion

Radiation sensitizers such as chemotherapy, monoclonal antibodies, and targeted agents, increase the local tumor effects of RT, without the need for higher RT doses and these have been clinically used in different cancer subtypes. These sensitizers increase the local and systemic control approximately with 10% to 20% .[54–57] However, with these regimens, radiation toxicity (such as oral mucositis) has been exacerbated. Many other common side effects such as myelosuppression, nausea, and vomiting have been observed.[11,54] In this review, immunotherapy was critically analyzed as a sensitizer for RT: different multiply sensitizing factors ranging from 0.4 to 84 have been derived from the reviewed literature for different subtypes of immunotherapy. This increase is enormous compared to the 0.1 increase found for the classical radiosensitizing drug cisplatin. When comparing the combination of RT-immunotherapy with RT in preclinical studies, mostly short-term responses were observed. The complete responses in all cases for more than 6 months were not documented in any preclinical setting. However, the mean life span of a mouse is 1.5 years, which means that this cutoff time will be barely observable in the pre-clinical setting. Most reports showed tumor growth delay, which meant that the optimal combination of specified immunotherapy is not yet known. Moreover, a wide range of different immunotherapy agents with different working mechanisms have been described. However, in the preclinical setting, the experimental set-up was generally not intended to quantify complete responses over a long time period: the sensitization effect therefore still needs to be demonstrated. Therefore, conscious decisions have been made to choose low RT doses in combination with different immunotherapy.

Local radiosensitization in patients

The local immunological potential of certain tumors also comes to the forefront in different case reports that described the combination of RT + immunotherapy treatment: a number of manuscripts have already confirmed the presence of *abscopal* effects of RT + immunotherapy.[12] Table 4 summarizes the *local* effect in patients and circumscribe the preclinical analyses, findings, and conclusions: immunotherapy is an extremely good local radiosensitizer in comparison with cisplatin or 5-Fluororacil. Ipilimumab is the most clinically cited immunotherapy in malignant melanoma. Ipilimumab causes CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) blockade leading to a decreased exhausted phenotype on CD8 T cells and decreased regulatory T-cell (Treg) activity.[58] This synergizes well with RT since Tregs lead to a suppressed immune response and tend to be more radio-resistant than other T cells .[59] These Treg inhibitions increase the CD8/Treg-ratio resulting in modest peripheral expansion of TCR (T-cell receptor)-clonotypes in the tumor. RT has the effect of diversifying the TCR repertoire of tumor-infiltrating lymphocytes and further shapes the repertoire of expanded clones, resulting in better local outcomes. Several reports of combinations of multiple immunotherapy have been are published reporting better overall survival than solely using immunotherapy: 5 years overall survival was 52% in the nivolumab-plus-ipilimumab group, in comparison with 44% in the nivolumab group, and 26% in the ipilimumab group.[60]

Timing and dose of radiotherapy

RT induces inflammation and necrosis, attracting in-field dendritic cells (DC) and other types of Antigen Presenting Cells (APC) into the tumor micro-environment.[61] Immune cells appear to be highly radiosensitive: in the body, naïve lymphocytes are one of the most radiosensitive among all cells: doses of 0.5 Gy have proven to be already cytotoxic.[62] DC and APC may survive higher RT doses, however, more rapid function loss has been observed.[63] Therefore, the choice of fractionation schedule and (consequently) the time point between different fractionations could impact on the availability of local immune effectors. The results are mainly dependent on the type of immunotherapy and the RT dose. In some reports, fractionation has been useful, while in others a single high-dose of RT appears to be best. High-dose RT seems to be good at producing immunogenic modulation of tumors resulting in intense $CD8^+$ T-cell tumor infiltration, and a loss of myeloid-derived suppressor cells (MDSC).[64] Due to the shorter period of treatment, this may avoid continued eradication of responding lymphocytes.[65] Furthermore, high-dose RT results in more vascular and stromal damage and increased apoptosis of tumor cells, thus creating a tumor microenvironment with increased levels of tumor-associated antigens.[66] When combining immunotherapy with RT, concurrent administration reveals a better superior sensitizing effect.

Limitations

This review has shown that different forms of immunotherapy have large potential to improve local tumor control within the radiation field. For the first time, systematic review has been performed to compare the effectiveness of different forms of immune treatment, and doing so in a quantitative way, using Radiation Enhancement Factors. An original approach was introduced enabling comparison of the results from different studies. This was done by extracting and digitizing the growth data of tumors from different experimental setups, determining the tumor growth delay for radiotherapy as well as for the

combined immune treatment. These data could then be used to determine the radiation enhancement factor as the ratio of the growth delay for combined treatment to that for radiation-only treatment. Since this methodology can be used to compare the potential of any kind or class of radiosensitizers, the methodology can be applied to address many alternative questions in this field. And, as growth delay experiments are the most widely used preclinical in vivo experiments assessing the efficacy of a radiosensitizer, our approach can move the field forward significantly in other areas, based on already available data.

However, this review has also some limitations.

Firstly, most reports were preclinical, including only small numbers of cases. The modeling of animals has biological and physical limitations, so this should be considered when interpreting preclinical RT trials. Murine tumor and normal tissue radiation response has been shown to vary from humans in regards to cellular and molecular pathways.[67] Secondly, as no randomized phase III trials were available, no good control groups have been reported to compare the combination therapy in the clinical reports. Thirdly, with the search strategy employed, abscopal reports were specifically excluded. Hence, it is possible that certain reports with a focus on abscopal effects but also reporting on local control have not been included in this review. Moreover, the search and screening method could be optimized.

Further, the evaluation of clinical local responses has not been consistent in every report: the disease progression is often reported without mentioning specific details of the local control. However, local control evaluation after extreme high-RT dose in combination with immunotherapy is obsolete: the tumor has already been destroyed by the RT itself. Response criteria are sometimes according to the traditional Response Evaluation Criteria In Solid Tumors (RECIST) criteria.[68] However, the evaluation criteria of the response of immunotherapy can differ from those with traditional therapies: a progression of known lesions or even the appearance of new lesions, before stabilization of the disease or even regression can be observed.[69] Therefore, consensus-based criteria for response to immunotherapy (iRECIST) have been developed recently for use in trials testing immunotherapy.[70] Moreover, a possible time delay could exist between the systemic treatment and the evaluation of the response to RT, and the presence or absence of control, in order to distinguish this effect of systemic treatment or RT.

Next, the levels of responses that we used to stratify the quality differences among the several preclinical studies consisted of only three levels. However, level 1 response was more a theoretical level, since no mice-related work had follow-ups of greater than 6 months which were as per our definition the highest demand for clinical work, which is described as a *knowledge* gap. Additionally, the review is based on a relatively small amount of papers with a broad amount of variables: seven different immune-competent mouse strains with a disease heterogeneity (cancer type and subtype) using radiation doses varying from conventional schedules to extreme hypo-fractionation, with the application of different immunotherapies at various time points during, before and after the RT. Response of immune-radiotherapy combinations further depends on total dose, and probably also other parameters like the treated tumor volume and the patients' condition or in preclinical studies the specified immunocompetence of the animal used. This study did take such parameters into account while comparing the different results over the described experiments.

Finally, the number of clinical studies is limited and varies in methodologies. This can definitely be extended toward parameters like total dose, dose fractionation, and timing as discussed.

Perspectives

More clinical and mechanistic knowledge is needed about the precise immune reaction created by RT. This additional information will give us supplementary knowledge to individualize the best sensitizing effect of immunotherapy on RT. This can ultimately lead to decreasing RT doses, with consequently decreasing toxicity levels, while preserving excellent local control, thus leading the way forward toward new organ preservation strategies. However, immunotherapy can also lead to increased toxicities like dermatologic (rashes), colitis (diarrhea), hepatotoxicity, pneumonitis, and endocrinopathies (such as thyroid, hypophysitis). More research is therefore needed to examine these combination treatment strategies.

Conclusion

We concluded that different forms of immunotherapy can act as a local sensitizer for RT with good local control rates. Local effects were observed in a variety of tumor types, with different RT doses and fractionation schedules. Further research is needed to confirm the optimal RT-immunotherapy combination.

Disclosure statement

All authors declare to have no conflict of interest.

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Author Contributions

BV and EvL did the systematic review and selection of all the publications. LD did the analysis using Plot Digitizer to obtain a tumor growth delayed for every specific Immunotherapy. BV, EvL, DDR wrote the first draft of the manuscript. All authors edited and contributed to the development of the final manuscript.

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