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 immunotherapies was varying from 1.7 to 9.1. There was no relationship observed either with subclasses of immunotherapy or RT 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.

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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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. The 6 months threshold was chosen because in several experiments this level is taken as a cutoff, i.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:

\[ TGD = \frac{T_{iv} \times 4 - T_{cv} \times 4}{T_{iv} - T_{cv}} \]

where \( T_{iv} \times 4 \) 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 (iv) and in untreated control tumors (cv), respectively.

When \( T_{iv} \times 4 \) 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:

\[ \text{REF} = \frac{TGD_{RT} + 10}{TGD_{RT}} \]

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:

\[ \text{REF} = \frac{\text{Median Survival}_{RT} + 10}{\text{Median Survival}_{RT}} \]

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:

\[ \text{REF} = \frac{\% \text{DFS}_{RT} + 10}{\% \text{DFS}_{RT}} \]

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 sub-classes 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.

### 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. 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

| Level of response | Study Endpoints | Clinical Relevance |
|-------------------|-----------------|--------------------|
| 1                 | Local Tumor Control > 6 months | Sustained complete Response = Cure |
| 2                 | Local tumor control < 6 months | Complete Response |
| 3                 | Growth Delay     | Partial Response |
### 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]) | Immunoetherapy | Observed Effect | Level of response | Suggested mediator |
|----------------------|------|----------------|-------------------|---------------|----------------------------|-----------------------------------------------|----------------|----------------|-------------------|-------------------|
| Piutz*               | 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               |
| Everse**             | 1997 | SL2 lymphoma MBO13 mammary carcinoma | s.c on one or both thighs | DBA/2Jco mice C57BL/6 JcoU 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                |
| Jürgenleimk-Schulz** | 1997 | SL2 lymphoma MBO13 mammary carcinoma | s.c on one or both flanks | DBA/2Jco mice C57BL/6 JcoU 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               |
| Meng†                | 2000 | 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†               | 2000 | 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§              | 2015 | C51 colon       | s.c. Flank       | C57Bl/6 + Balb/c mice | +/-                         | Flank – 10 Gy/1x L19-IL2 | CS1: CR: 75%  LL: additive effect 4T1: no effect | CR: 50%          | 2                | Upregulated expression of effector T cells (CD3, CD4, CD8, CD25) |
| Van den Heuvel**    | 2015 | Lewis lung      | i.m. right quadriceps muscle | C57Bl/6 mice | +/-                         | Leg – 3.6 Gy/2x NHS-IL2 | CR: 80 to 100%     | 2                | Upregulated antigen-presenting activity of dendritic cells + T cells |
| Schörlch²            | 2015 | pancreatic      | 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                |                 |
| Connolly³            | 2016 | Colon38, Gloma261, Linel | 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 |
| Wu⁴                  | 2018 | BNL-P2 HCC cells | s.c. in the right flank | Balb/c mice | +/-                         | Flank – 10 Gy/1x adenoviral vector +JL 12 | CR: 40%; PR: 50%  | 2                |                |
| Zhuang⁵              | 2018 | Lewis lung      | s.c. in the right leg | C57Bl/ 6mice | +/-                         | Leg – 8 Gy/1x CpG (intratumoral), Anti-PD-1 | CR: 100%         | 2                |                |

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 / Synergistic | Radiotherapy (Site – Total Dose [Gy] / Fractions) | Immunotherapy | Observed Effect | Level of response | Suggested mediator |
|--------------|------|------------|-------------------|--------|-------------------------------|-----------------------------------------------|--------------|----------------|------------------|--------------------|
| Buchegger | 1995 | Col 12 and LS174T | Transplants on the middle of their backs at 2 cm from the tail | nude mice | - / + | Back – 16 Gy/2x | monoclonal anti-CEA antibodies (mAB 35, CE25-B7, and B93) | Tumor growth delay | 3 | NA |
| Chiang | 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 | 2000 | 4T1 mammary tumor cFCH – 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 | 2003 | D5 melanoma or MCA 205 sarcoma | s.c. in the middle 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 | 2007 | renal cell carcinoma | s.c. in the right axilla | C57BL/6 mice | + / + | Right Axilla – 35 Gy / 5x | Right Leg – 6 to 12 Gy / 1x | Dendritic cell | Tumor growth delay | 3 | Down-regulation of Bcl-2, up-regulation of Bax, expression of TNFα, IL-2, IL-4 mIFN-γ, IgG, IgM. express immune stimulatory cytokines (CCL2, CCL5, CXCL9, CXCL10, and CXCL11) to activate cytotoxic T lymphocytes |
| Meng | 2012 | melanoma cell line B16SY | s.c. in the right leg | C57BL/6 mice | + / + | Right Leg – 6 to 12 Gy / 1x | poly(ADP-ribose) polymerase inhibitor verapilb | Tumor growth delay | 3 | |
| Wang | 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 IL-2 | Tumor growth delay | 3 | |
| Wei | 2013 | murine D5 melanoma | s.c. in the right flank | C57BL/6 (B6) and B6.AT-Thy1a/CyJ (CD90.1) mice | +/- | bilateral flanks – 8.5 Gy / 5x | | | | |
| Dovedi | 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 | |
| Lim | 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 | |
| Rekers | 2015 | F9 terato-carcinoma cells | s.c.in the flank | 129/SvHsd mice | +/- | Flank – 12 Gy / 1x | L19-IL2 | Tumor growth delay | 3 | |
| Blanchard | 2015 | B16-OVA melanoma | s.c. in the hind limb. | C57BL/6 mice | +/- | Lower limb – 20 Gy/1x | vesicular stomatitis virus-associated antigen viral immunotherapy | Tumor growth delay | 3 | |
| Mondini | 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 | |

(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      | 2015 | MC38-OVA cells; B16-OVA melanoma cells; 4T1HA breast carcinoma cell | s.c. in the right flank | C57BL/6, BALB/cJ, and MHC I knockout mice | +/+ | 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    | 2016 | B16 melanoma or 4T1 breast adenocarcinoma | 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       | 2016 | CT26 murine colorectal carcinoma | s.c. in the right hind limb | BALB/c and P88 mice | +/- | Limb – 20 Gy / 1x | OX40 (CD134) Anti-CTLA4 | Tumor growth delay | 3 | Depletion of CD4+ or CD25 |
| Zheng       | 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      | 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       | 2017 | SMA glioma cell lines | intracranial (the right striatum) | C57BL/6 | +/- | Cranial – 4 Gy / 1x | NKG2D-Based CAR T Cells | Tumor growth delay | 3 | high IFNg production and cytolytic activity in vitro |
| Choi        | 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        | 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
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) | Radiotherapy (Site - Dose (Gy/ Fractions)) | Immunotherapy | Local response | PFS after response |
|--------------|------|---------------------------|---------------------------------------------|---------------|----------------|-------------------|
| Brinkmann    | 2005 | RCC (Renal)               | Bone/Kidney – 45-50 Gy/ 25x                 | IL-2, IFN-a   | 15% CR, 15% PR | NA                |
| Jacobs       | 2005 | Nasopharyngeal Carcinomas | 70 Gy/35x                                  | IL-2          | Local control 77% | 63% 5 y          |
| Seung        | 2012 | RCC(renal) + Melanoma (skin) | 60 Gy /3x                                | IL-2          | LC: 100% M+ | 16 months        |
| Barker       | 2013 | Melanoma (Skin)           | 30 Gy /5x                                  | Ipilimumab    | Local response: 77% | 39 months | 6 months        |
| Abei         | 2013 | HCC (Hepato Celluar Carcinoma) | 52.8– 87.6 Gy /22-37x | In situ injection of “CaTUMP”(BCG extract + hydroxyapatite +microparticulated tuberculin) | Local response: 66% | 39 months | 6 months        |
| Kies         | 2015 | Melanoma (Skin)           | Brain – 15-24 Gy/1x                        | Ipilimumab    | 1-y LC 87 to 100% | NA                |
| Twyman-Saint Victor | 2015 | Melanoma Skin             | 12– 24 Gy/2-3X                            | Ipilimumab    | 5% CR; 28% PR; 41% | 3.8 months | 8% NA          |
| Nardin       | 2018 | Melanoma Skin             | 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%. 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. 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. Table 4 summarizes the local effect in patients and circumscribes the preclinical analyses, findings, and conclusions: immunotherapy is an extremely good local radiosensitizer in comparison with cisplatin or 5-Fluorouracil. 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. This synergizes well with RT since Tregs lead to a suppressed immune response and tend to be more radio-resistant than other T cells. 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 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.

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. 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. DC and APC may survive higher RT doses, however, more rapid function loss has been observed. Therefore, the choice of fractionation schedule and (consequently) the time point between different fractions 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). Due to the shorter period of treatment, this may avoid continued eradication of responding lymphocytes. 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. 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 inter-
preting preclinical RT trials. Murine tumor and normal tissue
radiation response has been shown to vary from humans in
regards to cellular and molecular pathways. Secondly, as no
randomized phase III trials were available, no good control
groups have been reported to compare the combination ther-
apy 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 con-

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. However,
the evaluation criteria of the response of immuno-
therapy can differ from those with traditional therapies:

progression of known lesions or even the appearance of
new lesions, before stabilization of the disease or even regres-
sion can be observed. Therefore, consensus-based criteria
for response to immunotherapy (iRECIST) have been developed
recently for use in trials testing immunotherapy. Moreover,
a possible time delay could exist between the systemic treat-
ment 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 immunocom-
petence of the animal used. This study did take such para-
meters 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 para-
eters 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 infor-
mation 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 con-

Control, thus leading the way toward toward new organ preser-
ervation strategies. However, immunotherapy can also lead to
increased toxicities like dermatologic (rashes), colitis (diar-
rhea), hepatotoxicity, pneumonitis, and endocrinopathies
(such as thyroid, hypophysis). 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.

**References**

1. Cooper JS, Guo MD, Herskovic A, Macdonald JS, Martenson, Jr
JA, Al-Sarraf M, Byhardt R, Russell AH, Beiterl JJ, Spencer S, et al.
Chemoradiotherapy of locally advanced esophageal cancer:
long-term follow-up of a prospective randomized trial (RTOG
Radiation-induced immunotherapeutic effects for cancer: Lessons from tumors treated with radiation therapy.

Weiss T, Weller M, Guckenberger M, Sentman CL, Roth P. NKG2D-Based CAR T cells and radiotherapy exert synergistic efficacy in Glioblastoma. Cancer Res. 2018 Feb 15;78(4):1031–1039. doi: 10.1158/0008-5472.CAN-17-1788.

Choi CW, Jeong MH, Park YS, Son CH, Lee HR, Koh EK. Combination treatment of stereotactic body radiation therapy and immature dendritic cell vaccination for augmentation of local and systemic effects. Cancer Res Treat. 2019 Apr;51(2):464–473. doi: 10.4134/crt.2018.186.

Wang H, Lin X, Luo Y, Sun S, Tian X, Sun Y, Zhang S, Chen J, Zhang J, Liu X, et al. α-PD-L1 mAb enhances the abscopal effect of hypo-fractionated radiotherapy by attenuating PD-L1 expression and inducing CD8+ T-cell infiltration. Immunotherapy. 2019 Feb;11(2):101–118. doi: 10.2217/imt-2018-0049.

Brinkmann OA, Bruns F, Gosheger G, Micke O, Hertle L. Treatment of bone metastases and local recurrence from renal cell carcinoma with immunomodulation and radiotherapy. World J Urol. 2005 Jul;23(3):185–190. doi: 10.1007/s00345-004-0479-8.

Jacobs JJ, Hordijk JJ, Gjurgjgeluck-Shulz IM, Terhaard CH, Kotev JW, Battemann JF, Den Otter W. Treatment of stage III-IV nasopharyngeal carcinoma with external beam radiation and local low doses of IL-2. Cancer Immunol Immunother. 2005 Aug;54(8):792–798. doi: 10.1007/s00262-004-0641-6.

Seung SK, Curti BD, Crittenden M, Walker E, Coffey T, Siebert JC, Miller W, Payne R, Glenn L, Bageac A, et al. Phase 1 study of stereotactic body radiotherapy and interleukin-2–tumor and immunological responses. Sci Transl Med. 2012 Jun 6;4(137):137ra74. doi: 10.1126/scitranslmed.3003649.

Barker CA, Postow MA, Khan SA, Beal K, Parkar PK, Yamada Y, Lee NY, Wolchok JD. Concurrent radiotherapy and ipilimumab immunotherapy for patients with melanoma. Cancer Immunol Res. 2013 Aug;1(2):92–98. doi: 10.1159/000366066.CIR-13-0082.

Abel M, Okumura T, Fukuda K, Hashimoto T, Araki M, Ishige K, Hyodo I, Kanimoto A, Numajiri H, Mizumoto M, et al. A phase I study on combined therapy with proton-beam radiotherapy and in situ tumor vaccination for locally advanced recurrent hepatocellular carcinoma. Radiat Oncol. 2013 Oct;16;8(239). doi: 10.1186/1874-714X-8-239.

Kies AP, Wolchok JD, Barker CA, Postow MA, Tabar V, Huse JT, Chan TA, Yamada Y, Beal K. Stereotactic radiosurgery for melanoma brain metastases in patients receiving ipilimumab: safety profile and efficacy of combined treatment. Int J Radiat Oncol Biol Phys. 2015 Jun 1;92(2):368–375. doi: 10.1016/j.ijrobp.2015.01.004.

Twyman-Smith VC, Rech AJ, Maity A, Rengan R, Pauken KE, Stelekati E, Bencic JL, Xu B, Dada H, Odorizzi PM, et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. Nature. 2015 Apr 16;520(7547):373–377. doi: 10.1038/nature14292.

Nardin C, Mateus C, Texier M, Laney E, Hibat-Allah S, Ammari S, Robert C, Dhermain F. Tolerance and outcomes of stereotactic radiosurgery combined with anti-programmed cell death-1 (pembrolizumab) for melanoma brain metastases. Melanoma Res. 2018 Apr;28(2):111–119. doi: 10.1097/CMR.0000000000000413.

Bourhis J, Sire C, Graff P, Grégoire V, Maiong P, Calais G, Gery B, Martin L, Alfonsi M, Desprez P, et al. Concomitant chemoradiotherapy versus acceleration of radiotherapy with or without concomitant chemotherapy in locally advanced neck node carcinoma (GORTCC 99-02): an open-label phase 3 randomised trial. Lancet Oncol. 2012;13(2):145. doi: 10.1016/S1470-2045(11)70346-1.

Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, Jones CU, Sur R, Raben D, Jassem J, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med. 2006 Feb 9;354(6):567–578. doi: 10.1056/NEJMoa0603342.

James ND, Hussain SA, Hall E, Jenkins P, Tremlett J, Rawlings C, Crudwell M, Sizer B,reenivasan T, Hendron C, et al. BC2001
investigators. Radiotherapy with or without chemotherapy in muscle-invasive bladder cancer. N Engl J Med. 2012 Apr 19;366(16):1477–1488. doi:10.1056/NEJMoa1106106.

57. Northover J, Glynne-Jones R, Sebag-Montefiore D, James R, Meadows H, Wan S, Jitlal M. Ledermann JChemoradiation for the treatment of epidermoid anal cancer: 13-year follow-up of the first randomised UKCCCR Anal Cancer Trial (ACT 1). Br J Cancer. 2010 Mar 30;102(7):1123–1128. doi:10.1038/sj.bjc.6605605.

58. Rudd CE. CTLA-4 co-receptor impacts on the function of Treg and CD8+ T-cell subsets. Eur J Immunol. 2009 Mar;39(3):687–690. doi:10.1002/eji.200939261.

59. Kachikwu EL, Iwamoto KS, Liao YP, DeMarco JJ, Agazaryan N, Economou JS, McBride WH, Schau D. Radiation enhances regulatory T cell representation. Int J Radiat Oncol Biol Phys. 2011;81(4):1128–1135. doi:10.1016/j.ijrobp.2010.09.034.

60. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Rutkowski P, Lao CD, Cowey CL, Schadendorf D, Wagstaff J, Dummer R, et al. Five-year survival with combined nivolumab and ipilimumab in advanced melanoma. N Engl J Med. 2019 Oct 17;381(16):1535–1546. doi:10.1056/NEJMoa1910836.

61. Serre R, Barlesi F, Muracciole X, Barbolosi D. Immunologically effective dose: a practical model for immuno-radiotherapy. Oncotarget. 2018 Aug 7;9(61):31812–31819. doi:10.18632/oncotarget.25746.

62. Yovino S, Kleinberg L, Grossman SA, Narayanan M, Ford E. The etiology of treatment-related lymphopenia in patients with malignant gliomas: modeling radiation dose to circulating lymphocytes explains clinical observations and suggests methods of modifying the impact of radiation on immune cells. Cancer Invest. 2013;31:140–144. doi:10.3109/07357907.2012.762780.

63. Merrick A, Errington F, Milward K, O’Donnell D, Harrington K, Bateman A, Pandha H, Vile R, Morrison E, Selby P, et al. Immunosuppressive effects of radiation on human dendritic cells: reduced IL-12 production on activation and impairment of naïve T-cell priming. Br J Cancer. 2005;92:1450. doi:10.1038/sj.bjc.6602518.

64. Filatenkov A, Baker J, Mueller AM, Kenkel J, Ahn GO, Dutt S, Zhang N, Kohrt H, Jensen K, Dejbaksh-Jones S, et al. Ablative tumor radiation can change the tumorimmune cell microenvironment to induce durable complete remissions. Clin Cancer Res. 2015;21:3727–3739. doi:10.1158/1078-0432.CCR-14-2824.

65. Honeychurch J, Illidge TM. The influence of radiation in the context of developing combination immunotherapies in cancer. Ther Adv Vaccines Immunother. 2017 Dec;5(6):115–122. doi:10.1177/2051013617750561.

66. Park HJ, Griffin RJ, Hui S, Levitt SH, Song CW. Radiation-induced vascular damage in tumors: implications of vascular damage in ablative hypofractionated radiotherapy (SBRT and SRS). Radiat Res. 2012;177(3):311–327. doi:10.1667/RR2773.1.

67. Koontz BF, Verhaegen F, De Raeymaeker D. Tumour and normal tissue radiobiology in mouse models: how close are mice to mini-humans? Br J Radiol. 2017;90:20160441. doi:10.1259/bjr.20160441.

68. Schwartz LH, Litière S, de Vries E, Ford R, Gwyther S, Mandrekar S, Shankar L, Bogaerts J, Chen A, Dancey J, et al. 1.1-Update and clarification: from the RECIST committee. Eur J Cancer. 2016 Jul;62:132–137. doi:10.1016/j.ejca.2016.03.081.

69. Wolchok JD, Hoos A, O’Day S, Weber JS, Hamid O, Lebbé C, Maio M, Binder M, Bohnsack O, Nichol G, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res. 2009;15(23):7412. doi:10.1158/1078-0432.CCR-09-1624.

70. Seymour L, Bogaerts J, Perrone A, Ford R, Schwartz LH, Mandrekar S, Lin NU, Litière S, Dancey J, Chen A, et al. RECIST working group. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. Lancet Oncol. 2017;18(3):e143. doi:10.1016/S1470-2045(17)30074-8.