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Combination of Radiation Therapy and Immunotherapy in the Treatment of Melanoma

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Abstract :

Melanoma is considered to be a very aggressive cancer due to its rapid growth, early and multiple metastases and limited response to standard treatment. Many researchers have hypothesized that the combination of radiation therapy and immunotherapy in the treatment of melanoma primary tumors and metastases improves the efficiency of these methods as compared to their use separately. Therefore, combined therapy is an increasingly popular topic in radiation oncology. Although the mechanism of immune response to ionizing radiation remains unclear, known are the factors involved in the immune response, including NK and CD8(+) T cells. Many studies have demonstrated the importance of inflammatory factors, primarily cytokines, in the response to ionizing radiation. In turn, many cytokines released in an irradiated organ, such as tumor necrosis factor α (TNFα), interleukins IL1 and IL6 and transforming growth factor beta (TGFβ), can induce the production of significant amounts of reactive oxygen species that are associated with the induction of DNA damage in tumor cells.

In relation to anticancer immunotherapy, the clinical data obtained to date can encourage future studies combining radiation therapy and the inhibitors of cell division checkpoints in the treatment of advanced melanoma. In a recent study, melanoma cell lines became more sensitive to radiation after BRAF inhibition, which provides a potential synergistic mechanism of BRAF inhibitor (BRAFi) combined with radiation therapy for better effects of treatment.

In this article, we present a systematic review of the literature on the use of the combination of radiation therapy and immunotherapy in the treatment of melanoma.

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Immune Surveillance

Homeostasis is a state of balance that every organism tries to obtain. The main goal of the immune system is to restore homeostasis once a pathogen enters the body and multiplies there, or upon neoplastic transformation. The genetic and epigenetic modifications that occur in tumors, despite the different antigenicity of tumor cells, do not initiate immune response[1]. Most patients diagnosed with a developing malignancy have abnormally functioning immune system with genetically and epigenetically distinct tumor cells as a result of variable antigen expression. Tumor cells develop a range of mechanisms that prevent their detection and destruction by the immune system. In their uncontrolled development, tumor cells escape the surveillance by the host's immune system or, in some cases, inhibit the development of the immune response as a result of:

1. Lack of major histocompatibility complex antigens, such as HLA-A (*Human Leukocyte Antigen-A*) or HLA -B, on tumor cells[2];
2. Presence or overexpression of major histocompatibility complex antigens, such as HLA-G or HLA-E, on tumor cells[3];
3. Change in the profile of tumor microenvironment leading to the inhibition via activation of the enzyme IDO and secretion of Th2 cytokines, such as IL-10, IL-4, TGF-β[4];
4. Expression of factors capable of inhibiting activated T and NK cells on tumor cell surface[5];
5. Accumulation of regulatory immune cells, such as Treg cells, in tumor microenvironment[6].

In patients with malignancies, a decrease in immune response is observed, particularly in that of the cellular type. An important role in this respect is played by the CTLA-4 molecule whose expression is not observed on naïve T cells, while it is present only on T cells activated by an antigen. Under physiological conditions, the molecule limits the response of T cells to foreign antigens, as well as autoantigens, and therefore constitutes an important element of negative feedback in immune response. It has been shown that in tumor microenvironment CTLA-4 inhibits T cells by raising their activation threshold or inhibiting their proliferative activity[7]. Administration of anti-CTLA-4 antibodies blocks the interaction between CTLA-4 and CD80/86, costimulatory molecules that are necessary for activation on both the APC and the lymphocyte. Once this receptor has been blocked, DNA replication and cell division in T cells can occur without obstacles.

Immune Response to Melanoma Radiation Therapy.

Infiltration of the tumor site by the cells of the immune system can be an indicator of the host response and treatment success after therapy administration. For some time it has been known that radiation therapy induces an advantageous systemic response in patients with melanoma[8,9]. Ionizing radiation destroys tumor cells through different mechanisms, e.g., cell death by apoptosis or necrosis or immunogenic cell death.

The phenomenon called abscopal effect was originally proposed by R.H. Mole in 1953. It describes the effect of radiation therapy at sites remote from the original tumor after local irradiation of the same organism[10]. Putatively, it constitutes evidence that radiation therapy can cause a stronger systemic antitumor response. However, the existing reports indicate that the abscopal effect occurs rarely in both pre-clinical and clinical trials, which suggests that the exact regimen of radiation therapy that leads to this effect is not fully understood. Therefore, further studies are suggested in order to elucidate the immune antitumor mechanisms after radiation therapy.

Many studies have demonstrated the importance of inflammatory factors, primarily cytokines, in the response to ionizing radiation[11]. In turn, many cytokines released in an irradiated organ, such as tumor necrosis factor α (TNFα), interleukins IL1 and IL6 and transforming growth factor beta (TGFβ)[12], can induce the production of significant amounts of reactive oxygen species[13,14], also from the systemic point of view. Moreover, recent studies have demonstrated that ionizing radiation leads to leukocyte activation[15], which may lead to respiratory burst[16] and production of significant quantities of reactive oxygen species associated with the induction of DNA damage in the target cells[17].

In their breast cancer and melanoma studies, Müller et al. demonstrated that chemokines—factors regulating the migration and integration of leukocytes into specific organs—play an analogous role with respect to tumor cells in the process of their dissemination[18,19].

Although the exact or unambiguous mechanism of immune response to ionizing radiation remains unclear, known are the factors involved in the immune response, including NK and CD8(+) T cells. In several reports[20,21], authors revealed clinical evidence that increasing the ablative dose can increase the immune response, leading to an increased antitumor effect. Evidence provided by preliminary results has encouraged combining radiation therapy with the available immunotherapies and conducting numerous studies in this field.

Principles of Combining Radiation Therapy with Immunotherapy

In the recent years, most studies were focused on melanoma immunotherapy. The most effective of these immunotherapies to date have been immune checkpoint inhibitors (ICIS), such as ipilimumab, nivolumab and pembrolizumab[22,23].

As regards anticancer immunotherapy, two immune checkpoints appear to be important: programmed cell death protein 1 (PD-1; CD279) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA4). The use of antibodies that block PD-1 and CTLA4 results in an increased activity of T cells against tumor cell[24,25]. Despite the experimentally confirmed effectiveness of ICIS, antitumor response due to the migration of T cells to the tumor regression site occurs in only a small group of patients.

In order to improve the effectiveness of melanoma therapy and increase progression-free survival, attempts to combine immunotherapy and radiation therapy have been made.
Radiation therapy is intended to cause a pro-inflammatory effect and lead to changes in tumor microenvironment to promote the antitumor phase of immune response[26].

Lugade et al. in their pioneer study[27] initiated immune response against a tumor by intramuscular injections of B16-F0 cells into mice with subsequent radiation therapy. In the first study group, the mice were not subjected to radiation therapy; in the second group, one dose of 15 Gy was administered; in the third group, 3 fractions of 5 Gy were administered. The groups were followed up and it was demonstrated that in mice subjected to radiation therapy, as a result of cell damage by radiation, induction of inflammatory signals necessary for APC maturation and migration to lymph nodes occurred, which allowed the activation of T cells. The irradiated mice had a greater ability to present tumor antigens and T cells. It was also demonstrated that for each type of the analyzed cells, the average number of immune cells per mg of tumor was greater in mice that received a single dose of radiation than in those that received fractionated doses[27].

Knisely et al. examined 77 patients with brain metastases treated with stereotactic radiation therapy, who additionally received ipilimumab. The median overall survival was 21.3 months compared to 4.9 months in the patients who did not receive ipilimuma[28].

In their retrospective clinical study, Barker et[29] presented the results of combination treatment of inoperable or metastatic melanoma with ipilimumab and radiation therapy. The results of treatment of 333 patients were analyzed. Ipilimumab was used in all patients and additional ionizing radiation was administered to half of them. The authors concluded that the occurrence of local or systemic immune-related adverse events (ir-AES) did not increase with the concurrent use of radiation therapy and ipilimumab. It was also demonstrated that the combination of these treatments increased the overall survival of the patients.

Schoenfeld et al.[30] studied patients with advanced melanoma and brain metastases who received ipilimumab and SRS (Stereotactic Radiosurgery). The patients who received SRS before ipilimumab administration had better overall survival (OS) values (median OS = 26 months) compared to the patients who received ipilimumab before radiation therapy (median OS = 6 months; $p<0.001$). The authors, based on their own research and other available studies, suggest that ipilimumab and SRS are well tolerated and can improve the overall survival of patients with advanced melanoma. Moreover, the authors demonstrated a tendency for a positive systemic response following radiation therapy, which encourages further studies aimed to detect the potential additional synergistic effects between irradiation and immunotherapy.

The clinical data obtained to date can encourage future studies combining radiation therapy and ipilimumab in the treatment of advanced melanoma.

In a recent study, melanoma cell lines became

Table: Efficacy of combining immune checkpoints inhibitors with radiation therapy in melanoma brain metastases patients.

				Primary outcome measure / Overall survival	
Study	**Radiotherapy**	**ICIS – immune checkpoints inhibitors**	**Targeted site**	**Only radiotherapy**	**Radioterapy and ICIS**
Knisely et al. 2012 [28]	SBRT (Stereotactic Body Radiation Therapy)	ipilimumab	Non-brain lesions	4,9 month	21,9 month
Barker et al. 2013 [29]	Radical radiotherapy	ipilimumab	inoperable and metastatic melanoma	9 months after radiotherapy during induction ipilimumab	39month (undergoing RT during maintenance)
Schoenfeld et al. 2015 [30]	SRS (Stereotactic Radiosurgery).	ipilimumab	Non-brain lesions	26 month / radiotherapy before ICIS	6 month / ICIS before radiotherapy
Hecht et al. 2015 [34]	WBRT (whole-brain radiotherapy)	vemurafenib	melanoma scin cancer	Not reported	increase radiosensitivity
Tazi et al.2015 [38]	SRS (Stereotactic)	ipilimumab	brain metastases/ non brain metastases	29,3	33,1
Silk et al.2013 [39]	SRS (Stereotactic) or WBRT (whole-brain radiotherapy)	ipilimumab	melanoma brain metastases	5.3	19.9
Mathew et al. 2013 [40]	SRS (Stereotactic)	ipilimumab	melanoma brain metastases	6 month - 42%	6 month -56%
Stinauer et.al 2011 [41]	SRS (Stereotactic)	ipilimumab	melanoma	no negative effects after radiotherapy and ipilimumab	
Bot et.al 2012 [42]	WBRT (whole-brain radiotherapy)	ipilimumab	melanoma	radiotherapy before ipilimumab enhancing the immune activation	

more sensitive to radiation after BRAF inhibition, which provides a potential synergistic mechanism of BRAF inhibitor (BRAFi) combined with radiation therapy for better effects of treatment[31]. BRAF inhibitors are standard treatment of patients with metastatic melanoma carrying mutated BRAF gene[32]. Active BRAF mutations are diagnosed in approx. 50% of melanomas characterized by tumor hyperproliferation[33]. Drugs targeting BRAF mutations introduced in the treatment of metastatic melanoma give hope for the improvement of treatment outcome. Many cases of metastatic melanoma involve brain metastases that are associated with poor prognosis due to limited response to treatment.

Hecht et al.[34] summarized a multi-center study aimed to generate reliable data regarding treatment outcomes in patients with melanoma treated with radiation therapy accompanied by BRAF inhibitors. A total of 161 melanoma patients from 11 European oncology centers were evaluated for acute and late toxicity, among whom 70 patients received radiation therapy combined with a BRAF inhibitor. In order to further characterize and quantify the possible sensitivity to radiation caused by the use of BRAF inhibitors, blood samples from 35 patients with melanoma were used for individual radiation sensitivity tests via *in situ* fluorescent hybridization of chromosomal breaks after *ex vivo* irradiation. In patients treated with a BRAF inhibitor, sensitivity to radiation increased. This effect was clearly visible in the group that received vemurafenib (VMF). The study gives hope that BRAFi combined with radiation therapy can improve the antitumor response in the body. The results are very important for patients with melanoma brain metastases (MBM) as they often undergo radiation therapy alone, without BRAFi. The results can have a significant impact on improving the prognosis in these patients. Radiation therapy, when administered concurrently with BRAF inhibitors, is conducted with an acceptable increase in toxicity. Vemurafenib appears to be a more effective radiosensitizer than dabrafenib[35].

Regulatory T Cells:

CD4+, CD25+, FOXP3 (Treg) T cells constitute components of the immune system that are directly responsible for most of the autoimmune tolerance in the body. Preliminary data on Treg cells reported an increase in their number in patients with developing cancer. Treg cells, especially CD4+, CD25+, Foxp3+ Treg cells, negatively regulate immunity by promoting the growth of tumor cells and directly attenuating CD8+ and CD4+ T cells. Alternatively, they can promote the spread of tumor cells by releasing interleukin-10 (IL-10) and transforming growth factor TGF-β.

Yang Yu et al.[36] used an *in vivo* tumor model generated in C57BL/6 J mice by subcutaneous injection of a suspension of B16 melanoma cells into the upper left flank. In this experiment, the mice were divided into five groups (n = 6) which were then treated using: radiation therapy (RT), chemotherapy (CT), radiochemotherapy (RCT) and interferon alpha (IFN**α**). It was demonstrated that tumors in mice treated using various methods were smaller compared to the tumors in mice from the control group. Detailed analysis of the study results revealed that the increase in tumor mass was inhibited when mice were subjected to CT and RCT (40.91% and 41.83%, respectively) compared to the tumors from the RT and IFN**α** groups (15.10% and 13.15%, respectively). Radiation therapy alone has a limited ability to inhibit melanoma growth, but can be used effectively in synergy with chemotherapy. It has also been demonstrated that IFN**α** therapy can modify the functioning of the immune system and thus slow down melanoma development. A reduction in the number of CD4+, CD25+, Foxp3+ Treg cells in the spleen of mice occurred after IFN**α** treatment. A significant reduction in the number of CD4+, CD25+, Foxp3+ Treg cells was observed in the peripheral blood. These results suggest that IFN**α** in immunotherapy has the ability to reduce the CD4+, CD25+, Foxp3+ Treg cell count. The results indicate that tumor growth can be significantly inhibited by properly selected treatment[36].

Ma et al.[37] attempted to study the relationship between modulation of the immune system and the sentinel lymph node (SLN) in patients with melanoma. They examined 84 patients with melanoma for the quantities of immune modulators, such as Treg cells (Treg: Foxp3+), dendritic cells (myDCs: CD11c+) and mature dendritic cells (maDCs: CD86+), in lymph nodes (LN) and primary tissues. Reduced immune response is defined as increased Treg, decreased myDC or decreased maDC counts. The authors demonstrated that the antitumor immune response was reduced with metastases present in the sentinel lymph node SLN(+) (n=31) compared to SLN(-) (n=53), and a considerable

increase in Treg (p=0.0002) and decrease in myDC counts in the group of 31 patients with SLN(+). The study showed that the advance of cancer (due to the invasion of the sentinel node SLN(+) by tumor cells) is accompanied by a decrease in antitumor response in favor of the development of the disease, thus helping the growth of immunosuppressive cells.

Conclusion:

For best results in the treatment of melanoma, it is necessary to demonstrate how ionizing radiation affects the functioning of the immune system. There is increasing evidence that radiation has a wide range of immunomodulatory activities. The combination of radiation therapy and immunotherapy is the reason for the increased curability and threshold of sensitivity to treatment. This combination has been shown to improve progression-free survival and objective response rate compared with either agent alone as monotherapy in patients with advanced melanoma. The data obtained to date encourage creating new study protocols that would combine immunotherapy and radiation therapy and give new hope to improve the outcomes of treatment of advanced melanoma. Preclinical and clinical evidence suggests that radiotherapy may enhance the cancer therapeutic benefit of ipilimumab.

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