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Review

Biological therapy in the treatment of melanoma

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Abstract

Melanoma is one of the most aggressive tumors and its incidence is on the rise. The low rates of survival in metastatic melanoma has led to the development of new drugs for this type of patient, such as biological therapy which has shown remarkable results. This therapy is based on stimulation of the immune system to fight tumoral cells through: injection of cytokines with immunomodulatory properties (interleukin-2, alpha-interferon), vaccination with tumor antigens or immune cells that process tumor antigens, adoptive immunotherapy, inhibition of immune checkpoints (PD-1, CTLA-4), inhibition or stimulation of immune modulator molecules (OX-40, LAG-3), inhibition of signaling pathways involved in cell proliferation (Raf/MAPK/ERK signaling pathway), or administration of oncolytic viruses. Biological therapy in melanoma has shown promise in laboratory and clinical studies, with more therapeutic targets to be revealed as new molecular and cellular mechanisms of the disease are discovered.

Keywords

: metastatic melanoma, biological therapy, immunotherapy

Highlights

- ✓ Biological therapeutic options for melanoma have considerably increased life expectancy of the patients.
- ✓ Current therapeutic approaches are related to the tumors associated immunosuppression, as well as messengers related to cell growth.

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Introduction

Melanoma is one of the most aggressive malignant tumors. The incidence is on the rise - from 2005 to 2014, the rate increased by 3% per year among men and women older than 50, but remained stable among those younger than age 50. At the same time, given early diagnosis and therapeutic progress, the 5-year relative survival rates have also increased from 80% for the period 1975-1977 to 92% nowadays. Nevertheless, 5-year relative survival rate in locally invasive or metastatic melanoma is 20%, which has led to the development of therapies which target this last group of patients (1). The introduction of biological therapy, also called immunotherapy, in the treatment of melanoma has shown remarkable results. This outcome is not surprising since melanoma is considered one of the most immunogenic tumors. Exposure to ultraviolet wavelengths promote mutagenesis and formation of neoantigens which then are recognized as "non-self" by the host's immune system (2).

Biological therapy can trigger or help the immune system fight tumoral cells in different ways: (I) stimulation of non-specific antitumoral response via cytokines, (II) vaccination, (III) and adoptive immunotherapy (2, 3).

Discussions

I Cytokines

a) IL-2

IL-2 is a cytokine secreted mainly by antigen-stimulated CD4+ T helper cells, but it can also be secreted by CD8+ T cells, natural killer (NK) cells, NKT cells, activated dendritic cells (DC), and mast cells. IL-2 has pleiotropic effects on the immune system: it is a trophic factor for lymphocytes, enhances the proliferation, activation, and differentiation of T lymphocytes and NK cells, and plays a key role in immune regulation via its effects on regulatory T cells (Treg). IL-2 was first termed "T-cell growth factor" upon its discovery and it revolutionized immunology research as it was one of the first cytokines tested in patients and approved to treat cancer. IL-2 was FDA approved in 1998 for the treatment of metastatic melanoma (3-6). In a study using high dose (HD) bolus IL-2 to treat melanoma patients, complete regression of the tumor was observed in 6.6% of the patients and this condition was maintained from 12 to more than 148 months. Partial regression was obtained in 15% of the patients and was maintained from 2 to 35 months (7). IL-2 therapy is recommended by guidelines as a second-line systemic treatment in metastatic or unresectable

melanoma or as local therapy, injection in the tumor after surgical treatment in stage III melanoma with in-transit metastasis or satellites without metastatic nodes (8).

There are several limitations to the use of IL-2 as immunotherapy. It acts on both effector T cells (Teff) and on Treg cells, thus having the ability to modulate the Teff/Treg ratio resulting in either stimulation or diminution of the immune response. It was demonstrated that low doses of IL-2 dampen the auto-immune responses by supporting the maintenance of Treg cells while high doses boost the anti-tumoral immune responses by stimulating effector T cells. The amount of cytokine needed for a certain type of immune response is dictated by the fact that different immune cells express different types of IL-2 receptors. Nevertheless, the in vitro effect is hard to translate in vivo, on laboratory animals and especially on patients. This may be one reason why IL-2 works only on a fraction of patients (4, 6). One of the disadvantages is the toxicity of high-dose therapy. IL-2 stimulates the production of other cytokines which leads to lymphoid infiltration and results in a capillary leak syndrome. The immediate effect is hypovolemia and accumulation of fluid in the extravascular space. Thus, systemic IL-2 use is limited to patients with good performance status and is administered under strict observation (9, 10).

b) IFN- α

Interferons (IFNs) are cytokines with immunomodulatory properties. IFNs were first described for their antiviral effect but recently, it was discovered that they also play a role in immune responses to cancer by their effect on dendritic cells. Dendritic cells are sentinel antigen-presenting cells. Encountering of DC with tumor antigens leads to their activation, migration into the lymph nodes, cooperation with other immune cells, and initiation of the antitumoral immune response. Type I IFNs (alpha and beta) promote the activation, migration, and antigen-presentation of DC. Moreover, type I IFNs have direct effects on tumor cells inducing their death through a series of intracellular signals and indirect effects on tumor cells through its anti-angiogenetic function (11,12). IFN-alpha is recommended by guidelines in (a) stage IIB and stage IIC melanoma with negative sentinel lymph node as adjuvant therapy after wide excision of the tumor, (b) clinical stage III as adjuvant therapy after wide excision and complete therapeutic lymph node dissection, and (c) stage III clinical satellite or in-transit as primary local therapy if the tumor cannot be surgically removed (8).

II Vaccination

Melanoma vaccines are vaccines containing tumoral antigens. Despite the significant success of microbial vaccines, cancer vaccines have demonstrated relatively little clinical benefit. Amongst the reasons for this are the difficulty of finding a target antigen and the capacity of the tumor cells to escape immune surveillance. Tested vaccines have focused on several kinds of antigens: (a) tumor lysate, (b) non-mutated self antigens - shared by tumoral cells, early embryogenetic cells and healthy cells in low amounts, and (c) neoantigens - mutated „non-self” antigens expressed only by tumor cells which can be shared by more patients or personalized for each patient. Vaccines exist in 2 main forms: (a) vaccines that comprise the antigen peptides in their composition, thus initiating the immune response upon immune cells’ encounter with the antigen or (b) vaccines can be administered in the form of dendritic cells loaded with antigen peptides. In this form, dendritic cells are challenged with the antigen in vitro, they process the antigen and then are administered to the patient (13). Murine models, as well as clinical trials, have demonstrated that vaccines alone are not sufficient but they can increase the efficiency of other therapies. For example, gp100 vaccines increase the efficacy of IL-2 therapy (14).

A recent study used vaccines targeting up to 20 personalized neoantigens per patient following curative surgical removal in patients with untreated high-risk melanoma (stage IIIB/C and IVM 1a/b). Four of the six vaccinated patients had no recurrence at 25 months post-vaccination. Two had progressive disease and were treated with anti-PD-1 therapy (pembrolizumab) which led to complete tumor regression (15).

III Adoptive immunotherapy

Adoptive immunotherapy is a technique by which T-cells are harvested from the patient and reinfused into the patient after expansion in cultures. Cells can be harvested either (a) in the form of tumor infiltrating lymphocytes, which are considered antigen-specific or (b) in the form of peripheral blood mononuclear cells which are then engineered to become tumor antigen-specific. The technique is based on the fact that T-cell responses are specific, robust, and have memory, suggesting that this type of immune response can target tumor antigens from throughout the body (including metastasis), promising a therapeutic effect that may last several years (16).

Adoptive cell therapy trials using T-cells from tumor-infiltrating lymphocytes show 40-50% response rates of adoptive T-cell therapy following IL-2 treatment

with a significant proportion of patients alive and free of disease 3-5 years after the treatment (17).

Considering that T-cells recognize antigens through interaction between their TCR receptor and the epitope presented on MHC molecules of the antigen-presenting cell, several techniques have been developed in order to genetically engineer TCR to become antigen-specific. One of the most promising techniques is the construction of chimeric antigen receptors for T cells (CAR-T). CAR-T therapy with CD19 as target antigen used in B-cell malignancies resulted in complete remission in 50-90% of the patients. Nevertheless, clinical trials using CAR-T with GD2 as target antigen on solid tumors, including melanoma, have not been very promising, being limited by the immunosuppressive microenvironment of solid tumors. Moreover, scarcity of tumor-specific antigens in solid tumors highlights the need to identify new tumor-specific target antigens in melanoma (18, 19).

IV Immune Checkpoint inhibitors and immune modulator molecules

a) PD-1 and CTLA-4

T cell activation after encountering a tumor antigen is a two-step process. Antigen-presenting cells present the antigen epitope to T-cells on the MHC molecules. This is the first step towards activation of T cells. Completion of the activation is done through co-stimulation, a process that requires interaction between B7 molecules on the APC and CD28 molecules on the T cell. Conversely, co-inhibitory signaling occurs between PD1/PD2 ligand (on APC) and PD-1 receptor (on T cells) or CTLA4 which competes for B7 binding (20). Inhibitory molecules are physiologically upregulated during high antigenic loads in order to limit immunopathology when the immune system faces persistent infection. This phenomenon is called immune exhaustion.

A side effect of this particular physiological phenomenon is that tumors are also situations with high loads of antigen which leads to immune exhaustion and loss of the anti-tumoral effect of T-cells (21). Therefore, anti-CTLA-4 and anti-PD-1 antibodies block the inhibition of T-cell activation, restoring the function of T-cells (20).

A Phase III clinical trial on stage III/IV melanoma patients comparing nivolumab (anti-PD-1 antibody) and ipilimumab (anti-CTLA-4 antibody) showed 44% response and 6.9 months progression-free survival among patients treated with nivolumab compared to 19%- and 2.8-months progression-free survival among those treated with ipilimumab. Administration of both

nivolumab and ipilimumab resulted in higher response rates (58%) and survival (11.5 months) (22).

Limitations of the treatment include, besides mild side effects, severe side effects related to immune stimulation such as inflammation pneumonitis, interstitial nephritis, colitis, and exacerbation of pre-existing auto-immune disorders (psoriasis) or onset of new ones (diabetes mellitus) (20).

b) CD40

CD40 is a molecule expressed mainly on certain subsets of antigen presenting cells, such as monocytes, macrophages, and dendritic cells. CD40 ligand (CD40-L) is expressed on a large number of cell types, including activated CD4 T cells, activated B cells, memory CD8 T cells, activated natural killer cells, granulocytes, endothelial cells, and macrophages. CD40 ligation has been demonstrated to enhance the capacity of dendritic cells to present antigens to CD8 T cell antigen by increasing the expression of co-stimulatory molecules such as CD80 and CD86 and by increasing the expression of immunostimulatory cytokines. Thus, activating CD40 has the potential to bridge innate and adaptive immunity by triggering an antitumor T cell response (23).

Several CD40 agonists have been developed for use in clinical trials for various types of cancer. CP 870,893 monoclonal antibody is a CD40 agonist used in a phase 1 clinical trial for recurrent and stage IV melanoma in combination with tremelimumab (anti CTLA-4). This research is still ongoing and results have not yet been published (23, 24). CP 870, 893 has also been used in combination with polyIC: LC TLR-3 ligand, an immunostimulant and vaccine peptides in a phase 1 clinical study on stage III and IV melanoma also with unpublished results (25).

c) LAG-3 (*Lymphocyte Activation Gene 3*)

LAG-3 is an immune checkpoint molecule. It has a role in antigen processing and presentation via MHC class II protein binding, negative regulation of T cell activation and IL-2 biosynthesis, and positive regulation of cytotoxicity through NK cells (26).

In a clinical trial that combined therapy with nivolumab and anti-LAG antibody in patients refractory to previous immunotherapies, the overall response rate was 13%. The results were preliminary, as the clinical trial is still ongoing (27).

d) Oncolytic viruses

Oncolytic viruses have 2 mechanisms of action: (a) infection of the tumor cells which results in the direct killing of tumor cells, and (b) the fact that tumor cells

are also infected cells will additionally trigger the innate and adaptive immune response. Oncolytic viruses are genetically engineered in order to decrease their pathogenicity and increase their immunogenicity (28–30).

Talimogene laherparepvec (T-VEC)

T-VEC is the first used oncolytic virus. It is a genetically manipulated Herpes Simplex Virus – 1. Due to genetic engineering, the virus is unable to replicate in normal cells, has completely lost the tropism for neurons, and replicates preferentially in tumor cells. Further changes in its genome have made it possible to prevent the rapid clearance of the virus, resulting in more profound oncolytic effects and making the virus lose the capacity to avoid the immune response, resulting in a stronger immune response against infected tumor cells. Moreover, T-VEC has inserted in its genome copies of the human Granulocyte Macrophage-Colony Stimulation Factor gene. Thus, inside a tumor cell, the virus replicates and secretes GM-CSF leading to the lysis of the tumor cell which is followed by the release of tumor antigens, GM-CSF and viruses. GM-CSF release leads to tumor site infiltration with dendritic cells and generation of T cell immune response against infected tumor cells (28–30).

A phase III clinical trial on patients with unresected stage IIIB to stage IV melanoma has shown an overall response rate of 26.4% and a median survival rate of 23.3 months (29). The encouraging results and the fact that T-VEC was well tolerated has led to T-VEC being the first oncolytic virus approved for melanoma therapy (28).

e) OX40

OX40 (CD134) is a co-stimulatory molecule on the antigen presenting cells and its ligand, OX40L (CD252), is expressed on a variety of cells, mostly activated CD4 and CD8 T cells. It increases clonal expansion of effector T-cells and generation of memory T-cells specific for a certain antigen. It also diminishes the immunosuppressive effect of T regulatory cells, which leads to amplification of the immune response (31–33).

Currently, five molecules are being used for targeting of OX40. A phase I clinical trial on patients with metastatic cancers (melanoma, renal cancer, prostate cancer, ureteral cancer and cholangiocarcinoma) showed regression of at least one metastasis in 30% of the patients (33).

V Therapy based on Raf/MAPK/ERK signaling pathway inhibition

Raf/MAP-kinase/ERK signaling pathway is a membrane-to-nucleus group of proteins that

communicates a signal from a receptor on the surface of the cell to the nucleus; it is involved in a wide variety of cellular functions, such as cell proliferation, cell-cycle arrest, and apoptosis (34). BRAF gene is a proto-oncogene that encodes for a protein (B-raf) involved in cell proliferation, differentiation, and survival by regulating the Raf/MAP-kinase/ERK signalling pathway. BRAF mutations were discovered in various types of cancers, including 40-60% of melanomas. The mutation results in the substitution of valine with glutamic acid at codon 600 (V600E). Vemurafenib and dabrafenib are BRAF V600E inhibitors approved by Food and Drug Administration (FDA) (35). A clinical study on vemurafenib in patients with BRAF V600E mutation—with unresectable or metastatic melanoma— included 337 patients that received vemurafenib treatment and 338 patients that received dacarbazine treatment. The overall response rate was 48.4% in the vemurafenib treatment group compared to 5.5% in the dacarbazine treatment group (36).

Other molecules acting on Raf/MAPK/ERK signaling pathway are drugs that inhibit MAPK kinase MEK1 and/or MEK2 (MEK inhibitors). The combination of the two types of molecules acting on the same signaling pathway is useful, as resistance to BRAF-inhibitors can develop. A double-blind phase 3 clinical study treated 212 patients with dabrafenib and trametinib (MEK inhibitor) and 213 patients with dabrafenib only. Overall survival was 74% at 1 year and 51% at 2 years in the combined therapy group versus 68% and 42%, respectively, in the dabrafenib only group (37).

Conclusions

Biological therapy for the treatment of melanoma has increased life expectancy in melanoma patients. Current options are mainly based on overcoming the immunosuppression that accompanies the tumor by enhancing the anti-tumoral immune response as well as regulating signal transduction related to cell growth. The immune system and cellular signaling cascades represent very complex jigsaw puzzles with some pieces that are still unknown and some pieces that are known but are not yet entirely assembled. The more pieces of the puzzle are discovered and assembled, the more biological therapeutic targets are revealed and new therapeutic agents are developed, thus making biological therapy a very promising therapeutical area for the treatment of metastatic melanoma.

Conflict of interest disclosure

The authors declare that there are no conflicts of interest to be disclosed for this article.

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