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Review article

Immunological Checkpoints: CD137 and LAG3 Receptors as a Target for Cancer Immunotherapy - Literature Review

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Abstract

In recent years, the search for more efficient and less toxic anti-cancer therapies focuses on monoclonal antibodies targeting cells' immune checkpoints. Stimulation of the immunological response against tumor cells is potentially safer than chemotherapy. However, the currently used anti-PD1, anti-PD-L1 or anti-CTLA4 antibodies are effective in a limited population of patients and also immunotherapy may induce autoimmune toxicity. The aim of this study was to present the latest information on anti-CD137 and anti-LAG3 monoclonal antibodies in anticancer therapy. The overview was made with the use of pubmed.com and clinicaltrials.gov databases, searched between the years 2000 – 2016. CD137 activating molecule provides signal to activation and proliferation of T-cells, recruitment of inflammatory cells, transformation of monocytes into macrophages and dendritic cells and the secretion of proinflammatory cytokines. LAG3 is an inhibiting protein constitutively expressed on surface of Treg cells and induced on CD4+, CD8+, NK cells, B cells and dendritic cells. It maintains the state of exhaustion of CD8+ T cells and reduces synthesis of many cytokines e.g. IFN-α. The results of the current studies indicate that the immunological checkpoints blockade or stimulation is the right approach towards the development of anticancer therapy.

Keywords: Cancer Immunotherapy; CD137; 4-1BB; LAG-3; Immunological Checkpoints

Introduction

An increase in cancer incidence observed for years prompts scientists to deal with the problem of carcinogenesis and immunological mechanisms involved in the pathogenesis and resistance to cancer treatment. Leading cancer therapies are based on the aim to irreversibly damage the DNA of cancer cells, either by ionizing radiation or chemotherapeutic agents. Unfortunately, the high toxicity and considerable damage to healthy tissue are the limitations of these methods of treatment. The search for new, more efficient and less toxic therapies focuses in recent years on the monoclonal antibodies against cells' immune checkpoints. However, the currently used anti-PD1, anti-PD-L1 or anti-CTLA4 antibodies are effective in a limited population of patients and may induce autoimmune toxicity [1].

Stimulation of the immunological response against tumor cells offers great opportunities for treatment, potentially safer than current methods. Mechanisms of immunity allow the regular destruction of cancer cells spontaneously formed in the body. There are many pathways of this immunity, among which the most significant are the cell-mediated immunity of cytotoxic lymphocytes (CTL) and natural killers (NK), the antibody-dependent cell-mediated cytotoxicity (ADCC) and the complement-dependent cytotoxicity (CDC), as well as the activity of cytokines, including chemokines secreted by the tumor's cells and surrounding tissues. The impact of chemokines on the tumor may also have significant adverse effects like stimulation of angiogenesis, proliferation of tumor cells, or even inhibiting cells' apoptosis. Chemokines also attract regulatory T cells (Treg) inhibiting cellular response in the tumor [2,3].

The activation of lymphocytes is crucial for cellular immunity development, however, requires forming immunological "synapse" between T cells and antigen-presenting cells (APC). Antigens presented by main histocompatibility complex (MHC) molecules on APC are recognized by T-cell receptor (TCR) on T lymphocyte. TCR contains the CD3 molecule, which is the marker of T cells, and CD4 or CD8 molecules specific for the helper T cells (Th) or cytotoxic T cells (Tc), respectively. The interaction between TCR and MHC is the first signal for lymphocytes activation. The second signal is the interaction of co-stimulatory molecules on T lymphocytes and APCs. Co-stimulatory signal is necessary to produce a cellular response on naive T lymphocytes. The third signal, which multiples the cell's response, directs its development and prevents apoptosis, T cells receive from cytokines [4].

Aim: The aim of this study was to present the novel information on anti-CD137 and anti-LAG3 monoclonal antibodies in anticancer therapy.

Material and Methods: The overview of pubmed.com and clinicaltrials.gov databases from the years 2000 - 2016 was made with the use of following terms: immunotherapy, immunological checkpoints, CD137, 4-1BB, LAG3, CD223, Urelumab, IMP 321.

Results and Discussion: Second T cell activation signal derives from binding CD28 on the surface of T cells to B7-1 or B7-2 molecules (CD80 or CD86) on the surface of the APC. However, there was found a large number of molecules involved in the formation of the immunological "synapse". These molecules can be divided into activating proteins, e.g. previously mentioned CD28 and inhibiting proteins, an example of which is CTLA-4 (CD152), the CD28 competitor to binding to CD80/CD86. Inhibition signal for T cell derives also from cooperation of PD-1 on the surface of T cells with PD-L1 or PD-L2 molecules on the surface of the APC and tumor cells. Moreover, the activating molecules are i.a. CD137 (4-1BB), CD134 (OX40), CD357 (GITR), ICOS, and CD 40 and the inhibiting are LAG3, BTLA and TIM-3. [5] In this article we will present two of the aforementioned molecules, activating CD 137 and inhibitory LAG3 as a target for monoclonal antibodies in anticancer therapies.

CD137 (4-1BB) receptor

CD137, also called 4-1BB is a co-stimulating receptor protein belonging to the TNFR superfamily (TNFRSF9) [6]. It is predominantly expressed on activated $CD8^+$ T cells, but also on $CD4^+$, B cells, NK cells, monocytes, macrophages and dendritic cells [2,7]. Its ligand is 4-1BBL (CD137L) constitutively present on many cells in small amounts. In larger quantities, it is expressed on APCs, mainly DCs, monocytes/macrophages and on activated B cells [8]. CD137 after binding to its ligand provides an activation and proliferation signal to T-cells. It is also important in the formation of immunological memory [2,7]. The connection of CD137 with CD137L on endothelial cells triggers signaling (on NF-κB pathway), which is the point of convergence for the recruitment of inflammatory cells, transformation of monocytes into macrophages and dendritic cells, and the secretion of pro-inflammatory cytokines [8].

Effects of CD137 activation are different depending on the cells' type on which it is located. 4-1BB signaling causes the upregulation of anti-apoptotic genes and prevents the activation-induced cell death (AICD). On $CD8^+$ T cells stimulates proliferation by increasing the INF-α and IFN-γ secretion and cytotoxicity by raising granzymes secretion. It also affects the secretion of IL-2 and its receptor – sIL-2Rα (CD25). Among the TNFRSF proteins family, which include OX40, GITR, CD30 and CD27, CD137 has the greatest ability to stimulate the expression of IL-2Rα on $CD8^+$ lymphocytes [2,9,10]. Its impact on $CD4^+$ cells and on synthesis of IFN-γ and proliferation is uncertain. In relation to Treg, the CD137 effect is bi-directional. It seems that CD137 may, on the one hand, inhibit the conversion of T cells to Treg, and on the other potentiate their inhibitory activity on other T cells.

Studies in mice depleted $CD8^+$ cytotoxic cells showed that therapy with CD137 agonists may reprogram Treg cells into the $CD4^+$ T cells with cytotoxic activity directed against tumor cells. Regulatory T cells, after stimulation with agonistic monoclonal antibody against CD137, were reprogrammed to remove tumor cells, expressed the marker characteristic for Th cells – CD154, as well as produced TNF-α, eomezodermin and granzymes B, without loss of Foxp3 expression [2,11]. In case of NK cells, CD137 stimulation increases their cytotoxicity by increasing ability to destroy tumor cells. CD137 signaling raises the capacity of $CD3^+$ $CD56^+$ cells to activate $CD4^+$ Th1 cells, increases the production of pro-inflammatory cytokines, especially IFN-γ, TNF-α and IL-2, while decreases the amount of TGF-β, IL-4 and IL-10. CD137 agonists also affect dendritic cells by stimulating their maturation and potentiating antigen presentation. They also intensify DCs to produce IL-6, IL-12 and IL-27. It is worth noting that interleukin 12 on the one hand stimulates cytotoxic T lymphocytes and NK cells, induces Th1 response by increasing the secretion of IL-2, IFN-γ and granulocyte-macrophage colony-stimulating factor (GM-CSF), and on the other, leads to inhibition of angiogenesis when in relevant level. Due to these properties, IL-12 is linked to the potent anticancer properties. Macrophages with CD137 stimulated with agonists present an increased activity as APCs, and produce more IL-8, but also the IDO enzyme (indoleamine 2,3-dioxygenase) responsible for decomposition of tryptophan to i.a. kynurenine, which has immunosuppressive properties. IDO impairs T cell proliferation and promotes synthesis of immunosuppressive TGF-β [2,10,12].

Robust tumor-repressing effect of CD137 agonist monoclonal antibodies is particularly evident in attempts to combine it with other antitumor methods of therapy such as radiotherapy or active, non-specific immunotherapy with IL-12, leading to regression of non-immunogenic or low-immunogenic tumor

types [10]. In this way $CD8^+$ T cells and NK cells were recruited and stimulated. In mice with low-immunogenic melanoma B16F10 such therapy significantly reduced tumor growth and in 50% of mice a complete regression was observed. In the lung metastasis model, this treatment yielded significant protection against the spread of metastases and resulted in their quantitative reduction [13,14]. Other studies demonstrated that in case of that combination therapy, a 18-fold reduction in the dose of IL-12 was possible, which still gave better results than monotherapy with maximal IL-12 dose or a monoclonal antibody against CD137 [14].

Many clinical trials in human carcinomas are currently recruiting. Most studies focus on evaluation of the effectiveness of activating monoclonal antibody that binds to CD137 – urelumab, in combination with existing chemotherapeutic agents and other immunomodulators, such as nivolumab (anti-PD1). Clinical trials NCT02534506 and NCT02253992 focus on finding a safe dosage, possible side effects and efficiency of urelumab and are carried out in patients with malignant solid tumors and B-cell non-Hodgkin's lymphoma. Other phase I study is to determine the safety and the best dose of anti-LAG3 monoclonal antibodies or urelumab alone and in combination with nivolumab in patients with recurrent glioblastoma (NCT02658981).

LAG3 molecule

Lymphocyte-activation gene 3 protein (CD223) belongs to immunoglobulin superfamily and contains 4 extracellular Ig-like domains. The LAG3 gene contains 8 exons. The sequence data, exon/intron organization, and chromosomal localization indicate a close relationship of LAG3 to CD4. LAG3, PD-1, and CTLA-4 are one of many receptors that inhibit immune responses, expressed on T-cells associated with the tumor environment.

LAG3 is constitutively expressed on the surface of Treg cells and induced on the surface of $CD4^+$, $CD8^+$, NK cells, B cells and plasmacytoid dendritic cells (pDCs), after their activation. LAG3 expression is rdegulated by two surface disintegrins/metalloproteinases: ADAM10 and ADAM17. Its close relationship with CD4 molecule results in the ability to bind to MHC class II molecules even with greater affinity than CD4. This results in negative regulation of T cells by yet unknown mechanism. It is known so far that a single lysine residue in the cytoplasmic tail of LAG3 plays an important role in this process.

LAG3 inhibits the primary T-cell activation in vivo and in vitro, and blockade of this receptor abolishes the inhibitory effect of Tr1 cells. The role of LAG3 and PD-1 was also demonstrated in maintaining the state of exhaustion of $CD8^+$ T cells. Blockade of those two receptors gives a synergistic effect in the form of reversal of exhausted $CD8^+$ phenotype [15,16]. Two discovered additional ligands for LAG3 allowed to prove the role of this receptor in regulating response to tumor cells. The first is the Liver Sinusoidal Endothelial Cell Lectin (LSECtin). The interaction with LAG3 in melanoma cells results in a decrease of IFN-γ production by effector T cells and an increase of inhibiting IL-10 synthesis by Treg. The second ligand is Galectin 3 (Gal-3) that is produced on the surface of various types of cancers. After binding to LAG3 the suppression of tumor-specific $CD8^+$ lymphocytes occurs [16].

Dendritic cells, in addition to their activity as APCs, also produce a number of cytokines including IFN-α. The classic pathway of plasma dendritic cells activation leads through binding to the toll-like receptor (TLR). LAG3 and pDCs interaction can lead to pDCs alternative incomplete activation, resulting in a reduced synthesis of IFN-α. Attempts to increase the activation of pDCs in the tumor microenvironment with TLR agonists alone proved ineffective, therefore attaching monoclonal antibodies against LAG3 may increase the antitumor activity of plasmacytoid dendritic cells [17].

Immunocompetent mouse model suggests a potential use of monoclonal antibodies against LAG3 and PD-1 in reversing the state of exhaustion of CTL [18]. Studies on patients with non-small cell lung cancer (NSCLC) revealed elevated levels of $CD4^+$, $CD25^+$, $FoxP3^+$ Treg cells and increased expression of CTLA4, PD-1 and LAG3 on their surface. Treg cells found in the tumor environment had higher expression of these molecules than Treg from peripheral blood. Interestingly, the percentage of Treg and expression of inhibitory receptors increase with the progression of the tumor. Elevated levels of cytokines with immunosuppressive properties: TGF-β and IL-10 have been also observed in patients' serum when compared to the healthy group [19].

Many phase I and II clinical trials with anti-LAG3 antibodies are currently at the stage of selection of candidates but some of them have already ended and the results are quite promising. In the phase I study (NCT00324623) in patients with advanced stage IV melanoma, an increase of specific $CD8^+$ T cells percentage occurred in 83% of patients treated with the combination of MART-1 protein vaccine and monoclonal anti-LAG3 antibody (IMP321), whereas in the group receiving only vaccination the increase was observed in only 17% of patients. The authors noted that with increasing dose of IMP321 the percentage of specific $CD8^+$ T cell multiplies, yet they did not show signs of exhaustion [20].

Phase I/II study (NCT00349934) carried out in women with metastatic breast cancer (MBC), using paclitaxel in combination with IMP321, showed a steady increase in the number and activity of APCs (monocytes and DCs). The patients achieved an increase in the percentage of NK and $CD8^+$ memory T cells. The clinical benefits were observed in 90% of patients. Only 3 out of 30 patients were diagnosed with progression within six months. The authors acknowledge the possibility of widespread use of monoclonal antibodies against LAG3 in combination with the first line chemotherapy in clinical practice [21].

IMP321 used in the phase I clinical trials (NCT00351949) in patients with renal cell carcinoma gave satisfactory results similarly as in the aforementioned study in the form of increased level of active $CD8^+$ lymphocytes. In most patients an inhibition of tumor growth and at least a 3-month period of stable disease without progression was observed [22].

The next step in studies on IMP321 should be to prove beyond all doubt its efficacy in tumor treatment and determine how to combine agonistic and antagonistic monoclonal antibodies against immune checkpoints with chemotherapeutic agents already used in clinical practice.

Conclusions

• The results of the current studies indicate that the immunological checkpoints turned out to be the right approach towards the development of cancer therapy.

• In phase 1 and 2 clinical trials the use of monoclonal antibodies against CD137 and LAG3 proved to be effective in raising the immune response against tumor cells, in the absence of evidence of therapy toxicities.

• There is a need for further research into the clinical use of the described immunological checkpoints agonists and antagonists used in anticancer monotherapy or combination treatment methods.

Author Contributions

All authors made substantial contributions to acquisition and interpretation of data and participated in drafting of the article and revising it critically for important intellectual content. All authors gave final approval of this and any revised versions to be submitted.

References

1. Michot JM, Bigenwald C, Champiat S, Collins M, Carbonnel F et al. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. Eur J Cancer. 2016, 54(S4): 139-148.

2. Bartkowiak T, Curran MA. 4-1BB agonists: multi-potent potentiators of tumor immunity. Front Oncol. 2015, 5: 117.

3. Houot R, Kohrt H. CD137 stimulation enhances the vaccinal effect of anti-tumor antibodies. OncoImmunology. 2014, 3(7): e941740.

4. Nassef Kadry Naguib Roufaiel M, Wells JW, Steptoe RJ. Impaired T-cell function in B-cell lymphoma: a direct consequence of events at the immunological synapse? Front. Immunol. 2015, 6: 258.

5. Brzostek J, Gascoigne NRJ, Rybakin V. Cell Type-Specific Regulation of Immunological Synapse Dynamics by B7 Ligand Recognition. Front Immunol. 2016, 7: 24.

6. Zhang GB, Dong QM, Hou JQ, Ge Y, Ju SG et al. Characterization and application of three novel monoclonal antibodies against human 4-1BB: distinct epitopes of human 4-1BB on lung tumor cells and immune cells. Tissue Antigens. 2007, 70(6): 470-479.

7. Kim JA, Averbook BJ, Chambers K, Rothchild K, Kjaergaard J et al. Divergent Effects of 4-1BB Antibodies on Antitumor Immunity and on Tumor-reactive T-Cell Generation. Cancer Research. 2001, 61(5): 2031-2037.

8. Kwon B. Is CD137 Ligand (CD137L) Signaling a Fine Tuner of Immune Responses? Immune Netw. 2015, 15(3): 121-124.

9. Oh HS, Choi BK, Kim YH, Lee DG, Hwang S et al. 4-1BB Signaling Enhances Primary and Secondary Population Expansion of CD8+ T Cells by Maximizing Autocrine IL-2/IL-2 Receptor Signaling. PLoS ONE. 2015, 10(5): e0126765.

10. Vinay DS, Kwon BS. Immunotherapy of cancer with 4-1BB. Mol Cancer Ther. 2012, 11(5): 1062-1070.

11. Akhmetzyanova I, Zelinskyy G, Littwitz-Salomon E, Malyshkina A, Dietze KK et al. CD137 Agonist Therapy Can Reprogram Regulatory T Cells into Cytotoxic CD4+ T Cells with Antitumor Activity. J Immunol. 2016, 196(1): 484-492.

12. Zhu BQ, Ju SW, Shu YQ. CD137 enhances cytotoxicity of CD3(+)CD56(+) cells and their capacities to induce CD4(+) Th1 responses. Biomed Pharmacother. 2009, 63(7): 509-516.

13. Xu D, Gu P, Pan PY, et al. NK and CD8+ T cell-mediated eradication of poorly immunogenic B16-F10 melanoma by the combined action of IL-12 gene therapy and 4-1BB costimulation. Int J Cancer. 2004, 109(4): 499-506.

14. Chen SH, Pham-Nguyen KB, Martinet O, Huang Y, Yang W et al. Rejection of disseminated metastases of colon carcinoma by synergism of IL-12 gene therapy and 4-1BB costimulation. Mol Ther. 2000, 2(1): 39-46.

15. Turnis ME, Andrews LP, Vignali DA. Inhibitory receptors as targets for cancer immunotherapy. Eur J Immunol. 2015, 45(7): 1892-1905.

16. Le Mercier I, Lines JL, Noelle RJ. Beyond CTLA-4 and PD-1, the generation Z of negative checkpoint regulators. Front Immunol. 2015, 6: 418.

17. Castelli C, Triebel F, Rivoltini L. Lymphocyte activation gene-3 (LAG-3, CD223) in plasmacytoid dendritic cells (pDCs): a molecular target for the restoration of active antitumor immunity. Oncoimmunology. 2014, 3(11): e967146.

18. Hasegawa K, Tanaka S, Fujiki F. An Immunocompetent Mouse Model for MLL/AF9 Leukemia Reveals the Potential of Spontaneous Cytotoxic T-Cell Response to an Antigen Expressed in Leukemia Cells. PLoS One. 2015, 10(12): e0144594.

19. Wei T, Zhang J, Qin Y, et al. Increased expression of immunosuppressive molecules on intratumoral and circulating regulatory T cells in non-small-cell lung cancer patients. Am J Cancer Res. 2015, 5(7): 2190-2201.

20. Romano E, Michielin O, Voelter V. MART-1 peptide vaccination plus IMP321 (LAG-3Ig fusion protein) in patients receiving autologous PBMCs after lymphodepletion: results of a Phase I trial. J Transl Med. 2014, 12(1): 1-12.

21. Brignone C, Gutierrez M, Mefti F, Brain E, Jarcau R et al. First-line chemoimmunotherapy in metastatic breast carcinoma: combination of paclitaxel and IMP321 (LAG-3Ig) enhances immune responses and antitumor activity. J Transl Med. 2010, 8: 71.

22. Brignone C, Escudier B, Grygar C, Marcu M, Triebel F, et al. A phase I pharmacokinetic and biological correlative study of IMP321, a novel MHC class II agonist, in patients with advanced renal cell carcinoma. Clin Cancer Res. 2009, 15.