Advances in Molecular Imaging Strategies in Immune Checkpoint Therapy

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Abstract Tumor cells avoid being detected and eliminated by the innate immune system, and as a result, are able to proliferate in the body. Immune checkpoint inhibitor therapies eliminate cancer cells by activating immune cells in the body; however, this treatment is not suitable against all cancer types. This reflects a dire need for the development of non-invasive molecular imaging tools in the visualization of immune checkpoints expression, which will then allow practitioners to improve clinical assessments, screen interest groups and provide therapeutic predictions, furthering the development of personalized medicine. Therefore, the analyzing the efficacy of various tracers and the optimization of antibodies have recently become popular topics of research in the field of antibody-based imaging. This review summarizes the current mechanism of immune checkpoint-based treatments, and preclinical studies on immune checkpoint imaging.

Keywords Immune checkpoints; PD-1/PD-L1; Molecular imaging

Background
Under normal circumstances, immune checkpoints serve as protective agents in the immune system. However, tumor cells avoid being detected and eliminated by the body’s immune system by overexpressing immune checkpoint molecules; this allows them to proliferate in the body. In an era when personalized cancer medicine is advocated, immune checkpoint inhibitor therapy (ICT) stands out uniquely amongst other cancer therapies by virtue of its nature as a ‘common denominator’. This therapeutic strategy suppresses tumors by inhibiting the activity of immune checkpoints, while activating the T-cell immune response towards tumor cells. At present, the most extensively researched immune checkpoint molecules include: cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1) and PD-L1, as well as B- and T-lymphocyte attenuator (BTLA), V-domain Ig-containing suppressor of T-cell activation (VISTA), T-cell immunoglobulin and mucin domain-3 (TIM3), lymphocyte-activation gene 3 (LAG3), etc (Le Mercier et al., 2015). The field of molecular imaging itself is on the rise, as immune-based molecular imaging of monoclonal antibodies (mAbs) is a prospective, non-invasive molecular imaging technique. In particular, Immuno-PET imaging uses positron-emitting isotopes to non-invasively trace and quantify the expression of mAbs, and is capable of producing high-quality images.

1 Role of T-cell Co-stimulation during the Immune Response
Complete activation of T-cells requires dual signaling, as well as cytokine activity. Initial T-cell activation occurs during the specific binding of T-cell receptors (TCR) to major histocompatibility complexes (MHC)-antigenic peptide complexes, constituting the first signal for T-cell activation; the second signal for T-cell activation comes from the interaction between co-stimulatory molecules expressed on the surface of antigen presenting cells (APCs) and the corresponding receptors or ligands expressed on the surface of T-cells. Many of these molecules are members of the B7 family, and act as regulators of immune checkpoints, to either activate or suppress T-cell activity during the immune response by modulating signaling thresholds. Positive co-stimulatory molecules function by facilitating T-cell proliferation and acquisition of effector function. CD28 is one such positive co-stimulatory molecule (immune checkpoint) that facilitates T-cell activation, as the recognition of the CD80 and CD86 ligands occur on the surfaces of mature APCs. Negative checkpoint regulators (NCRs), on the other hand,
are molecules that downregulate the immune response and prevent the over-activation of T-cells, thereby playing a critical role in regulating peripheral tolerance.

2 Preclinical Advances in Tumor Treatment Mechanisms and Pharmacological Approaches in Immune Checkpoint Therapy

Many patients involve gene mutations or genetic changes. These changes affect special T-cell populations by inducing tolerance to tumors, cause a high expression of inhibitory receptors and related ligands and suppressing T-cell activation which caused anti-tumor T-cells useless in tumor microenvironments. Thus, tumor cells are not recognized by adaptive immunity or innate immunity systems, and can avoid attack from the host immune system (Topalian et al., 2015; Parra et al., 2016). In an era when personalized medicine is strongly advocated in the field of oncology, the sheer diversity of cellular mutations that occur in different cancers pose a significant challenge to the personalization of cancer treatment. Here, the ‘common denominator’ nature of immune checkpoint blockades allow it to stand out amongst other conventional cancer treatments. At present, the most extensively studied immune checkpoints are cytotoxic T-lymphocyte-associated protein 4 (CTLA-4, also known as CD152), and programmed cell death protein 1 (PD-1, also known as CD279). These operate at different levels, and regulate the immune response through different mechanisms (Duraiswamy et al., 2013). CTLA-4 is mainly expressed on the surface of activated T-cells, and shares a high degree of commonality with CD28, another molecule that is present on the surface of T-cells, and provides co-stimulatory signals. CTLA-4 and CD28 competitively bind to the same ligands CD86 and CD80; CTLA-4 and CD28 have opposing effects on T-cell activation. The former inhibits the initial stages of T-cell activation (Walker and Sansom, 2015). Studies on variety cell lines in mouse tumor models have proven that treatment with CTLA-4 mAbs results in elevated cell line specificity. After CTLA-4 mAb treatment, complete degeneration or stunted development of tumor tissue can be observed in several types of tumor xenograft models, including ovarian cancer, bladder cancer, brain cancer and fibrosarcoma (Fecchi et al., 2007; Grauer et al., 2007; Grosso and Jure-Kunkel, 2013). The PD-1 immune modulation pathway is similar to the CTLA-4 pathway in a number of ways, PD-1 does not exist on the surface of either quiescent T-cells or memory T-cells, and is mainly expressed on activated T-cells, B-cells, and other cells of the immune system. Cytoplasmic agents in PD-1 include Immunoreceptor tyrosine-based inhibitory motif (ITIM) and immunoreceptor tyrosine-based switch motif (ITSM), which bind ligands (PD-L1 and PD-L2), then undergo phosphorylation and recruit the intra-cellular signaling molecules Src homologous protein tyrosine phosphatase (SHP-2), thereby triggering the dephosphorylation of the antigen receptor complex. This mechanism allows PD-1 to inhibit signals and control the effects of the T-cell immune response (Parry et al., 2005; Honda et al., 2014; Topalian et al., 2015). PD-1 mAbs can prevent recognition between PD-1 and PD-L1, thus restoring the cytotoxic effects of T-cells to a certain extent. Preclinical studies on treatment using PD-1 mAbs have led to important breakthroughs in the field of oncology. Pembrolizumab, a PD-1 inhibitor, has been proven to decrease the mortality of patients with melanoma, and has been used in the treatment of other cancer types, such as non-small-cell lung carcinoma, renal cell carcinoma, head and neck cancers, bladder cancer, breast cancer, and Hodgkin’s lymphoma (Kantoff et al., 2010; Hodi et al., 2010; Gangadhar and Salama, 2015; Li et al., 2016). The targeted therapeutic application of PD-1 mAbs has revolutionized conventional treatments in oncology. Future work will be focused on designing personalized strategies for combination therapy of immunosuppressant, to strengthen antitumor immune responses and overcome the limitations of tumor resistance.

3 Preliminary Studies in Molecular Imaging of Immune Checkpoints

3.1 Non-invasive selection of patient populations likely to benefit from immune checkpoint therapy

With respect to PD-1/PD-L1 blockade therapy, the use of targeted PD-1 and PD-L1 blockers has achieved significant success in treating patients with cancer that overexpress PD-L1. However, not all cancer cells express PD-L1, and as a result, not all patients are suitable candidates for immune checkpoint blockade therapy. To better realize the aim of personalized medicine, clinicians are experiencing an urgent need for a selection method to accurately predict which patients stand to benefit from the administration of pharmacological immune checkpoint blocker agents. Immunohistochemical (IHC) studies have shown that the expression of PD-L1 proteins on CD8+ T-cells in infiltrated tumors can be used as a predictive marker for selection (Gros et al., 2015). However, the
results of PD-L1 staining can be affected by differential sensitivity towards different antibodies, difficulty in interpreting positive staining value, uneven expression of PD-L1 in tumors, sampling time and sampling position, etc., thus decreasing the reliability and accuracy of immunohistochemical methods for detecting PD-L1. Therefore, dynamic assessments of tumor microenvironment invivo, and the use of biological or radioactive markers to perform live imaging will allow for an improved selection of patients that are likely to benefit from immune checkpoint therapy (Wang et al., 2016). Immuno-PET imaging, on the other hand, is a non-invasive method used to evaluate the pharmacokinetics of immune checkpoint mAbs and quantification the expression levels of tumor markers (Mayer et al., 2016). Immuno-PET imaging uses radiolabeled antibodies to target and identify cells; however, designing immuno-PET imaging tracers is not an easy task. Radiotracer options, antibody design, and imaging kinetics, etc., are factors that need to be taken into consideration.

3.2 Choosing tracers for immuno-PET imaging of immune checkpoints

Immuno-PET is an advantageous imaging method, in that it is both high-resolution and high-sensitivity, and can be used to quantify the invivobehavior of administered mAbs and their interactions with target tissues. Determining an ideal immune checkpoint tracer involves many factors pertaining to the cancer itself, such as its localization, size, morphology, physiology, and sensitivity to radiation. Under normal circumstances, optimal tumor-to-background ratio can be obtained 2-4 days after the injection of mAbs, which have limited the application of long-lived radionuclides (van Dongen and Vosjan, 2010). Common metal positrons such as 99mTc, 86Y, 68Ga, or 64Cu have half-life duration of 15 hours, and will decay well before the radionuclide-tagged mAbs have reached optimal tumor-to-background ratios. However, recently, the imaging of 64Cu and 68Ga-tagged mAbs 1 hour following injection have achieved some success in animal research. The use of long-lived radionuclides will allow the imaging to be performed at optimal tumor-to-background ratios before decay of the mAb tag. Examples of radionuclides with long half-life duration are 124I and 89Zr, which have half-lives of 4.18 and 3.27 days, respectively, making those better choices for use as tracers in Immuno-PET imaging. Different nuclides have their own respective shortcomings; for instance, iodine (I) markers are relatively simple to use and to acquire, but its decay process involves the activity of high energy positrons, which decreases its image resolution. Zirconium (Zr) is a residual radionuclide which remains stable inside the body, and remains in target cells after catabolism. As a result, 89Zr markers are often used with mAbs in Immuno-PET imaging. At present, 89Zr-tagged mAbs have been used in many preclinical studies, include cetuximab, ibritumomabrituxetan, rituximab, bevacizumab, and trastuzumab, etc (Wang and Yang, 2016). Choosing an ideal radionuclide is an important step while performing immuno-PET; in addition, to allow the radionuclide to more efficiently localize and reach target tissues, researchers have invented engineered antibody fragments, dual-function antibodies, small-molecule antibodies, and other mAb fragments. In the future, studies will use improved antibody fragments to target proteins and increase imaging resolutions (Wright and Lapi, 2013).

3.3 Preliminary advances in PET, SPECT, and optical imaging methods in the visualization of immune checkpoint molecules

At Stanford University, Dr. Mayer A.T. and colleagues (Mayer et al., 2017) have developed 64Cu and 68Ga radionuclide-tagged murine PD-1 mAbs, and imaged the expression of PD-1 on tumor-infiltrating lymphocytes in murine CT26 colon carcinoma models, using Immuno-PET methods. Targeting these types of high-affinity protein scaffolds (HAC-PD1), the group designed 6 HAC-PD1 radiotracer variants, and imaged tumor regions 1, 2, 4, 18 and 24 h after the injection of radioactive tracers, reporting that imaging tumor-bearing CT26 colon carcinoma cell lines 1h postinjection resulted in the most prominent signal. These small proteins not only increase tumor penetration, but also have biological characteristics that allow them to be used in conjunction easily with other immunotherapy agents. For instance, the effects of co-administration of Nivolumab (a PD-1 blocker) and Ipilimumab (a CTLA-4 blocker) have been proven in patients with melanoma (Wolchok et al., 2013). In addition, many preclinical studies have been done to support their targeted synergistic effects on multiple types of immune checkpoints, such as PD-L1, TIM-3, LAG-3, etc. These results shed light on the potential of small-protein mAbs in research on immune checkpoint blockades, as well as their applicability in the modulation of the immune
response (Maute et al., 2015). Therefore, these molecular imaging methods are able to establish a basis for the non-invasive quantification of temporal or spatial features of immune checkpoints.

Whole mAbs are relatively large, have poor penetrative abilities, slow metabolic turnover, and may carry the risk of false-positive results due to the activity of metabolic residues in the blood pool. This results in low imaging efficiency, especially in target regions where the expression of PD-L1 is relatively low. To increase imaging efficiency, researchers need to raise effective antigen binding fragments. Nanobodies (Nbs) are single-domain antibodies with a variable heavy chain first identified in serum taken from camels, and are currently the smallest possible antigen-binding fragments that can be obtained, making them the most promising alternative to mAbs. Being relatively stable, soluble, and high-affinity, Nbs can quickly enter tissues, specifically bind antigens; unbound Nbs can be rapidly cleared by the kidneys (Muyldermans, 2013). Dr. Broos K and his colleagues (Broos et al., 2017) used 99mTc markers to tag 4 types of high-affinity PD-L1-specific nanobodies (Nbs) (C3, C7, E2, and E4, respectively), and used SPECT/CT imaging to assess the expression of PD-L1 on lung epithelial cells in murine TC-1 cancer models. The results showed that 99mTc-tagged NbsC3 and E2 had good antigen binding ability, and therefore, SPECT/CT imaging of 99mTc-tagged Nbs C3 and E2 may serve as a non-invasive method to assess tumor PD-L1 expression levels, with signal intensities that correlate with PD-L1 expression levels. At Johns Hopkins University, Professor S. Chatterjee and his colleagues (Chatterjee et al., 2016) made use of MPDL3280A, a mouse and human cross-reactive PD-L1 antibody, to develop a new radiotracer, 111In-PD-L1-mAb. Consequent SPECT imaging of various cell lines in murine tumor models with different PD-L1 expression levels demonstrate the specificity of 111In-PD-L1-mAb in identify PD-L1 expression. Results show that in murine models of PD-L1-positive breast cancer (MDAMB231) and non-small cell lung cancer in particular, 111In-PD-L1-mAb shows higher specificity and sustained high uptake levels, which prove that 111In-PD-L1-mAb can be used to evaluate the expression levels of PD-L1 in tumor tissues.

The analysis of fluorescent protein markers has the advantages of high-sensitivity, broad selection, high affinity, dynamic imaging, and higher resemblance to physiological conditions. Near-infrared spectroscopy (NIR) technology has been used in the detection of tumor border zones in preoperative staging processes in ovarian cancer and breast cancer. In addition, targeting MPDL3280A, the mouse and human cross-reactive PD-L1 antibody, S. Chatterjee et al. simultaneously (Chatterjee et al., 2016) developed a Near-Infrared Fluorescent dye (NIR) to mark PD-L1 mAbs, and assess the expression of PD-L1 in tumor tissues. Optical imaging results show that NIR-PD-L1-mAb tracer have high specific uptake in murine models of PD-L1 positive breast cancer (MDAMB231) and non-small cell lung cancer (H2444). This also shows that optical imaging methods can be used to non-invasively assess PD-L1 expression in tumors, to guide further clinical advances.

3.4 Issues faced by molecular imaging of immune checkpoints

As Immuno-PET has a few clinical shortcomings, including relatively long clearance time and high background signal, and the fact that patients are asked to return to the hospital for follow-up imaging a few days after the injection of tracers. Recent studies have also shown that whole mAbs have limited penetrability (Wittrup et al., 2012). In addition, the optimization of small-molecule high-affinity protein binding agents has become a major challenge in Immuno-PET imaging (Hettich et al., 2016). We should attempt to continuously improve imaging techniques of mAbs, allowing for improved clinical applications.

3.5 Future prospects and outlook

Following the continuous development of novel immune checkpoint drugs, and the validation of tracers marking different mAbs, the visualization of primary tumors and metastatic tumor targets has become a dire need of doctors from different clinical departments. The evaluation of molecular imaging for preclinical studies, especially using Immuno-PET, allows for non-invasive, dynamic live imaging, that provides a comprehensive and accurate assessment of immune checkpoints expression in tumor tissue. Thus, it aids preclinical evaluation prior to the administration of immune checkpoint therapy, as well as patient selection. If molecular imaging can indeed be used in a clinical setting in the future and provide accurate patient selection and treatment tracking in targeted immunotherapies, it will serve as a beacon of hope for patients with cancer.
Authors’ contributions
Huijie Jiang conceived and designed the work that led to the submission. Mingyu Zhang and Hailong Xu evaluated images and analyzed data. Mingyu Zhang and Hao Jiang measured and acquired data. All authors participated in paper writing and approved the final manuscript.

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