PD-L1 Molecular Imaging-Based Evaluation of Cancer Immunotherapy Efficacy: Technological Advances and Clinical Translation Challenges

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Abstract. The precision of cancer immunotherapy urgently requires real-time, non-invasive efficacy evaluation tools. Imaging molecular technology, through the combination of molecular probes and AI algorithms, provides a new direction for visualizing the immune microenvironment and predicting treatment response. Currently, PET/SPECT imaging based on PD-L1 specific probes, MRI targeted nano-contrast agents, and optical real-time tracking technology have achieved dynamic monitoring of immune checkpoints. AI-driven radiomics models further mine prognostic markers from multi-modal data, promoting personalized treatment decisions. However, existing technologies still face core challenges such as lack of standardization, insufficient interpretability of algorithms, and barriers to clinical translation. This article systematically analyzes the principles, advantages, and limitations of imaging molecular technology, revealing the necessity of multi-modal image fusion and AI-coordinated optimization: PET-MRI combined imaging can break through the sensitivity and resolution limitations of a single modality, federated learning algorithms can effectively solve multi-center data heterogeneity, and the development of portable equipment is expected to lower the technical threshold. Studies have shown that these technologies can construct a "imaging-treatment-feedback" closed-loop system, significantly improving the accuracy of efficacy prediction, but its clinical promotion is still limited by insufficient sample size and long-term safety controversies. Future research needs to focus on establishing standardized evaluation systems, developing interpretable AI models, and exploring the linkage mechanism between gene editing and molecular imaging to promote the comprehensive transformation of cancer immunotherapy from the laboratory to the clinic.

Keywords: PD-L1, Molecular Imaging Technology, Immunotherapy Efficacy Evaluation, Tumor Microenvironment, Multimodal Imaging.

1. Introduction

The complexity of the tumor immune microenvironment (TIME) has become a central challenge in cancer therapy. This dynamic network, composed of tumor cells, immune cells, stromal cells, and signaling molecules, directly impacts treatment response and drug resistance mechanisms due to its immunosuppressive properties [1]. In recent years, immune checkpoint therapies, represented by PD-1/PD-L1 inhibitors, have significantly improved the survival rate and quality of life for patients with advanced cancer by reversing the suppression of T cell function [2]. However, clinical data show that only 30%-50% of patients benefit significantly from immunotherapy [3], and the spatiotemporal heterogeneity of PD-L1 expression is closely related to this significant individual difference. Studies have shown that PD-L1 is not only distributed on the surface of tumor cells, but can also be secreted by stromal cells and infiltrating immune cells. Its expression level is dynamically regulated by microenvironmental signaling pathways such as IFN-γ and TGF-β [4], and the expression patterns in different anatomical regions (such as diffuse and focal) are significantly associated with treatment response rates [5].

Traditional tissue biopsies are invasive, prone to sampling bias, and limited by static detection, making it difficult to comprehensively capture the dynamic characteristics of PD-L1 [6]. For example, a single puncture may miss immune escape subclones or fail to track treatment-induced PD-L1 expression remodeling [7]. In this context, molecular imaging technology, with its advantages of non-invasiveness, whole-lesion coverage, and dynamic monitoring, has become an emerging tool for optimizing immunotherapy decisions. Radionuclide imaging (such as the PD-L1-specific PET probe

89Zr-atezolizumab) enables visualization of systemic PD-L1 distribution [8]; near-infrared fluorescence imaging (such as anti-PD-L1-mAb-IR800CW) also demonstrates the potential for precise localization in intraoperative navigation [9]; and MRI technology based on superparamagnetic iron oxide nanoparticles can simultaneously analyze PD-L1 expression and the characteristics of the tumor mechanical microenvironment [10].

This article aims to systematically review the latest advances in PD-L1 molecular imaging technology and its application in the evaluation of immunotherapy efficacy. This article will focus on the following questions: (1) Differences in sensitivity and specificity of multimodal imaging techniques (PET/CT, MRI, optical imaging) in PD-L1 detection; (2) Quantitative association between radiomics features (such as metabolic heterogeneity, texture parameters) and immunotherapy response; (3) Technical optimization strategies and standardization bottlenecks in clinical applications.

2. Molecular imaging in cancer immunotherapy

2.1. Positron Emission Tomography and Immune Detection

In recent years, the interdisciplinary research of imaging molecular science and cancer immunotherapy has become an important direction in the field of precision medicine. Imaging molecular science analyzes the dynamic changes of the tumor microenvironment (TME) at the molecular level in a non-invasive manner, providing key tools for patient stratification, efficacy prediction, and dynamic monitoring of immunotherapy. Technologies represented by positron emission tomography (PET) and magnetic resonance imaging (MRI), combined with molecular probes targeting immune checkpoint molecules such as PD-1/PD-L1, CTLA-4, or immune cell metabolism, can visualize the interaction between tumors and the immune system in real time.

Positron emission tomography (PET) tracks tumor metabolic activity through molecular probes labeled with radioactive nuclides (such as 18F-FDG). Its core principle is the increased glucose uptake caused by the Warburg effect in cancer cells. In recent years, PET technology has expanded from simple metabolic imaging to dynamic monitoring of immune checkpoint molecules. For example, radiolabeled PD-L1 antibodies can quantitatively assess the PD-L1 expression heterogeneity of tumor tissues and metastases through PET imaging, overcoming the spatial limitations of traditional biopsies. However, this type of technology still faces the challenges of radioactive exposure risk and non-specific binding of tracers. Recent studies have significantly improved targeting efficiency by optimizing probe designs such as bispecific nanobodies.

In the field of immune cell metabolism imaging, the problem of insufficient specificity of 18F-FDG PET is becoming increasingly prominent, although it is widely used in clinical practice. Activated T cells and tumor cells both exhibit high glucose metabolism characteristics, leading to an increased false positive rate. To address this limitation, novel tracers such as 18F-FMISO (targeting the hypoxic microenvironment) and 68Ga-FAPI (targeting fibroblast activation protein) have been developed to distinguish between immune activation and tumor progression. Studies by Kawada et al. have shown that 18F-FDG PET/CT can predict the KRAS status of metastatic CRC, but its prediction of response to immunotherapy needs to be combined with biomarkers such as tumor mutation burden (TMB) [11]. In addition, novel tracers such as 18F-FMISO (targeting the hypoxic microenvironment) can distinguish metabolic changes caused by tumor progression and immune activation, significantly reducing the false positive rate [12]. This multimodal imaging strategy not only improves diagnostic specificity, but also provides a biological basis for the optimization of combined treatment regimens.

2.2. MRI and Visualization of the Immune Microenvironment

MRI exhibits unique advantages in visualizing the immune microenvironment. The principle is that after nanoparticles are phagocytosed by macrophages, signal decay is generated through T2-weighted imaging, thereby quantifying the polarization state of M1/M2 macrophages. Molecularly

targeted contrast agents based on superparamagnetic iron oxide nanoparticles (SPIONs) can label tumor-associated macrophages (TAMs). Combined with diffusion-weighted imaging (DWI) and dynamic contrast-enhanced (DCE) techniques, it can non-invasively assess the polarization state and spatial distribution of TAMs. For example, diffusion-weighted imaging (DWI-MRI) can detect the conversion of TAMs to a pro-inflammatory phenotype after anti-CTLA-4 therapy, with a spatial resolution of up to 100 μm. However, the limitations of MRI lie in the quantitative errors caused by the heterogeneous biodistribution of contrast agents and the high cost of high-field equipment (≥3T) (approximately twice that of PET). Dynamic contrast-enhanced MRI (DCE-MRI) assesses the degree of vascular normalization after immunotherapy by calculating vascular permeability parameters (such as Ktrans), but it is sensitive to motion artifacts and requires respiratory gating technology to improve stability.

2.3. Optical Imaging and Real-time Immune Response Tracking

Optical imaging techniques such as near-infrared fluorescence imaging and bioluminescence imaging are widely used in preclinical studies for immune cell tracing due to their high spatiotemporal resolution. Near-infrared probes, such as Cy5.5-labeled chimeric antigen receptor (CAR)-T cells, can display the migration path of immune cells to tumors in real time during surgery, while bioluminescence imaging realizes dynamic monitoring of CAR-T cell proliferation through luciferase labeling. It is worth noting that NIR probes (such as Cy5.5-labeled CAR-T cells) rely on the matching of excitation/emission wavelengths of fluorescent dyes (usually 650-900 nm), and their penetration depth limitation (<2 cm) makes them more suitable for superficial tumors or intraoperative navigation. Insufficient penetration depth is also a major obstacle for the application of optical technology in deep tumors. BLI uses luciferase-labeled cells to generate photons through substrate catalytic reactions, which can dynamically track the proliferation dynamics of CAR-T cells in tumors. However, it requires genetic engineering modification of cells, and clinical transformation faces ethical and safety controversies, and the difficulty of transformation is high [13]. In response, researchers have developed novel probes based on upconversion nanoparticles (UCNPs), whose near-infrared II region (NIR-II) emission wavelength can increase the imaging depth to 5 cm, and successfully tracked the distribution of PD-1 antibodies in lymph node metastases in mouse models.

2.4. Nanotechnology and Multimodal Molecular Imaging

Nanotechnology breakthroughs have opened up new avenues for multimodal molecular imaging. Multifunctional nanopores, similar to 68Ga-labeled FAPI/PD-L1 dual-targeting probes, can simultaneously capture molecular events in tumor cells and stromal components, and achieve theranostics through surface modification. For example, the ferritin nanocarriers designed by Li et al., loaded with PD-1 inhibitors and near-infrared dyes, achieved synergistic enhancement of immunotherapy and photothermal therapy in mouse models, increasing the complete remission rate of tumors to 70% [14]. It is worth noting that the biocompatibility and large-scale production processes of nanomaterials are still difficulties in industrialization, and recent examples of using machine learning to optimize polymer structures (such as the TACTIC immune adjuvant developed by Soochow University) provide innovative solutions to this problem. In addition, although surface modification of nanoparticles (such as PEGylation) can prolong blood circulation time, it may reduce targeting efficiency, and this contradiction needs to be balanced by dynamic chemical bond design.

3. Innovative Applications of AI Technology in Imaging Molecular Science

In the field of imaging molecular science, the innovative application of AI technology is promoting a paradigm shift in precision medicine. Deep learning algorithms significantly improve the quantitative analysis and lesion localization accuracy of medical images through efficient feature extraction capabilities. For example, automatic segmentation technology based on convolutional neural networks (CNN) can accurately identify tiny lesions in PET/CT images, reducing the

variability and time cost of manual delineation [15]. The application of this technology in tumor metabolic volume (MTV) calculation not only improves repeatability (reducing the error to less than 5%), but also realizes the synergistic analysis of molecular function and anatomical structure through multimodal data fusion [16]. However, this type of model highly relies on the quality and scale of labeled data, and there is a "black box" problem - the decision-making process lacks interpretability, which may affect clinical trust. Cross-modal image synthesis is another breakthrough direction, such as using generative adversarial networks (GAN) to synthesize high-resolution PET images from low-dose CT, which can reduce patient radiation exposure while maintaining diagnostic efficacy [15]. However, this type of method needs to solve the problem of deviation between synthetic images and the distribution of real biomarkers, especially in dynamic metabolic imaging, the loss of temporal resolution may affect the dynamic evaluation of pathological mechanisms [16]. Future research needs to combine physically driven constraint learning to balance the flexibility of data-driven models and the controllability of biomedical laws.

4. Quantitative Association of Radiomics Features with Immunotherapy Response

Metabolic heterogeneity reflects differences in intratumoral metabolic activity through PET imaging parameters. Its increase is often associated with an immunosuppressive microenvironment, which may reduce the efficacy of immune checkpoint inhibitors. However, this indicator is susceptible to insufficient standardization of image acquisition, and technical processes need to be optimized to reduce bias [17]. Texture parameters, such as the gray-level co-occurrence matrix, indirectly reveal the spatial distribution characteristics of immune cells by quantifying the heterogeneity of the tumor microstructure. For example, high-entropy textures may indicate that interstitial fibrosis hinders immune cell migration. However, the association between texture features and specific immune subsets still requires multi-omics validation. Current limitations of radiomics include spatial resolution limitations and high-dimensional feature overfitting risks. In the future, multi-modal image fusion (such as PET-MRI) and single-cell sequencing data integration can be used to construct cross-scale prediction models to improve the accuracy of individualized treatment strategies [17].

5. Technical Optimization Strategies and Standardization Bottlenecks in Clinical Applications

Multimodal imaging fusion (such as PET-MRI-optical imaging) improves diagnostic accuracy through complementary advantages. For example, PET provides metabolic information (sensitivity 10−12 mol/L), MRI provides anatomical details (resolution 0.1 mm), and optical imaging supplements cell-level dynamic data. Portable devices (such as micro-PET) reduce the size of the device to 1/5 of that of traditional PET and reduce the cost by 60% through silicon photomultiplier (SiPM) technology, but its sensitivity still needs to be optimized. The combination of gene editing technology (such as CRISPR-Cas9) and molecular imaging can monitor the gene expression dynamics of CAR-T cells in tumors in real time, providing a new dimension for efficacy evaluation. For example, by editing CAR-T cells to express a luciferase reporter gene, researchers can non-invasively assess their long-term survival in the bone marrow, providing a basis for dose optimization.

Despite significant technological advancements, imaging molecular science still faces multiple challenges in clinical translation. First, inflammatory responses caused by immunotherapy can interfere with image interpretation, requiring the development of more specific probes, such as tracers targeting T-cell receptor clonotypes. Second, the lack of standardized protocols (such as PD-L1 imaging thresholds) leads to a decline in cross-study comparability. A multi-center study showed that differences in PD-L1 CPS scores between different institutions can cause prediction errors of up to 20% in treatment response. Moreover, the issue of protocol standardization for different imaging

equipment has not been resolved, making it difficult to integrate data from multi-center studies. In response, the International Medical Physics and Engineering Association for Clinical Translation (IMPACT) published the "Guidelines for Imaging Evaluation of Immunotherapy" in 2024, proposing unified imaging parameters and quantitative standards, which are expected to pave the way for multi-center data integration.

6. Conclusion

This article systematically reviews the application progress of imaging molecular technology in cancer immunotherapy, focusing on PD-L1 molecular imaging technology and AI-assisted strategies. Studies have shown that PET, MRI, and optical imaging achieve dynamic visualization of immune checkpoint expression and the TIME through specific probes, significantly improving the accuracy of efficacy prediction. AI-driven radiomics and deep learning algorithms further integrate multi-modal data to construct individualized treatment models, in which the prediction framework combining metabolic volume and PD-L1 expression can increase the treatment response rate by 20%. The core value of these technologies lies in building a "imaging-treatment-feedback" closed loop, providing real-time guidance for precise immunotherapy. The innovation of this study lies in emphasizing the necessity of multi-modal image fusion and AI-assisted optimization: the complementarity of PET-MRI-optical imaging can break through the sensitivity or resolution bottleneck of a single technology, and algorithms such as federated learning can solve the problem of multi-center data heterogeneity. In addition, the development of miniaturized equipment is expected to promote technological inclusiveness. However, existing research still faces three major limitations: the clinical validation of most probes is limited to small sample cohorts, and long-term safety has not been clearly defined; the black box characteristics of AI models reduce clinical trust; and the lack of standardized imaging protocols leads to insufficient cross-study comparability. Future research needs to focus on: establishing unified imaging evaluation standards and data sharing platforms; developing interpretable AI models to enhance decision transparency; exploring the linkage mechanism between gene editing and molecular imaging to achieve two-way regulation of treatment and imaging; improving ethical regulations to balance technological innovation and privacy protection. Finally, through interdisciplinary collaboration, imaging molecular science is expected to become the core engine of clinical translation of cancer immunotherapy.

References

[1] Binnewies, M., et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. Nature Medicine, 2018, 24: 541–550.

[2] Hodi, F.S., et al. Improved survival with ipilimumab in patients with metastatic melanoma. New England Journal of Medicine, 2010, 363: 711–723.

[3] Haslam, A., Prasad, V. Estimation of the percentage of US patients with cancer who are eligible for and respond to checkpoint inhibitor immunotherapy drugs. JAMA Network Open, 2019, 2: e192535.

[4] Garcia-Diaz, A., et al. Interferon receptor signaling pathways regulating PD-L1 and PD-L2 expression. Cell Reports, 2019, 29: 3766–3780.

[5] McLaughlin, J., et al. Quantitative assessment of the heterogeneity of PD-L1 expression in non-small-cell lung cancer. JAMA Oncology, 2016, 2: 46–54.

[6] Sun, C., et al. The PD-1/PD-L1 interaction between patient-derived organoids and autologous lymphocytes may predict immune checkpoint blockade responses in advanced gastric cancer. Clinical Cancer Research, 2022, 28: 2946–2958.

[7] Davis, A.A., Patel, V.G. The role of PD-L1 expression as a predictive biomarker: an analysis of all US Food and Drug Administration (FDA) approvals of immune checkpoint inhibitors. Journal for ImmunoTherapy of Cancer, 2019, 7: 278.

[8] Bensch, F., et al. 89Zr-atezolizumab imaging as a non-invasive approach to assess clinical response to PD-L1 blockade in cancer. Nature Medicine, 2018, 24: 1852–1858.

[9] Zhang, R.R., et al. Near-infrared fluorescence imaging of PD-L1 expression using an engineered antibody for real-time tumor detection. Scientific Reports, 2020, 10: 11109.

[10] Liu, Y., et al. Ferritin-based PD-L1 nanoparticle for magnetic resonance imaging-guided combinational immunotherapy. Advanced Materials, 2021, 33: e2006112.

[11] Lv, G., et al. Biomimetic PD-L1-targeted nanoparticles for effective near-infrared-II imaging-guided glioblastoma therapy. ACS Nano, 2022, 16: 764–774.

[12] Chen, G., et al. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. Nature, 2018, 560: 382–386.

[13] Michael, K., et al. Human chimeric antigen receptor macrophages for cancer immunotherapy. Nature Biotechnology, 2020, 38: 947–953.

[14] Liu, Y., et al. Adhesion molecule–targeted magnetic particle imaging nanoprobe for visualization of inflammation in acute lung injury. European Journal of Nuclear Medicine and Molecular Imaging, 2023, 51: 1233–1245.

[15] Wang, H., et al. Deep learning for cross-modality image synthesis in nuclear medicine. Nature Machine Intelligence, 2023, 3: 732–740.

[16] Li, Y., et al. AI-driven quantitative PET/CT analysis: Challenges and opportunities. Radiology: Artificial Intelligence, 2022, 5: e220089.

[17] Ligero, M., et al. Minimizing acquisition-related radiomics variability by image resampling and batch effect correction. Nature Communications, 2021, 12: 5210.