Imaging-based Biomarkers for Predicting and Evaluating Cancer Immunotherapy Response

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Over the past decade, substantial advances in cancer immunotherapy with immune checkpoint blockade (ICB) have changed therapeutic strategies for advanced cancer (1,2). Due to multiple mechanisms of immunotherapy (3), tumor response patterns differ from traditional treatments, such as chemotherapy and radiation therapy (4). Therefore, traditional response criteria cannot adequately capture atypical patterns produced by treatment (eg, durable and/or delayed responses, pseudoprogression, and hyperprogression) (5), which renders the interpretation of changes in tumor burden a challenging issue (6). Meanwhile, expensive ICB therapy currently shows limited rates of success (no more than 30%) and serious immune-related adverse events (irAEs, such as pneumonitis) (7–10). To meet the growing demand for prediction and evaluation of response to immunotherapy, more accurate and rapid methods are needed. Imaging plays an essential role in medical practice, cancer research, and clinical trials. However, current response evaluation criteria based on imaging measurements of tumor size have inherent limitations in specifying effects of tumor immunotherapy.

Investigators have proposed several different clinical approaches to predict and monitor response to immunotherapy, including Food and Drug Administration–approved biomarkers based on expression of programmed cell death-ligand 1 (PD-L1) and status of microsatellite instability-high and/or mismatch repair (11). However, existing markers fail to reliably distinguish responders from nonresponders and overall response to ICB due in part to tumor heterogeneity and evolution over time. Imaging-based biomarkers offer potential solutions to identify appropriate patients and to monitor response to ICB (12). Compared with traditional biopsy-based assays, images reflect the entire tumor burden. This is of particular importance in predicting and evaluating the response to cancer immunotherapy, which strongly depends on the tumor microenvironment. To date, radiomics-based artificial intelligence (AI) and molecular imaging are currently two of the most promising diagnostic image technologies. Radiomics offers high-throughput extraction of quantitative imaging features from a radiologic image, frequently providing more detailed characterization than possible by visual inspection of images (13,14). In addition, recent advances in biochemistry, protein engineering, and nanotechnology have promoted development of multifunctional molecular imaging agents. A variety of targeted imaging agents have been used to demonstrate the response to cancer immunotherapy.

In this review, we will systematically discuss imaging biomarkers in predicting and evaluating the response to cancer immunotherapy. There are already numerous excellent reviews that cover traditional morphologic evaluation criteria such as Response Evaluation Criteria in Solid Tumors (RECIST), imaging of peculiar patterns of response, and irAEs (15). However, radiomics and molecular imaging in cancer immunotherapy are relatively new fields that have not previously been comprehensively reviewed to our knowledge. In light of recent increased research activities in cancer immunotherapy, especially in radiomics and molecular imaging, an up-to-date account of the status of imaging biomarkers has become necessary. The present review provides a discussion on the critical features, traditional
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Abbreviations
ADC = apparent diffusion coefficient, AI = artificial intelligence, AUC = area under the receiver operating characteristic curve, CTLA-4 = cytotoxic T-lymphocyte associated protein 4, FDG = fluorine 18 fluorodeoxyglucose, ICB = immune checkpoint blockade, irAEs = immune-related adverse events, NSCLC = non-small cell lung carcinoma, PD-1 = programmed cell death protein 1, PD-L1 = programmed cell death protein ligand 1, RECIST = Response Evaluation Criteria in Solid Tumors, TILs = tumor infiltrating lymphocytes

Summary
Imaging-based biomarkers are being developed as a way to predict and evaluate the response to cancer immunotherapy, and techniques involving radiomics and molecular imaging may aid in optimizing predictions.

Essentials
- Limited success rates and immune-related adverse events of immunotherapy exemplify the need for more accurate and efficient methods to identify patients who could benefit from immune checkpoint blockade early.
- Traditional evaluation criteria and biomarkers cannot adequately capture atypical response patterns of tumor immunotherapy, which renders the interpretation of changes in tumor burden a challenging issue.
- Biomarker detection methods relying on needle biopsy may not capture dynamics of the complex immune response and tumor heterogeneity.
- Currently, radiomics-based artificial intelligence and molecular imaging nearly dominate research hotspots in the field of image diagnosis and may ultimately improve evaluation of response to immunotherapy.

Critical Features, Traditional Evaluation Criteria, and Challenges of Cancer Immunotherapy
Immune checkpoints are stimulatory and inhibitory signals from the ligand-receptor pairs among tumor cells, T cells, dendritic cells, and macrophages. These signals play a critical role in maintaining immune homeostasis by downregulating cytotoxic T cell activity in healthy tissue. However, immune checkpoints (eg, PD-L1 and/or cytotoxic T-lymphocyte antigen-4 [CTLA-4]) are overexpressed on many types of tumor cells, which results in suppression of antitumor immune responses (16). Cancer immunotherapy by ICB as a promising therapeutic strategy in medical oncology has fueled developments in the field of immune cancer therapies (17). Cancer vaccines (18), chimeric antigen receptor T cell and adoptive T cell transfer (19), as well as oncolytic viruses (20) also show promising clinical results across a variety of malignancies (21). ICB, as one of the main strategies in cancer immunotherapy, acts by preventing inhibitory interactions between immune checkpoint molecules expressed on T cells and their ligands in tumor microenvironment, rather than by producing direct cytotoxic or targeted effects to tumor cells. However, so far only a fraction of patients (20%–50%) have shown durable clinical responses to immunotherapy (22). Recently, use of combination strategies may increase the number of patients who benefit from immunotherapy (23). Various combinations of ICB with many established therapies, such as antiangiogenesis agents, targeted therapies, chemotherapy and radiation therapy, are being evaluated in clinical trials. However, these trials require an unprecedented number of patients and major financial investments. Moreover, the amplified T cell activation triggered by ICB can lead to a broad range of adverse and off-target effects (24). Therefore, it is important to establish early predictive biomarkers for response to ICB in an individual patient, which may modify or change treatment strategies.

It is important to emphasize that immunotherapy can generate irAEs involving various organs, such as dermatologic toxicity, pneumonitis, colitis, thyroiditis, hepatitis, pancreatitis, and myositis (25). The irAEs are defined as complications related to immunotherapy and may result from either autoimmunity or a proinflammatory state. These events tend to resolve with discontinuation of therapy, suggesting that they are not true autoimmune processes but result from general immunologic enhancement (26). Patterns of irAEs may be varied with the type of ICB. Hypophysitis is more frequent in patients treated with an anti-CTLA-4 antibody, while pneumonitis is more prevalent in patients treated with anti-programmed cell death protein 1 (PD-1) or anti-PD-L1 antibodies (27,28). The presence of irAEs may represent a good sign of response to immunotherapy (29), but irAEs such as pneumonitis may lead to treatment-related deaths and long-term respiratory morbidity. Therefore, it is important for radiologists to recognize the unique imaging manifestations of irAEs and distinguish these findings from metastatic progression of disease (30).

Last, unique mechanisms of ICB therapy are postulated to lead to peculiar patterns of response (eg, durable or delayed responses, pseudoprogression, and hyperprogression). The initial response to tumor immunotherapy may show an apparent transient increase in total tumor burden or development of new lesions followed by regression, which is called pseudoprogression (Fig 2) (31–34). The mechanism of the phenomenon is thought to be infiltration of immune cells into tumors (35). Hyperprogression is defined as a more rapid increase (minimum two-fold) in tumor growth rate after immunotherapy as compared with before immunotherapy (Fig 3) (36). Pseudoprogression or hyperprogression occur in up to 10% of patients after ICB treatment (36). Current clinical evaluation criteria, RECIST version 1.1 (RECIST 1.1), cannot adequately capture the previously described atypical patterns and recognize irAEs. To address this issue, several response criteria have been developed to evaluate tumor immune response, such as immune-related response.
criteria, the recently updated immune RECIST, and immune-related RECIST (37). Although immune-related response criteria, immune-related RECIST, and immune RECIST represent continual improvements over the conventional World Health Organization and RECIST criteria for evaluating response to immunotherapy, these measurements based on tumor size still cannot specify other characteristics of a tumor in the field of immunotherapy. Because of the inherent defects and limitations, these criteria only provide a consistent standard for management of data collected in clinical trials about immunotherapy rather than clinical practice or treatment decisions (38).

Clinical Biomarkers and Challenges of Cancer Immunotherapy

Development of biomarkers for early response to ICB in individual patients remains an ongoing need in precision medicine (39). Because multiple mechanisms of immune suppression exist in cancer, a better understanding of the molecular, cellular, and treatment components at all stages of cancer development is necessary to devise improved monitoring techniques. Different biomarkers have been investigated with variable success, especially two Food and Drug Administration–approved biomarkers, including PD-L1 measured with immunohistochemistry and microsatellite instability-high or mismatch repair deficient status measurement by immunohistochemistry and polymerase chain reaction–based assays. With growing evidence that a higher tumor mutational burden results in a higher probability for response, it is a predictive biomarker that has been included in the latest National Comprehensive Cancer Network guidelines for immunotherapy. Since immune checkpoint antibodies primarily target T cells, tumor infiltrating lymphocytes (TILs) and tumor immune microenvironment play an important role in response to ICB. Analysis of TILs has demonstrated that the presence of cytotoxic CD8+ T cells can predict overall survival in breast, lung, ovarian, melanoma, and colorectal cancers (40,41). Interferon-γ, a proinflammatory cytokine produced predominantly by natural killer T cells, activated T cells, and natural killer cells, plays a substantial role in regulating anticancer immunity (42). Although the necessity of interferon-γ signaling in response to tumor immunotherapy has been demonstrated, the predictive value of interferon-γ to ICB remains controversial and unconfirmed (43). Specific oncogenic mutations (epidermal growth factor receptor [EGFR]/ KRAS mutation or anaplastic lymphoma kinase [ALK] fusion) within tumors may be associated with response to ICB, but these correlations remain insufficient to direct therapy (44). Finally, another recent study showed that acidic microenvironments induced by highly glycolytic tumor cells promoted noninflammatory polarization of tumor-associated macrophages, which resulted in immunoevasion (45). Thus, assessment of tumor acidification may be important for predicting response to tumor immunotherapy.

However, the complexity and dynamics of the tumor immune response restrict the development and implementation of these biomarkers (38). ICB can sometimes induce tumor immune responses without these biomarkers or fail to induce responses despite presence of them (46). Low response rates of ICB have been noted even in tumor with high expression of PD-L1 (47). A possible reason is that a single biopsy may not capture the dynamics of the complex immune response and heterogeneity within a single tumor or among primary and metastatic lesions (48). Moreover, different kits and antibodies also produce different results for expression of PD-L1, which results in no uniform PD-L1 reaction threshold (eg, commonly 1%, 20% and 50%) (49). A recent report showed that cancer cells release extracellular vesicles, known as exosomes, that contain surface PD-L1, which suppresses T cell activity (50). In addition, another recent study demonstrated no significant relationship between TILs and anti-PD-1 inhibitor response, and only about 10% of tumor-infiltrating T have the ability to recognize surrounding cancer cells (51). Thus, based on the currently available methods for evaluating these biomarkers, prediction of response to immunotherapy remains challenging.

Given the increasing use of ICB with low efficacy and unique patterns of irAEs, there has been a push to develop validated and consistent biomarkers and approaches for better characterization and prediction of tumor immunotherapy response to guide ICB utilization in a personalized or stratified manner of treatment.
et al reported that ¹⁸F FDG PET/CT had success staging and assessing response and prognosis of patients with melanoma, especially for detection of irAEs (53). Cho et al evaluated ¹⁸F FDG PET/CT by combining functional and anatomic imaging parameters to predict early response to immunotherapy in 20 patients with advanced melanoma (54). However, the role of ¹⁸F FDG PET/CT in evaluation of immunotherapy is uncertain. One of the substantial challenges with ¹⁸F FDG PET/CT is the high false-positive rate that results from increased FDG uptake by activated immune cells (12). Some studies demonstrated the inability of ¹⁸F FDG PET/CT to differentiate between pseudoprogression resulting from inflammatory infiltrate versus progressive disease (55). Nevertheless, Ito et al recently reported that ¹⁸F FDG PET/CT had success staging and assessing response and prognosis of patients with melanoma, especially for detection of irAEs (53). Cho et al evaluated ¹⁸F FDG PET/CT by combining functional and anatomic imaging parameters to predict early response to immunotherapy in 20 patients with advanced melanoma (54). However, the role of ¹⁸F FDG PET/CT in evaluation of immunotherapy is uncertain. One of the substantial challenges with ¹⁸F FDG PET/CT is the high false-positive rate that results from increased FDG uptake by activated immune cells (12). Some studies demonstrated the inability of ¹⁸F FDG PET/CT to differentiate between pseudoprogression resulting from inflammatory infiltrate versus progressive disease (55). Nevertheless, Ito et al recently

Emerging Imaging-based Methods for Prediction and Evaluation of Cancer Immunotherapy

Emerging Qualitative and Quantitative Imaging Features in Immune-related Tumor Response

PET/CT is the most used technique in clinical practice for evaluation of tumor metabolism. The most frequently used radiotracer in clinical practice is 2-deoxy-2-(¹⁸F)fluoro-D-glucose (¹⁸F FDG). Previous studies were performed with small cohorts of patients. For example, Kaira et al showed that metabolic response by ¹⁸F FDG was effective in predicting early therapeutic response at 1 month after anti-PD1 therapy (nivolumab) in treated non–small cell lung carcinoma (NSCLC) (52). Perng

Figure 2: Pseudoprogression with initial increase in tumor burden followed by subsequent tumor shrinkage due to immune-related response in a 64-year-old man with lung squamous carcinoma of the right lower lobe treated with pembrolizumab (anti-PD-1). Images on the right are mediastinal window, images on left are pulmonary window. A. Baseline axial CT images obtained before therapy reveal a right lower lobe mass near pleura (arrow). B. Follow-up of axial CT images at 2 months of therapy show an interval increase in the size of the tumor (arrow) and in pleural effusion. Moreover, lytic destruction of the adjacent rib and multiple enlarged lymph nodes in the right hilum and mediastinum appear. C. Other follow-up axial CT images at 4 months of therapy reveal a marked interval decrease in the size of the mass (arrow). In addition, the destruction of the adjacent rib was substantially relieved, and the right hilum and mediastinal lymph nodes were substantially reduced. IO = immunotherapy.

First IO dose 2/5/2018

Baseline scan 1/31/2018

Evaluation scan 4/4/2018

Evaluation scan 6/7/2018
immune-related response criteria and the recently proposed immune RECIST criteria have been developed for the effective evaluation of ICB therapy on CT images (3). Dercle et al introduced a simple baseline three-point CT scan score to identify patients with metastatic lesions with long-term survival on anti-PD-1 or anti-PD-L1 therapy, including tumor burden, skeletal muscle index, and presence of nonpulmonary visceral metastases (58). Shrot et al emphasized that the appearance of a CT halo sign around pulmonary melanoma metastases following adoptive T cell therapy might indicate antitumoral effect and hence predict response (59). A recent study found that tumors likely to respond to immunotherapy were typically characterized with more heterogeneous response within the tumor mass (57).

CT is one of the most frequently used diagnostic imaging techniques in the follow-up of patients (57). The association between tumor response at 18F FDG PET/CT and prognosis in patients with metastatic melanoma who received anti-CTLA-4 antibody (ipilimumab) (56). Their data showed that tumor response according to PET Response Criteria in Solid Tumors (PERCIST) was associated with overall survival. They suggested that progressive metabolic disease should not be defined by the appearance of new lesions, but rather by an increase in the sum of the highest average standardized uptake value peak. In general, due to unresolved challenges, the role of 18F FDG PET/CT imaging in ICB therapy still requires further validation in larger cohorts.

Figure 3: Hyperprogression in a 58-year-old man with lung squamous carcinoma of a right upper lobe treated with pembrolizumab. Images on the right are mediastinal window, images on left are pulmonary window. A, Prebaseline axial CT images obtained before therapy reveal a right upper lobe mass (arrow). B, Baseline axial CT images before therapy reveal no substantial increase compared with prebaseline (arrow). C, Follow-up axial CT images at 1.8 months of therapy show a substantial increase in size of a right upper lobe lung lesion (arrow) and intralesion cavitary components. IO = immunotherapy.
morphologic profiles with nonuniform density patterns and compact borders (60).

MRI is another widely used technique for imaging cancer, especially in the central nervous system. As more ICBs enter clinical trials for patients with glioblastoma, there will be an urgent need to understand MRI changes induced by these agents for reliably evaluating response to immunotherapy (61). Guidelines for continuing immunotherapy in patients with neurotumors prior to follow-up imaging and confirmation of progression have recently been published as the immunotherapy response assessment in neuro-oncology (iRANO) criteria (62). However, some patients whose early clinical and/or imaging findings raise questions of pseudoprogression may be observed with serial MRI during therapy for 6 months or more. Due to the morbidity and the short life expectancy of patients with recurrent glioblastoma, delayed surveillance follow-up is not optimal. Qin et al revealed intermediate apparent diffusion coefficient (ADC) volumes of interest in patients with glioblastoma receiving ICB might better predict therapeutic benefit than conventional imaging (63). Similarly, Wieduwilt et al reported the tumor minimum ADC (ADC_min) derived from diffusion-weighted imaging provided better prognostic information for patients with primary central nervous system lymphoma treated with immunochemotherapy than established clinical risk scores (64). In addition, Vrabec et al also supported ADC_min as a potential radiologic marker to differentiate immunotherapy-induced inflammatory response and glioblastoma progression (65). The study found ADC_min levels were lower in progressive than in stable lesions. Several other MRI techniques have shown effectiveness regarding assessment of treatment-related heterogeneity, including perfusion-weighted imaging (66) and magnetic resonance spectroscopy (67). Diffusion tensor imaging has not been proposed in the follow-up of glioblastoma with immunotherapy because this method is limited by the frequent presence of substantial edema due to inflammation (68). Arterial spin labeling and susceptibility-weighted imaging data in immunotherapy have not yet been reported. In clinical practice, a combination of different MRI techniques may be necessary to differentiate between pseudoprogression and actual tumor progression. Advanced MRI techniques would have great potential in prediction of treatment response and overall evaluation of immunotherapy.

Although conventional immune-related qualitative and quantitative imaging features can provide important information on response and toxicity for cancer immunotherapy in the clinical setting, there are still many substantial questions that remain to be addressed (12). To address unmet needs in the immunotherapy field, various techniques for evaluation of response to immunotherapy have been explored, such as radiomics and molecular imaging techniques.

Al and Radiomics

Recent advances in radiomics-based AI have achieved impressive initial successes in automatically quantifying radiologic patterns of tumors (69) and characterizing tumor microenvironments (13). Radiomics is defined as the quantification of phenotypic features, some of which may not be readily apparent on visual or qualitative assessment. Radiomics can transform digitally encrypted medical images holding relevant information about pathophysiology of tumors into mineable data for quantitative imaging features (70). The relationship between radiomic measurements of a region of interest on radiologic images and the presence and aggressiveness of disease is established by computational algorithms (71). The process performed in radiomics is roughly five steps: (a) image acquisition; (b) identification of the volume of interest; (c) storage of data in federated databases; (d) quantitative feature extraction; and (e) selection of features to predict aspects of disease biology and/or response to therapy (72). The heart of radiomics is the extraction of high-dimension data features to quantitatively analyze attributes of volumes of interest. In practice, “semantic” and “agnostic” features are the two kinds of features extracted in radiomics (72). Semantic features are those commonly used in the radiology lexicon to describe regions of interest, mainly including shape, location, vascularity, spiculation, necrosis, and attachments or lepidics (72). Agnostic features are those that attempt to capture lesion heterogeneity through quantitative descriptors, which can be divided into first-, second-, or higher order statistical outputs (72). These first-order statistics describe the distribution of values of individual voxels regardless of spatial relationships. These are usually histogram-based methods and reduce mean, median, maximum, minimum, and uniformity or randomness (entropy) of the intensities on the image to single values, as well as the skewness (asymmetry) and kurtosis (flatness) of the histogram of values (72). Second-order statistical descriptors, generally described as “texture” features, describe statistical interrelationships between voxels with similar (or dissimilar) contrast values (72). Higher-order statistical methods impose filter grids on the image to extract repetitive or nonrepetitive patterns (72). These include fractal analyses, wherein patterns are imposed on the image and the number of grid elements containing voxels of a specified value is computed: Minkowski functionals, which assess patterns of voxels whose intensity is above a threshold; wavelets, which are filter transforms that multiply an image by a matrix of complex linear or radial “waves”; and Laplacian transforms of Gaussian bandpass filters that can extract areas with increasingly coarse texture patterns from the image (72). Radiomics as a diagnostic and predictive tool has been applied to a wide range of questions in oncology (73).

Tumor immune phenotypes defined by histology and immunostaining fall broadly into immune-inflamed, immune-excluded, and immune-desert (74). Immune-inflamed tumors are characterized by dense TILs, expression of immune checkpoints (eg, PD-L1), and a high tumor mutational burden. These features relate closely to favorable response to immunotherapy (75). The immune-excluded and immune-desert tumors are characterized by low TILs and highly proliferating tumor cells (76). Noninvasive detection methods are now needed to quantify TILs and PD-L1 expression in predicting and monitoring tumor response to immunotherapy. To overcome these limitations, Sun et al developed a radiomic signature of immune infiltration of tumors to predict response to anti-PD-1 or anti-PD-L1 immunotherapy (77). In this retrospective multicohort study, contrast material–enhanced CT images and CD8 T
cell RNA expression data from 135 patients in four cohorts were analyzed to identify and validate a radiomic signature to predict immunotherapy response. The radiomic signature predicted the gene expression signature of CD8 cells and discriminated inflamed tumors from immune-desert tumors with an area under the receiver operating characteristic curve (AUC) of 0.67 and 0.74, respectively. In patients treated with anti-PD-1 and anti-PD-L1 antibodies, a high baseline radiomic score was associated with an objective response rate and improved overall survival. Similarly, Tang et al developed a NSCLC radiomics signature associated with PD-L1 expression and density of TILs to evaluate immune state (78). Two cohorts of patients with NSCLC treated with definitive surgical resection were used to establish and validate a radiomics signature by combining pretreatment CT with TILs (via counts of CD3 T cells) and PD-L1 expression in tissue samples from tumors. The radiomics signature correlated significantly with overall survival and immune pathology. Specifically, tumors characterized by a low radiomics signature and high heterogeneity exhibited low PD-L1 and high CD3 infiltration, which suggested a favorable immune-activated state. Recently, Trebesch et al evaluated the potential predictive value of a CT-derived radiomic biomarker by directly analyzing all visible cancer lesions (1055 primary and metastatic lesions) in 203 patients with advanced melanoma and NSCLC receiving immunotherapy (60). The radiomic biomarker reached significant performance on NSCLC lesions (up to 0.83 AUC, \( P < .001 \)) and on melanoma lymph node (0.64 AUC, \( P = .05 \)). Combining predictions made on individual lesions, significant performances in predicting immunotherapy response were also observed in both cancer types (0.76 AUC, \( P < .001 \)), with a 1-year significant survival difference (24\%, \( P = .02 \)). In addition, results of biologic validation showed significant associations with pathways involved in mitosis, which indicated a relationship between increased proliferative potential and preferential response to immunotherapy. These results suggested that radiomics could be an efficient way to predict response to cancer immunotherapy.

In addition, radiomics has been applied to predict risk of irAEs. Pneumonitis is a potentially fatal and common irAE. Besides serious clinical consequences, irAEs such as pneumonitis are increasingly considered as factors contributing to treatment noncompliance and dose modification or discontinuation (79). Thus, early detection is critical to improve treatment outcomes and optimize patient management. Colen and colleagues presented the first reported work that explored the potential of radiomics to predict development of immunotherapy-induced pneumonitis (79). Radiomic analyses were performed based on baseline chest CT images of patients who did (\( n = 2 \)) and did not (\( n = 30 \)) develop immunotherapy-induced pneumonitis. In the training cohort (32 patients), the most predictive radiomic features were skewness and angular variance of sum of squares. Maximum relevance and minimum redundancy feature selection method, anomaly detection algorithm, and leave-one-out cross-validation identified radiomic features that discriminated patients who subsequently developed immunotherapy-induced pneumonitis with 100\% accuracy (\( P = .0033 \)). Larger cohorts of patients with immunotherapy-induced pneumonitis are needed to further assess this method. With further joint collaboration of oncologists, radiologists, physicians, and computational scientists, radiomics is expected to allow doctors to better assess immunotherapy and improve systems for clinical decision-making.

### Molecular Imaging

Molecular imaging encompasses multiple technologies focused on detecting specific functional and molecular features of tumor microenvironments in addition to anatomy (80). These capabilities position molecular imaging methods to analyze tumor immune microenvironments (81). Various novel radioactive tracers that target key molecules (eg, PD-1 or PD-L1) of immune checkpoint pathways and cellular immune responses have been explored widely in preclinical settings (82). In addition, recent progress in nanotechnology has generated a variety of nanoparticles as combined diagnostic and therapeutic agents (theranostics) with potential applications in cancer immunotherapy.

**PET/CT imaging**—Initial efforts have focused on radiolabeling of antibodies against immune checkpoints such as PD-1 and PD-L1 (83,84). Natarajan et al developed zirconium 89 (\(^{89}\text{Zr}\)) copper 64 (\(^{64}\text{Cu}\))-labeled anti-PD-1 human antibodies and demonstrated in vivo imaging of TILs expressing PD-1 within tumors and lymphoid tissues in mouse xenografts (85). Hettrich et al developed \(^{64}\text{Cu}\)-conjugated anti-PD-1 and anti-PD-L1 murine antibodies with 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) tracers for high-resolution PET imaging of both PD-1 and PD-L1 (15). Li et al developed an anti-PD-L1 domain antibody (KN035) radiolabeled with \(^{89}\text{Zr}\) (\(^{89}\text{Zr}\)-Desferrioxamine) (86). KN035 was conjugated with p-isothiocyanatobenzyl-desferrioxamine B (DFO-Bz-NCs), followed by being labeled with \(^{89}\text{Zr}\) (Fig 4, A). Immuno-PET studies were performed to monitor PD-L1 levels in nude mice bearing LN229 xenografts with positive expression for PD-L1. \(^{89}\text{Zr}\)-Desferrioxamine produced high tumor uptake with favorable tumor-to-background ratios at 24 hours after injection with strong uptake persisting for up to 120 hours (Fig 4, B). Recently, Bensch et al presented initial results from a first-in-human study to assess feasibility of imaging with \(^{89}\text{Zr}\)-labeled atezolizumab (anti-PD-L1) and to test its potential to predict response to PD-L1 blockade. PET imaging showed that the imaging signal corresponded to PD-L1 expression at sites of inflammation and in various normal lymphoid tissues. Furthermore, PET/CT imaging results demonstrated generally high but heterogeneous uptake in tumors, varying within and among lesions, patients, and tumor types. Intriguingly, tracer uptake appeared to be stronger in predicting response to atezolizumab treatment than immunohistochemistry or RNA sequencing–based predictive biomarkers, which encourages further development of molecular PET imaging for assessment of PD-L1 status and clinical response (87). Besides targeting PD-L1, Tavaré et al developed an \(^{89}\text{Zr}\)-Desferrioxamine–labeled anti-CD8 cis-diabody (\(^{89}\text{Zr}\)-MalDFO-169cDb) and demonstrated sensitive detection of tumor-infiltrating CD8 expression in mouse models of tumor immunotherapy (88). Similarly, \(^{64}\text{Cu}\)-169cDb was developed to visualize and quantify changes of tumor-infiltrating...
CD8⁺ T cells in response to immunotherapy (89). Although these radiolabeled antibodies have advantages in imaging, such as naturally high avidity and antigen specificity, several drawbacks have also been noted. These drawbacks include long circulation times, high background signal, and low specific uptake. Thus, visualization of target molecules was usually performed more than 24 hours after the injection of tracers (90). To this end, Maute et al developed a high-affinity competitive nonantibody antagonist of PD-L1 by engineering the PD-1 ectodomain. The antagonist was conjugated with ⁶⁸Cu-DOTA for optimized immunotherapy and immuno-PET imaging of PD-L1. The tracer demonstrated superior tumor penetration with high tumor uptake and favorable tumor-to-background ratios occurring only 1 hour after injection in tumor-bearing mice, and persistent strong uptake in PD-L1-positive tumors remained for 24 hours (91). In addition to targeting PD-L1, there are other molecules and cells of interest that could respond to immunotherapies. Larimer and colleagues focused on in vivo visualization of granzyme B, a serine protease released by CD8⁺ T cells and natural killer cells during the cellular immune response (92). ⁶⁸Ga-NOTA-GZP, as a granzyme B-specific PET imaging agent, was developed to detect release of granzyme B in mouse xenografts (92). High ⁶⁸Ga-NOTA-GZP uptake after 12 days of ICB therapy occurred within 1 hour after injection in responders with low uptake of the radiotracer in nonresponders. These promising results in preclinical models support further investigations of these agents for clinical translation.

**CT imaging**—Recent advances in nanotechnology have promoted development of various nanoparticle contrast agents for CT (97). Gold nanoparticles (AuNPs) serving as CT contrast agents have been intensively designed and fabricated owing to favorable properties of x-ray attenuation, tunable surface chemical modifications, easy control of the size, and biocompatibility (98). Meir et al fabricated AuNPs with a PD-L1 antibody (aPD-L1) to achieve fast image-guided prediction of therapeutic response to ICB in mouse xenografts (Fig 5, A). Although the dosage of conjugated aPD-L1 was equivalent to only a fifth of standard dose, aPD-L1-GNPs achieved a similar therapeutic effect. The gold nanoparticle core enabled noninvasive, longitudinal tracking of PD-L1 by CT in vivo. CT findings revealed maximum accumulation of nanoparticles within a mouse tumor 24 hours after injection with aPD-L1-GNPs. The quantitative CT signal 48 hours after injection relat-

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**Figure 4:** A. Schematic illustration shows the synthesis of ⁶⁸Zr-Df-KN035. B. Whole-body PET images of BALB/c nude mice bearing LN229 xenografts (arrows) after injection of ⁶⁸Zr-Df-KN035. Blocking was performed by injection of unlabeled KN035. (Reprinted, with permission, from reference 86.)
ed linearly with inhibition of tumor growth, which enabled fast image-guided stratification between responders or nonresponders (Fig 5, B,C). In addition to ICB, AuNPs were also used to evaluate dendritic cell or T cell–based immunotherapy (100). These promising results in preclinical models support further investigations of nanoparticle-based nanomedicines in predicting and evaluating response to cancer immunotherapy.

Conclusions and Outlooks

In conclusion, prediction and evaluation of responses to immunotherapy remains challenging. Atypical responses and the possible occurrence of irAEs should be considered. Current response evaluation criteria based on tumor size remain limited in predicting and evaluating response to cancer immunotherapy. Particularly, two key issues are limited success rates and serious toxicity of ICB, which make it quite necessary to establish imaging biomarkers for treatment outcome. In this review, we highlight exciting and innovative radiomics and molecular imaging methods as potential methods to predict response to ICB treatment and assess early response to immunotherapy. These techniques and developments show tremendous promise in the field of immunotherapy. For radiomics, quantitative image features, in conjunction with other genomic and clinical information, can be correlated with outcome data to generate algorithms to support clinical decision-making. Radiomics offers an almost limitless array of prospective imaging biomarkers that may contribute to cancer detection, diagnosis, assessment of prognosis, prediction of response to treatment, and monitoring of disease status. The combination of this subset of radiomic features with genomic data, known as radiogenomics, may prospectively increase diagnostic, prognostic, and predictive power. Because many tumors have indistinct borders, segmentation is the most critical, challenging, and contentious component of radiomics. Recent advances in AI methodologies have made great strides in automatically quantifying radiologic patterns in medical imaging data. Deep learning, a subset of AI, is an especially promising method that automatically learns feature representations from sample images based on three-dimensional convolutional neural networks. Compared with conventional radiomic methods, deep learning–based radiomics do not require precise annotation of tumor boundaries and can learn features automatically from image data (101). Therefore, deep learning–based radiomics have great potential in cancer diagnosis, monitoring, and prediction response to treatment. Molecular imaging methods may enable noninvasive detection of multicellular components of tumor microenvironments to better predict response to immunotherapy and monitor changes in tumor composition during therapy. These methods require further development and validation in clinical settings. Furthermore, as investigators identify additional molecules regulating immune checkpoints, developing approaches for multiparametric imaging of immune environments likely will help establish better predictive biomarkers.

Figure 5:  A, Nanomedicine predicts immune response via imaging.  B, Representative three-dimensional (top panels) and two-dimensional (bottom panels) CT images of mice with high, medium, or low CT signals.  C, Box-and-whisker plot of CT signal enables prediction of the signal intensity at 48 hours. (Reprinted, with permission, from reference 99.)
Assessing additional molecules regulating immune checkpoints may improve accuracy of imaging methods for outcomes in immunotherapy.

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