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Critical Review

Role of noninvasive molecular imaging in determining response

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Abstract

The intersection of immunotherapy and radiation oncology is a rapidly evolving area of preclinical and clinical investigation. The strategy of combining radiation and immunotherapy to enhance local and systemic antitumor immune responses is intriguing yet largely unproven in the clinical setting because the mechanisms of synergy and the determinants of therapeutic response remain undefined. In recent years, several noninvasive molecular imaging approaches have emerged as a platform to interrogate the tumor immune microenvironment. These tools have the potential to serve as robust biomarkers for cancer immunotherapy and may hold several advantages over conventional anatomic imaging modalities and contemporary invasive tissue acquisition techniques. Given the key and expanding role of precision imaging in radiation oncology for patient selection, target delineation, image guided treatment delivery, and response assessment, noninvasive molecular-specific imaging may be uniquely suited to evaluate radiation/immunotherapy combinations. Herein, we describe several experimental imaging-based strategies that are currently being explored to characterize in vivo immune responses, and we review a growing body of preclinical data and nascent clinical experience with immuno-positron emission tomography molecular imaging as a putative biomarker for cancer immunotherapy. Finally, we discuss practical considerations for clinical translation to implement noninvasive molecular imaging of immune checkpoint molecules, immune cells, or associated elements of the antitumor immune response with a specific emphasis on its potential application at the interface of radiation oncology and immuno-oncology.

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Introduction

The general premise of cancer immunotherapy is to induce, augment, or reinvigorate the host's antitumor immune response. The disruption of immune-inhibitory ligand-receptor interactions with immune checkpoint blockade (ICB) has been a transformative advancement in cancer immunotherapy.[1] In particular, the blockade of the programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) axis, which is an important mechanism of immune evasion within the tumor microenvironment (TME), has resulted in impressive clinical responses across a spectrum of malignancies. Despite this undeniable progress, the reality remains that only subsets of patients benefit from anti-PD-1/anti-PD-L1 ICB.

To address this issue, there is a pressing need to identify biomarkers that may guide patient selection and assist the evaluation of treatment response.[2] This has proven to be a challenging task given the complexity and dynamic nature of antitumor immune responses. Monotherapy with ICB is increasingly unlikely to be sufficient treatment for most patients, and combination approaches that involve immunotherapy with standard-of-care therapies (eg, radiation therapy, surgery, chemotherapy, and targeted therapy) or other immuno-oncology agents (eg, dual ICB, cancer vaccines, adoptive cellular therapy, T-cell agonists, and immunocytokines) are a future direction to improve clinical outcomes.[3] Radiation therapy has emerged as an appealing partner for cancer immunotherapy given its dual cytotoxic and immunomodulatory properties. However, much work is needed to optimize potential synergies, and additional validation of this approach in humans is desperately needed.[4–6]

Noninvasive molecular imaging has become an integral component of cancer diagnosis and management. Molecular imaging can provide additional tumor- and immune-specific information beyond conventional x-ray computed tomography (CT) or magnetic resonance anatomic imaging. Because of the capacity of molecular imaging to noninvasively assay in vivo biological processes with high resolution at a whole-body level, there is considerable interest to develop this technology to guide treatment in multiple oncologic indications.[7] Immune positron emission tomography (immuno-PET) describes a facet of molecular imaging in which radio-labeled monoclonal antibodies, engineered antibody fragments, or peptides permit the direct imaging of molecularly specific targets. An advantage of PET is its ability to quantitatively measure and monitor molecular expression. Together, these features hold tremendous promise as a platform for biomarker-driven ICB and other cancer immunotherapies.

Given the inextricable link between imaging and radiation oncology, there is a unique opportunity to explore immuno-PET and other molecular imaging tools to evaluate radiation/immunotherapy combinations. These emerging methods in PET may improve patient selection and target delineation and ultimately may become a useful tool for adaptive radiation planning as we collectively strive toward personalized medicine in radiation oncology.[8,9]

Biomarkers and cancer immunotherapy

At present, there is a paucity of validated biomarkers for cancer immunotherapy. Several candidates, including intratumoral cluster of differentiation (CD) 8+ T-cell density/infiltration,[10] tumor mutational burden,[11,12] T-cell-inflamed/interferon (IFN)-ɣ-related gene signature,[13] and intratumoral PD-L1 expression may help predict response to ICB, although each metric is imperfect. Ultimately, a single measure may fail to capture the tumor immune landscape.

Among these putative markers, assaying PD-L1 protein expression within the TME by immunohistochemistry (IHC) has received the most attention.[14] Unfortunately, baseline PD-L1 expression has not consistently predicted clinical response to PD-1/PD-L1 blockade in prospective studies of several cancer types. Increased PD-L1 expression levels have generally correlated positively with improved clinical outcomes, but other studies have failed to support this association.[15,16] As clinical responses are observed among PD-L1-negative patients and within PD-L1 unselected cohorts, the use of PD-L1 IHC as a patient selection criterion could exclude patients who may benefit from ICB and highlights the need for better tools to characterize the immune TME.

There are both technical and biological considerations for these discrepancies in clinical reports. From a technical standpoint, differences in tissue preparation, assays and detection antibodies, variable grading schemas, and arbitrary cutoffs to define PD-L1 positivity, each limit comparison and interpretation across studies and between different anti-PD-1/PD-L1 pharmaceuticals.[17] One relevant issue that remains unresolved is the relative importance of PD-L1 expression on tumor cells, tumor-infiltrating immune cells, or both.

Tumor cell expression is used by several assays to determine PD-L1 status; however, studies in refractory urothelial carcinoma have associated increased levels of PD-L1 expression on immune cells with an increased response to atezolizumab (anti-PD-L1).[18] This conundrum of expression by cell type will likely extend to radiation/immunotherapy combinations because radiation modulates PD-L1 expression within the TME.[19] Indeed, various preclinical models have attributed synergy with anti-PD-L1 therapies with either reversal of resistance that is mediated by PD-L1-expressing tumor cells,[20,21] or the

depletion of tumor-infiltrating PD-L1-positive immune cells[22,23] (myeloid-derived suppressor cells).

Along these lines, immune checkpoint expression is highly dynamic and context-dependent. This presents an obstacle for tissue-based approaches, which can only provide a static snapshot of the immune TME at the time of biopsy or surgery and cannot monitor response to therapy over time without additional invasive procedures. PD-L1 expression can arise in response to an active antitumor response as an adaptive immune resistance mechanism[24] and is also modulated/induced by various therapies (including radiation therapy); therefore, to surmise that PD-L1 expression at the time of tissue acquisition also reflects the current state of the TME and can be extrapolated to predict response may be an oversimplification.

Furthermore, significant spatiotemporal variation and discordance of immune checkpoint expression may exist between the primary tumor and metastatic foci or between metastatic lesions at multiple body sites/organs. To date, studies have demonstrated that clinically meaningful heterogeneity in PD-L1 expression can exist even within the same tumor.[25] This has clear implications for targeted biopsies because sampling error (or undersampling) as well as inter- and intratumor heterogeneity may yield dramatically different results and could influence treatment decisions if patients are selected based on PD-L1 IHC.

An ideal biomarker for cancer immunotherapy should be able to assess dynamic changes over time and provide whole-body real-time information. Noninvasive imaging could address many of the aforementioned limitations with contemporary approaches, and either circumvents the need for invasive tissue acquisition or augments the clinical value of invasive tissue acquisition. Further, serial imaging to assess modulation of a target of interest within the TME at baseline and in response to therapeutic intervention may provide insight into potential mechanisms of synergy. Although much of this discussion has been dedicated to PD-L1 IHC-based assays, many of these principles and considerations apply to other putative immunotherapy biomarkers that rely on tissue-based techniques and provide a rationale to explore immuno-PET in cancer immunotherapy.

Radiologic evaluation and imaging-based response criteria with immunotherapy

Conventional anatomic imaging is a reliable and convenient approach to evaluate treatment response. However, the traditional assessment criteria were designed for and validated in patients treated with cytotoxic systemic therapies. Given the unique mechanisms of action and distinct patterns of response that are associated with immunotherapeutic intervention, a need to refine existing methodologies and develop new metrics that more accurately capture clinically relevant responses is widely recognized.[26]

The Response Evaluation Criteria in Solid Tumors (RECIST) that were initially developed in 2000 and revised in 2009 (RECIST v1.1) remain the gold standard for response evaluation in clinical trials. Since then, our experience with ICB has matured, and RECIST is appreciated to possibly underestimate the clinical benefit that is associated with immunotherapy. This can lead to a misinterpretation of treatment-related radiological changes as progressive disease, which in turn leads to the premature discontinuation of effective therapy. Certain patterns, such as transient enlargement followed by stabilization/shrinkage (pseudoprogression) or the appearance of new lesions before subsequent response, are representative of delayed response kinetics that can be characteristic of immune-modulating agents. These patterns of response are at risk for misclassification by RECIST.[26]

The clinical benefit of treatment beyond RECIST-defined progression has been reported in patients with metastatic renal cell carcinoma[27,28] and melanoma[29] who are treated with anti-PD-1 ICB. Of note, pseudoprogression remains a relatively rare phenomenon, and an enlarging target lesion is much more likely to represent true clinical progression. Pseudoprogression is hypothesized to represent paradoxical tumor enlargement as a consequence of immune-infiltration; thus, noninvasive molecular imaging that can monitor T-cell trafficking might help distinguish this entity from clinical progression.

The immune-related response criteria (irRC)[30] were an important first step in the standardization of treatment response evaluation for patients treated with immunotherapy in an effort to facilitate therapeutic decision making. These criteria incorporate alternative methods to calculate tumor burden and, importantly, reclassify development of new or enlarging lesions so that they do not automatically constitute progressive disease. An eloquent comparison of the irRC and RECIST v1.1 in a cohort of patients with melanoma who were treated with pembrolizumab demonstrated that the conventional RECIST underestimate the benefit of immunotherapy in approximately 15% of patients. A subset of patients with discordant radiologic outcome (RECIST-defined progression without irRC-defined progressive disease) experienced improved overall survival rates compared with their counterparts with progressive disease with both metrics.[31]

The development of criteria for immunotherapy-treatment evaluation has been an iterative process because the immune-response RECIST, immune RECIST, and most recently the immune-modified RECIST have each attempted to further refine the guidelines to assess the clinical benefit of

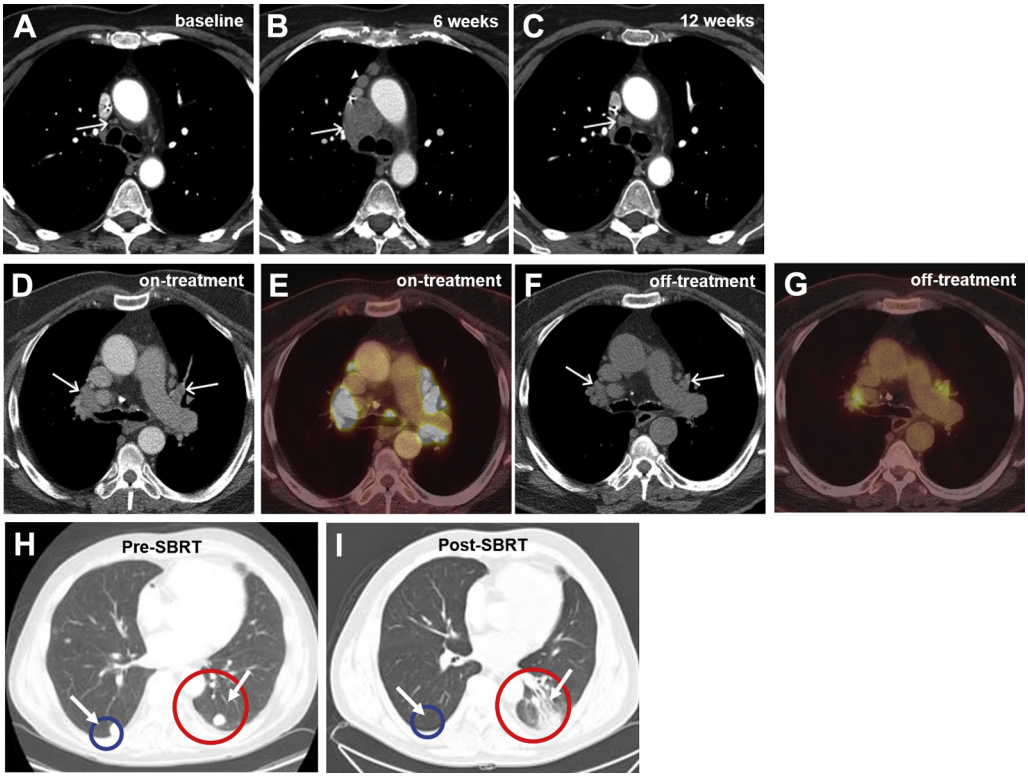

Figure 1 Imaging changes in response to immunotherapy. (A-C) Axial contrast-enhanced computed tomography (CT) scan demonstrating pseudoprogression in a patient treated with immunotherapy for advanced lung cancer. Marked interval enlargement of right paratracheal lymph node (arrow) and development of new prevascular mediastinal adenopathy (arrow head) 6 weeks after treatment compared with baseline (A-B). Follow-up CT scan 6 weeks later (12 weeks; C) demonstrates interval decrease in right paratracheal node and disappearance of other nodes. (D-G) CT and fused [^{18}F]FDG positron emission tomography/CT scan of metastatic melanoma patient on-treatment with immunotherapy with development of new FDG-avid mediastinal/hilar adenopathy (arrows, D-E). Discontinuation of therapy because of suspected immune-related adverse event. Repeat imaging 6 weeks after treatment termination (F-G) demonstrated substantial decrease in size and avidity of nodes. Biopsy test results demonstrated sarcoid-like reaction and no evidence of malignancy. (H-I) Pre- and posttreatment nonenhanced axial CT images in patient with metastatic renal cell carcinoma treated with stereotactic body radiation therapy to posterior left lung lesion, followed by high-dose interleukin-2 with development of fibrotic changes (red) in site of stereotactic body radiation therapy and resolution of nonirradiated nodule (blue) in contralateral lung. Treatment-related radiographic changes complicate assessment of local control.[26,96] Images adapted with permission for reuse ©2018 American Cancer Society and ©2012 American Association for the Advancement of Science.

immunotherapy.[32–35] Continued experience will likely further refine image-based approaches to monitor ICB therapeutic outcomes.

Recent research has been focused on extracting additional radiologic information beyond uni- and bidimensional measurements to better describe the host-tumor immune contexture and infer clinical outcomes. An intriguing report by Tang et al. has provided early evidence that pretreatment quantitative imaging metrics may be used as a surrogate for immune pathology-based approaches.[36] Using lung cancer surgical pathology specimens, patients were stratified by tumor PD-L1 expression and CD3+ T-cell density into 4 subgroups, and pretreatment CT radiologic features were associated with these immune TME parameters to develop an immune-pathology informed radiomics model.

This novel radiomics model was successfully able to discern subsets of patients with distinct survival outcomes and immune-pathology features, which alludes to the potential for this technology to be used as a prognostic or predictive tool for patients treated with immunotherapy. Similar efforts using magnetic resonance imaging (MRI) have been pursued to identify specific immune populations within the TME and might have future applications for patient stratification and selection for immunotherapy.[37] A small prospective study of ferumoxytol-enhanced MRI in patients with lymphoma or bone sarcoma demonstrated that tumor enhancement on T_2-weighted sequences was significantly associated with the density of CD68+/163+ tumor-associated macrophages within the TME, which is an immune subset that is generally correlated with adverse outcomes and immunosuppression.[38]

Functional imaging using positron emission tomography with 2-deoxy-2-[fluorine-18]-2-fluoro-2-deoxy-D-glucose integrated with computed tomography, or [^{18}F]FDG-PET/CT, which measures glucose metabolism as a surrogate for tumor activity, has modernized staging, treatment decision making, and response evaluation in lymphoma and other malignancies.[39,40] This technique has also demonstrated promise as a tool to predict response to immune-based treatments. Interestingly, in a cohort of 20 patients with melanoma who were treated with ICB, Cho et al. reported that an increase in [^{18}F]FDG uptake 3 to 4 weeks after initiation of therapy was associated with favorable clinical outcomes at 4 months, which prompted the authors to propose PET-based criteria for the early prediction of eventual response (PET/CT criteria for Early Prediction of Response to Immune Checkpoint Inhibitor Therapy).[39–41]

Ultimately, [^{18}F]FDG-PET/CT is not an optimal molecular imaging tool for immunotherapy because of its lack of specificity at multiple levels. Increased glucose utilization may be a function of tumor, stromal, or immune cell processes and uptake often cannot be differentiated from nonspecific inflammation or other nonmalignant inflammatory conditions (see Fig 1). However, other groups have argued a potential role for earlier detection of immune-related adverse events that could prevent avoidable treatment-related toxicity.[26,42,43] Accordingly, novel molecular imaging strategies that are more readily able to directly and indirectly assay the tumor-immune interaction are being developed.[44]

Noninvasive molecular imaging of immunotherapy

Immuno-PET uses radio-labeled antibodies or other targeting scaffolds such as engineered proteins and peptides that bind cell-surface molecular targets with molecular specificity. This provides high-resolution, quantitative, in vivo molecular imaging information to annotate target expression patterns and whole-body biodistribution. In the context of cancer immunotherapy, preclinical models of immuno-PET have predominantly focused on the expression of immunologically relevant targets within the TME (ie, immune checkpoints and effector molecules) or the detection and tracking of immune cell populations (ie, T-cells subsets and chimeric antigen receptor T-cells).

Imaging of PD-L1 has understandably attracted the most attention given the clinical emergence of several therapeutic antibodies that target the PD-1/PD-L1 axis and the evolving importance of PD-L1 expression as a putative biomarker of response to ICB. Encouragingly, preclinical models have generally shown good concordance between IHC- and imaging-based PD-L1 expression. Indeed, the ability to noninvasively detect PD-L1 could transform patient selection and treatment monitoring for ICB. Further, an assessment of dynamic changes in expression with serial imaging and the added spatiotemporal information can help clinicians make real-time decisions to guide therapy and provide a novel opportunity to more comprehensively evaluate PD-L1 as a biomarker.[45]

An appreciation for the radioisotope, scaffold type, and chelator and their ability to each alter the radiopharmaceutical properties, and thus the imaging result, is critical. Ultimately, the impact of targeting vector molecular weight, clearance characteristics, and binding affinity on in vivo pharmacokinetics and the complex's radiochemistry must be optimized with consideration for the scientific question at hand and the underlying immunobiology.[46] Radioisotope half-life dictates the temporal window for imaging whereas radiometals display different organ-specific tropism and patterns of normal tissue biodistribution. To date, Zirconium-89 (^{89}Zr; $t_{1/2}$ = 78.4 hr) and Copper-64 (^{64}Cu; $t_{1/2}$ = 12.7 hr) have been the most extensively studied radiometals used for full-IgG antibody-based imaging because the longer half-lives approach the biological half-life of these proteins in circulation. Likewise, Fluorine-18 ($t_{1/2}$ = 110 min) has been the radiolabel of choice for lower molecular weight (and therefore faster clearing) tracers. We summarize ongoing clinical molecular imaging/immuno-PET efforts in Table 1.[46]

Radiometal labeling of antibodies uses established chemical approaches to append isotope binding ligands (chelates) to the protein. These methods enable only minimal modification of the antibody, including approved ICB pharmaceutical formulations to be converted into immuno-PET tools for in vivo testing. These efforts have also extended to other immune checkpoints as ^{64}Cu or ^{89}Zr antihuman PD-1 (pembrolizumab, nivolumab) immuno-PET radiotracers have detected PD-1 expression on tumor-infiltrating lymphocytes (TIL) in humanized mouse models[47–49] and nonhuman primate studies.[50]

PD-L1 expression has also been successfully assayed with ^{89}Zr-C4 (IgG_1 targeting the extracellular epitope of human/mouse PD-L1) immuno-PET imaging in patient-derived xenograft models.[51] The first in-human PD-L1 immuno-PET experience was reported at American Association for Cancer Research 2017 Annual Meeting.[52] Sixteen patients underwent pretreatment ^{89}Zr-atezolizumab immuno-PET imaging to evaluate normal organ radiotracer uptake and correlate with baseline PD-L1 expression by IHC. The preliminary results demonstrated heterogeneous tumor uptake even among tumors with minimal PD-L1 expression by IHC as well as high background uptake in lymphoid tissues. Although these nascent clinical findings demonstrate clinical feasibility, they also indicate that substantial improvements are needed before PD-L1 immuno-PET is ready for routine clinical use.

Radiolabeled antibodies have been the most common scaffolds for PD-L1 immuno-PET studies,[53–55] but there

Table 1 Select clinical trials evaluating immuno-PET imaging for cancer immunotherapy

ClinicalTrials.gov identification number	Trial	Patient population phase/design	Enrollment status/ anticipated completion
NCT03520634 (opened May 2017)	PD-L1 PET imaging in patients with inoperable melanoma with brain metastases and eligible for treatment with nivolumab	• Metastatic melanoma with ≥1 brain lesion • Phase 1/2 feasibility study to determine optimal dose/timing of [^{18}F]PD-L1 immuno-PET and biopsy of accessible lesion to correlate PD-L1 expression by IHC after each scan.	• Recruiting (n = 15) • Estimated completion 10/2018
NCT03514719 (opened May 2018)	PD-L1 imaging in non-small cell lung cancer (PINNACLE)	• Metastatic NSCLC • Phase 1/2 study of [^{89}Zr]avelumab (anti-PD-L1) immuno-PET to assess tumor and systemic uptake of [^{89}Zr]avelumab and potential to predict avelumab treatment response	• Recruiting (n = 37) • Estimated completion 08/2021
NCT02453984 (opened Feb 2016)	Immuno-PET imaging with [^{89}Zr] MPDL3280A in Patients with locally advanced or metastatic NSCLC, bladder or TNBC before MPDL3280A	• Metastatic NSCLC, bladder cancer or TNBC • Phase 1 to describe pharmacokinetics by measuring SUV on [^{89}Zr]MPDL3280A-PET scans • Tumor and immune cell PD-L1 expression correlated to [^{89}Zr]MPDL3280A tumor uptake	• Recruiting (n = 30) • Estimated completion 09/2019
NCT03007719 (opened Mar 2017)	Functional imaging of T-cell activation with [^{18}F] AraG in urothelial carcinoma patients receiving neoadjuvant or SOC anti-PD-1/PD-L1	• Urothelial carcinoma eligible for NCT02451423 or planned anti-PD-1/PD-L1 per SOC • Phase 2 to assess changes in [^{18}F]AraG uptake in primary and metastatic tumors in patients treated with neoadjuvant atezolizumab or SOC anti-PD-1/PD-L1. • Secondary objective to correlate [^{18}F]AraG uptake with pathologic response in primary tumor.	• Not yet recruiting (n = 31) • Estimated completion 12/2018
NCT03065764 (opened Jan 2017)	89-Zr-pembrolizumab immuno-PET in patients with NSCLC	• Metastatic NSCLC refractory to platinum • Phase 2 study of 2 immuno-PET scan with nontherapeutic tracer dose (2 mg) of [^{89}Zr] pembrolizumab−scan ± cold therapeutic dose of pembrolizumab • Timing 1, 72, and 120 hours after tracer injection. • Safety assessment, describe SUV on [^{89}Zr] pembrolizuamb scan, characterize intra- and interpatient heterogeneity in uptake, grade uptake as positive/negative.	• Active, not recruiting (n = 10) • Estimated completion 12/2019
NCT02760225 (opened Sep 2016)	[^{89}Zr]pembrolizumab-PET imaging in patients with locally advanced/ metastatic melanoma or NSCLC	• Locally advanced/ metastatic NSCLC or melanoma • Description of whole body [^{89}Zr] pembrolizumab by measuring SUV on immuno-PET scan to determine optimal tracer dose and interval between tracer injection and scanning.	• Recruiting (n = 21) • Estimated completion 06/2018
NCT02591654 (opened Oct 2015)	Feasibility study of MRI and PET imaging to assess response to pembrolizumab in metastatic melanoma	• Metastatic melanoma • Early phase 1 using whole-body diffusion-weighted MRI and [^{18F}F]FLT PET to evaluate disease burden in patients treated with pembrolizumab • FLT-PET may be used as an early progressive disease biomarker given close association between FLT uptake and proliferative index	• Recruiting (n = 20) • Estimated completion 11/2024

(*continued on next page*)

Table 1 (*continued*)

ClinicalTrials.gov identification number	Trial	Patient population phase/design	Enrollment status/ anticipated completion
NCT03089606 (opened Jun 2017)	Pembrolizumab in treatment-naïve melanoma and use of [^{11}C] AMT PET as baseline imaging biomarker	• PD-1 inhibitor naïve unresectable/metastatic melanoma • Phase 2 study to associate 11-C-AMT PET at baseline (SUV_{max}) with objective response rate at 12 weeks to pembrolizumab • AMT = 1-methyl-D-tryptophan−exploratory endpoint to correlate [^{11}C]AMT PET with IDO pathway by IHC (IDO, tryptophan, CD4/CD8 T-cells, Treg, MDSC, PD-L1, LAG-3/TIM-3/ GITR)	• Recruiting (n = 25) • Estimated completion 06/2022
NCT02478099 (opened Feb 2016)	Evaluation of efficacy of MPDL3280A after investigation imaging as measure by objective response rate	• Locally advanced/metastatic NSCLC, TNBC, or urinary tract cancers • Phase 2 trial of therapeutic MPDL3280A (atezolizumab) with incorporation of 3 investigational imaging trials to assess baseline activation status of immune system − [^{89}Zr] MPDL3280A PET, [^{89}Zr]CD8 PET or [^{18}F]FB-IL2 PET	• Recruiting (n = 79) • Estimated completion 12/2020
NCT02922283 (opened Oct 2016)	[^{18}F]FB-IL2 PET imaging of T-cell response as biomarker to guide treatment decisions in metastatic melanoma	• Metastatic melanoma • [^{18}F]FB-IL2 PET scan at baseline and week 6 after treatment with ipilimumab, nivolumab, pembrolizumab or ipilimumab/nivolumab • Comparison of baseline and 6-week [^{18}F]FB-IL2 immuno-PET to evaluate if treatment-induced immune response is detected and correlates with clinical outcome. • [^{18}F]FB-IL2 tumor uptake correlation with IL2R-positive immune cells by IHC	• Recruiting (n = 30) • Estimated completion 08/2018
NCT03313323 (opened Feb 2017)	Uptake and biodistribution of ^{89}Zr-labeled ipilimumab in ipilimumab-treated metastatic melanoma patients	• Metastatic melanoma • Phase 2 trial of ^{89}Zr-labeled ipilimumab infused within 2 hours of 1st and 2nd therapeutic ipilimumab doses • Visually and quantitative asses [^{89}Zr]-ipilimumab immuno-PET uptake in tumor lesions and biodistribution at 2 timepoints (baseline, 3-weeks). Secondary objective to correlate tumor uptake with response, organ uptake with toxicity.	• Recruiting (n = 29) • Estimated completion 02/2020
NCT03107663 (opened Jun 2017)	[^{89}Zr]-Dfd-IAB22M2C PET/CT in patients with selected solid malignancies or Hodgkin lymphoma	• Selected solid tumors (NSCLC, small cell lung cancer, head and neck squamous cell cancer, TNBC, Merkel cell carcinoma, renal cell, bladder, hepatocellular, gastroesophageal cancer) and Hodgkin lymphoma • Phase 1 dose-escalation study to evaluate safety, tolerability, optimal time window/dose for imaging and dosimetry • Ability of [^{89}Zr]-Dfd-IAB22M2C to detect CD8+ T-cells − inert antibody fragment against human CD8-antigen (mini-body)	• Recruiting (n = 24) • Estimated completion 09/2018
NCT01082926 (opened May 2010)	Cellular immunotherapy for recurrent/refractory malignant glioma using intratumoral infusion of GRm13Z40-2 in combination with IL-2	• Recurrent high-grade glioma • Phase 1 to assess safety of GRm13Z40-2 CTL locoregional cellular immunotherapy • Allogeneic CD8+ T-cell line genetically modified to express IL-13-Zetakine and HyTK in combination with IL-2.	• Completed (n = 6) • Estimated completion 09/2013

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Table 1 (*continued*)

ClinicalTrials.gov identification number	Trial	Patient population phase/design	Enrollment status/ anticipated completion
		• Ability of [^{18}F]FHBG PET to image Grm13Z40-2 cytotoxic T-cells.	
NCT03029871 (opened Jan 2017)	Oncolytic adenovirus-mediated gene therapy for lung cancer (NSCLC)	• Inoperable stage I lung cancer (1-6cm) • Phase 1 study to determine MTD of Ad5-yCD/ mutTKSR39rep-ADP adenovirus • Intratumoral injection of virus. Prodrug and SBRT (48Gy / 4Fx) 48hrs after virus administration • [^{18}F]FHBG PET will be administered to quantify HSV-1 TK gene expression at baseline and postadministration	• Recruiting (n = 9) • Estimated completion 12/2022
NCT032813382 (opened Jul 2017)	Oncolytic adenovirus-mediated and IL-12 gene therapy in combination with chemotherapy for metastatic pancreatic cancer	• Metastatic pancreatic cancer • Phase 1 study to determine toxicity of combining IL-12 gene therapy with chemotherapy • Single intratumoral injection of oncolytic Ad5-yCD/ mutTKSR39rep-hIL12 adenovirus followed by prodrug • Optional [^{18}F]FHBG PET imaging to quantify intensity, persistence and biodistribution of HSV-1 TK gene expression in the pancreas	• Recruiting (n = 9) • Estimated completion 06/2021

^{11}C, Carbon-11; [^{18}F]FDG-PET, 2-deoxy-2-[fluoro-D-glucose [^{18}F]; ^{89}Zr, Zirconium-89; AMT, 1-methyl-D-tryptophan; CD, cluster of differentiation; FLT, fluorothymidine; IDO, indolamine 2,3-dioxygenase; IHC, immunohistochemistry; IL, interleukin; MRI, magnetic resonance imaging; MTD, maximum-tolerated dose; NSCLC, non-small cell lung cancer; PET, positron emission tomography; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; SBRT, stereotactic body radiation therapy; SOC, standard of care; SUV, standardized uptake value; TNBC, triple-negative breast cancer.

are also drawbacks to using full antibody constructs including long blood circulation time, slow clearance, and high background signal. As key immunological effector molecules themselves, these IgG tracers have inherent Fc region-dependent interactions with FcγR-expressing immune cells within the TME.[23,45,46] As an alternative, engineered antibody fragments including single-chain variable fragments (scFv; minibodies) or scFv dimers (diabodies) and high-affinity low-molecular weight peptides have been evaluated as potential immuno-PET radiotracers. The rapid clearance kinetics of non–IgG-based agents enhance their capacity to integrate into clinical workflow, can reduce the absorbed dose of ionizing radiation, and may provide a means to serially monitor immune targets of interest with greater frequency.

To that end, Chatterjee et al. have reported that ^{64}Cu-WL12, which is a high-specificity human PD-L1 binding peptide, rapidly detects PD-L1-expressing cells within 60 minutes of administration with favorable intratumoral accumulation and low background uptake.[56] Similarly, Mayer et al. introduced a small, engineered protein binder that is derived from the ectodomain of PD-1 with high-affinity for human PD-L1.[46] The small, engineered protein binder scaffold for PD-L1 immuno-PET exhibited rapid clearance and high specific uptake.

Immuno-PET for cancer immunotherapy has not been limited to the assessment of the PD-1/PD-L1 axis. The concept of using reporter gene transduced or directly labeled lymphocyte populations for in vivo imaging with PET is several decades old.[57–59] Preclinical immuno-PET studies have largely focused on the detection and monitoring of specific T-cell subsets. This has been accomplished by the specific targeting of cell-surface CD molecule expression (CD3, CD8), or the detection of molecules that represent an effector/activated T-cell phenotype (interleukin [IL] 2, granzyme B; see Table 2).

Preclinical models of ^{89}Zr-DFO-anti-CD3 immuno-PET have demonstrated the ability to visualize homeostatic T-cell populations in healthy mice as well as TIL in tumor-bearing counterparts.[60] These investigations demonstrated an 11.5-fold increase in tumor-to-blood signal relative to control and no change in the absolute CD3+ count, which allays concerns about potential lymphodepletion. However, DFO-anti-CD3 did skew the T-cell population toward a CD8+ T-cell central/effector memory phenotype with a relative decrease in CD4+ T-cells. Although not overtly deleterious to antitumor immunity, the impact of radiolabeling/chelation on the peripheral immunome should be further investigated. Molecular imaging that uses radiolabeled targeting molecules is generally assumed to operate

Table 2 Advantages and disadvantages of imaging biomarkers in cancer immunotherapy

	Examples	Pro	Con
Anatomic conventional imaging modalities	Magnetic resonance imaging and x-ray computed tomography.	Widely available; high resolution; standardized response criteria (ie, RECIST).	Lack of molecularly specific information; response readout of index lesions based upon anatomic changes may not reflect long-term response.
Approved molecular imaging modalities	[^{18}F]FDG positron emission tomography	Widely available; quantitative.	Uptake due to glycolytic activity due to cancer and microenvironmental cell sources.
Experimental radiolabeled ICB antibodies	[^{89}Zr]- or [^{64}Cu]-labeled anti-PD1; anti-PDL1; anti-CTLA4	Facile manufacturing; in vivo imaging performance can be validated by IHC.	Checkpoint expression level may not function as a correlate of response.
Experimental targeted imaging of immunomodulators	[^{68}Ga]-NOTA-GZP (granzyme B-targeted imaging); [18F]FB-IL2 (radiolabeled IL-2)	Functional measure of immune activity; improved imaging kinetics	Limited investigation at this time.
Reporter imaging	[^{18}F]FHBG and HSV1-sr39TK PET	High sensitivity; whole body; selected cell population can be imaged repeatedly.	Requires genetic manipulation of cells ex vivo to express reporter.

[^{18}F]FDG-PET, 2-deoxy-2-[fluoro-D-glucose [^{18}F]; ^{68}Ga, Gallium-68; ^{89}Zr, Zirconium-89; ICB, immune checkpoint blockade; IHC, immunohistochemistry; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; RECIST, Response Evaluation Criteria In Solid Tumors.

in the microdosing regime, which is a term used to refer to nonpharmacologic relevant mass doses of pharmaceuticals afforded by the pM sensitivity of PET imaging. This work demonstrates that, despite these assumptions, the careful validation of a given tracer is required when dealing with lymphocyte populations whose targeting may modulate status or lead to depletion.

Larimer et al. demonstrated that increased ^{89}Zr-DFO-anti-CD3 uptake correlated with improved tumor control in a CT26 colon model, which highlights a predictive role for anti-CD3 immuno-PET with anti-CTLA-4 ICB.[61] Tavaré et al. have extensively studied immuno-PET of endogenous CD8+ T-cells using a ^{64}Cu-radiolabeled minibody[62] as well as an ^{89}Zr-radiolabeled anti-CD8 cys-diabody.[63] Encouragingly, the monitoring of systemic and intratumoral CD8+ T-cell responses with immuno-PET appears feasible across various therapeutic models, including adoptive cellular therapy (ACT), T-cell agonistic antibodies, and anti-PD-L1 ICB. Camelid derived single-domain antibody fragments (so called nanobodies) are an emerging targeting vector class with several advantageous properties.[64] An ^{89}Zr-radiolabeled pegylated nanobody that specifically targets CD8 has shown similar capacity to detect the presence of CD8+ TIL. Interestingly, homogeneous intratumoral uptake, when compared with more heterogeneous patterns, correlated with improved response to anti-CTLA-4 therapy.[65]

Intratumoral Granzyme B expression indicates the presence of cytotoxic T-cells (or natural killer cells) within the TME and active tumoricidal effector function. To interrogate the presence of granzyme B, Larimer et al. hypothesized that a short-lived Gallium-68 (^{68}Ga; $t_{1/2}$ = 68 min) radiolabeled peptide-based (GZP) imaging-agent specific to this enzyme could function as a predictive biomarker for ICB.[66] A preclinical therapeutic model of dual ICB demonstrated that ^{68}Ga-NOTA-GZP immuno-PET on day 14 was able to differentiate responders and nonresponders based on Granzyme B expression patterns. The authors proposed that early quantitative imaging data may be used to guide treatment intensification for poor responders or discontinuation of ineffective therapy to mitigate unnecessary toxicity. OX40 has drawn attention as a potential biomarker for immunotherapy because OX40 expression is restricted to antigen-specific activated T-cells.[67]

Promising preclinical work from Alam et al. reported that ^{64}Cu-DOTA-AbOX40 immuno-PET can detect complex spatiotemporal trafficking patterns of activated T-cells after in situ vaccination with CpG oligodeoxynucleotides. Early (48 hours) OX40 radiotracer uptake was predominantly detected within the tumor and tumor-associated draining lymph nodes; however, OX40 expression patterns shifted to the spleen with persistent activity in the regional nodes at day 9.[67]

An understanding of the dynamics of T-cell activation and migration in relation to different immuno-oncology agents could help optimize combination approaches as timing and sequencing can dramatically influence synergy.[68,69] Nucleoside analogs, such as [^{18}F]-AraG, have also demonstrated specificity for activated T-cells in preclinical models[70,71] and is currently under clinical investigation (NCT03007719) as a noninvasive imaging

strategy to assay T-cell activation in patients undergoing anti-PD-1/PD-L1 ICB.

PET has a long-established role in the clinical evaluation of metabolism, most notably through the use of [^{18}F]FDG for glucose fixation. Immunometabolism is now increasingly appreciated as an important determinant of response to immunotherapy. The 1-methyl-D-tryptophan (AMT) radiolabel with short lived Carbon-11 (^{11}C; $t_{1/2}$ = 20 min) for ^{11}C-AMT PET is being explored as a surrogate for indolamine 2,3-dioxygenase (IDO) pathway activation (Table 1), which has been associated with immunosuppression and a potential immune evasion mechanism after radiation therapy.[72]

The unprecedented success of chimeric antigen receptor T-cells in several hematological malignancies has revolutionized ACT and ignited interest in this approach for solid tumors. Accordingly, there is an unmet need to noninvasively monitor the trafficking of tumor-specific T-cells.[73] An early proof-of-principle study demonstrated the ability of immuno-PET to detect antigen-specific antitumor T-cells after ACT.[57,74] A series of adoptive transfer experiments with either antigen-naïve or tumor-antigen experienced T-cells engineered to express the herpes simplex virus type 1 thymidine kinase (HSV1-sr39TK) PET reporter gene demonstrated that only antigen-experienced T-cells successfully localized intratumorally. This was denoted by increased tumor uptake of radiotracer [^{18}F]-FHBG, which is a high-affinity substrate for HSV-sr39TK, and the PET findings were corroborated by an increased CD3+ T-cell infiltrate observed by IHC.

Recently, this approach has been successfully translated for patients with recurrent glioma who undergo IL-13-targeted ACT.[75] In this study, CD8+ cytotoxic T-cells were engineered to express both HSV1-TK and IL-13-zetakine gene constructs to allow the IL-13-directed cytotoxic T-cells to be monitored with [^{18}F]-FHBG PET/MRI. Other PET-based approaches to monitor in vivo T-cell trafficking for ACT include the ex-vivo direct radiolabeling of antigen-specific T-cells before infusion.[76,77]

Integrating molecular imaging into radiation/immunotherapy combinations

An early indication of the promise of immuno-PET for radiation oncology is supported by preclinical studies of ^{89}Zr-radiolabeled anti-PD-L1 to evaluate radiation-induced PD-L1 upregulation in syngeneic HPV + HNSCC (MEER) and melanoma (B16F10) models.[78] In these experiments, pre- and postradiation therapy immuno-PET/CT demonstrated both a histology-dependent and radiation dose-dependent upregulation of PD-L1 expression after fractionated radiation therapy (2 Gy in 4 or 10 fractions). Multiparametric flow cytometry confirmed enhanced PD-L1 expression in the irradiated TME, which was mediated predominantly via infiltrating PD-L1-expressing CD11b+ myeloid cells. These findings suggest that this immune subset could be a target of interest for future molecular imaging endeavors and therapeutic combination studies.

Using a similar ^{89}Zr radiolabeled anti-CTLA-4 IgG2a immuno-PET approach, our group demonstrated that stereotactic radiation (12 Gy in 1 fraction) enhances the intratumoral accumulation of immunosuppressive CTLA-4-expressing regulatory T-cells (Tregs) and may be an important therapeutic target for radiation/immunotherapy combinations.[79,80] A recent proof-of-principle study lends further validation to immuno-PET for preclinical combination studies and has provided insight into mechanisms of tumor localization of activated T-cells after local tumor irradiation (14 Gy in 1 fraction).[81]

As T-cells express CD25 (IL-2Rα) upon activation, Hartimath et al. developed an [^{18}F]FB-IL-2 agent as a means to noninvasively detect and monitor in vivo tumor-infiltration of activated (CD25-expressing) T-cells. Mice bearing HPV-related TC-1 tumors underwent local radiation with or without subsequent vaccination with an E6/E7-encoding virus (SFVeE6,7). Intratumoral [^{18}F]FB-IL-2 uptake was significantly enhanced among irradiated mice compared with sham-irradiated controls, and the addition of immunization further increased radiotracer uptake. Interestingly, irradiated mice that were treated with a CXCR4 inhibitor had dramatically diminished intratumoral [^{18}F]FB-IL-2 activity, which suggests the reduced infiltration of activated T-cells and was corroborated by reduced CD8+ TIL on IHC.[81]

Ultimately, these preclinical immuno-PET studies provide a platform to improve our understanding of mechanisms of synergy between radiation therapy and various immunotherapeutic-based approaches to specifically evaluate dose-fractionation schedules, relative timing or sequencing, and dynamic spatiotemporal uptake patterns in response to combination therapy.

As evidence of the clinical benefit of metastasis-directed therapy in the oligometastatic state continues to mount,[82–85] the role and utilization of focal radiation therapy applied in the metastatic setting will become increasingly important. Consequently, there is a growing need for companion imaging tools to help assess both local and systemic responses to radiation therapy. This has received increased attention, given the advent of ICB and the potential for radiation therapy to potentiate a systemic antitumor immune response in addition to a local control benefit when combined with immunotherapy.[86]

A growing union of molecular imaging and radiation oncology is exemplified by the evolution of prostate-specific membrane antigen (PSMA)-based PET imaging in prostate cancer. Radiation target delineation that is informed by PSMA-PET imaging can plausibly be a near-term reality in the definitive, adjuvant/salvage, and

oligometastatic settings.[87–89] A similar molecularly oriented immuno-PET approach could be envisioned to help guide radiation target selection for radiation/immunotherapy combinations. Indeed, radiation therapy is postulated to be one of several therapeutics to introduce inflammation into the TME and theoretically convert an immunologically cold (non-T-cell infiltrated) tumor into a hot tumor that is characterized by a T-cell inflamed phenotype with a type I IFN signature and concordant chemokine expression.[90] The ability to noninvasively detect and target cold tumors with radiation therapy could help enhance immune recognition and boost antitumor immunity. Therefore, the development of noninvasive imaging strategies that target radiation-driven immunomodulation and immune-related molecular processes could offer substantial gains to our existing understanding of radio-immuno-oncology and ultimately alter therapeutic management.

Similar to immunotherapy, radiologic changes associated with radiation treatment effect can be challenging to interpret at times and pseudoprogression is a well-documented phenomenon, particularly after ablative radiation therapy regimens.[91–94] Functional imaging with [^{18}F]FDG PET for radiation/immunotherapy combinations has demonstrated some promise but remains nonspecific.[95]

A phase 1 trial of stereotactic body radiation therapy (SBRT; 16-20 Gy in 1-3 fractions) followed by high-dose IL-2 in a cohort of patients with renal cell carcinoma and melanoma demonstrated an impressive 66.6% response rate per RECIST (8 of 12 patients with partial or complete response; Fig 1).[96] Among the 8 responders, 7 partial responses were reported per the CT-based RECIST evaluation. However, 6 of 7 patients with a CT-defined partial response were considered to have complete responses per the [^{18}F]FDG PET evaluation. These findings highlight potentially clinically relevant differences between anatomic and functional imaging in the determination of response.

A report of an abscopal response by Golden et al. in a patient with metastatic non-small cell lung cancer who was treated with hypofractionated radiation therapy (6 Gy in 5 fractions) to a hepatic metastasis with concurrent or sequential ipilimumab demonstrated an increased [^{18}F]FDG uptake in an isolated nonirradiated supraclavicular node on a surveillance [^{18}F]FDG scan.[97] The residual tumor was also noted in this subsequently excised hypermetabolic node, but a comparison of this specimen with adjacent nodal tissue resected several years before radio-immunotherapy demonstrated the development of a robust lymphocytic infiltrate that was characterized by the presence of cytotoxic CD8+ T-cells and FoxP3 + Tregs with an enhanced CD8+/FoxP3+ ratio relative to the pretreatment specimen. As such, there are caveats in the interpretation of nonspecific functional imaging as increased avidity could represent progressive or recurrent tumor versus inflammation because of an evolving antitumor immune response or immune-related adverse event (Fig 1).

Significant strides have been made in preclinical models to decipher immunological mechanisms of synergy. The available prospective clinical data have provided some insight into potential immuno-PET molecular imaging targets via interrogation of the peripheral immunome and evaluation of activation markers on circulating T-cells or serum chemokines/cytokines.[86,96,98–100] Ultimately, a better understanding of the human immune TME in response to radiation therapy in irradiated and nonirradiated lesions and in comparison with pretreatment baseline will be essential to elucidate immunologically relevant targets for immuno-PET studies in the future. Recent work by Luke et al. in a cohort of patients undergoing multisite SBRT and sequential pembrolizumab included paired pre- and post-SBRT biopsies, and the test results demonstrated an upregulated IFN-ɣ-associated gene signature in a subset of nonirradiated tumors after SBRT. These findings represent a step forward for this field and identify candidate tissue-based biomarkers that could be developed for future noninvasive biomarker assessments.[101]

Conclusions

Immuno-PET holds outstanding potential to transform the biomarker landscape for immunotherapy, and efforts are underway to translate this molecular imaging technology into the clinical setting to noninvasively monitor responses to various immuno-oncology agents. The interdependence of oncologic imaging and radiation oncology suggests that noninvasive molecular imaging will play a key role in an effort to deliver individualized, biology-driven, precision radiation therapy.[102,103] Combining radiation therapy with immunotherapy is a potential game changer in oncology, but this potential is yet to be realized. Immuno-PET imaging may hold a critical role in delivering the valuable insight needed to help unravel this complex partnership.

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