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Exploring molecular imaging to investigate immune checkpoint inhibitor-related toxicity

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ABSTRACT

Immune checkpoint inhibitors (ICI) boost the endogenous anticancer immunity, evoking long-lasting anticancer responses in a subset of patients with solid tumors. Simultaneously, ICI are also associated with serious toxicities, impacting treatment duration and the quality of life. The proposed processes underlying ICI-related toxicity include T-cell activation and recruitment to non-tumor tissues, involvement of other immune cells and fibroblasts and the host' microbiome composition. However, the exact mechanisms of these processes remain incompletely understood, hindering clinicians' ability to predict and identify ICI-related toxicity in the early stages of treatment. Molecular imaging may play a role as a non-invasive biomarker, providing a tool to study ICI-related toxicity. This review discusses the applications of molecular imaging to answer questions regarding the mechanisms, detection, and prediction of ICI-related toxicity. Potential targets and the current state of development of suitable imaging techniques are discussed.

INTRODUCTION

Immune checkpoints

Since their introduction in the clinic, immune checkpoint inhibitors (ICIs) have had tremendous impact on the treatment landscape of solid tumors. Boosting endogenous anticancer immunity by blocking the interaction of immune checkpoint molecules, can evoke long-lasting responses in subsets of patients with cancer.[1 2] Immune checkpoint molecules are expressed on T cells and antigen-presenting cells (APCs) as part of a complex regulatory network to maintain tissue integrity by preventing autoreactivity and exaggerated inflammatory responses to exogenous stimuli.[3] The checkpoint molecule cytotoxic T lymphocyte associated protein 4 (CTLA-4) is expressed by T cells and engagement of CTLA-4 with its ligands B7-1 and B7-2 negatively regulates T-cell activation.[4 5] In the thymus, CTLA-4 interaction is crucial for the elimination of autoreactive T cells, thereby regulating central tolerance.[6] Consequently, loss of function of CTLA-4 caused by genetic deficiencies results in an autoimmune phenotype.[7 8] Moreover, CTLA-4 is expressed on regulatory T cells (T reg), maintaining immune homeostasis by down-tuning effector T cells.[9] Programmed cell death protein-1 (PD-1) regulates peripheral immune tolerance, by negatively regulating T-cell cytotoxicity on interaction with its ligands programmed death ligand-1 (PD-L1) or programmed death ligand-2 expressed on APCs.[3 10] Cancer cells are known to upregulate these inhibitory checkpoint molecules as part of their strategies to evade the immune system, rendering it a target for immunotherapy.[11] In the last decade, clinical trials have primarily focused on inhibitors of CTLA-4 and PD-(L)1.[12] However, the focus is currently broadening to various other checkpoints, such as LAG-3, OX40, and TIM-3.[11 12]

Immune checkpoint inhibitor-related toxicity

Intrinsic to their physiological role, blocking the interactions of immune checkpoints can result in disproportional inflammatory responses in normal tissues, referred to as immune-related adverse events (irAEs). These irAEs can develop within a few days, but also occur throughout the course of treatment, and even after completion.[13] irAEs occur more frequently with dual ICI combination therapy compared with monotherapy.[14] More than 50% of patients treated with ICI combination therapy (CTLA-4 and PD-(L)1) develop grade 3 or higher irAEs,[15] leading to treatment discontinuation, significant morbidity, and even treatment-related mortality.[16] Generally, grade 3 or higher non-endocrine irAEs require high-dose systemic immune suppression[17 18] and impact treatment duration as well as quality of

life.[19 20] High-grade toxicity is often observed in patients with a favorable antitumor response to therapy which often continues after therapy cessation because of irAEs.[21]

Investigating ICI-related toxicity

Molecular mechanisms underlying ICI-related toxicity are challenging to investigate in preclinical models for multiple reasons. Murine models incompletely represent the heterogeneous and dynamic processes that occur in patients, regarding immunological mediators, cellular composition and host factors, such as age, comorbidity, comedication, and microbiome composition.[22 23] Observational and translational studies in patients have proposed mechanisms of irAEs based on tissue biopsies, soluble biomarkers, and metagenome analyses.[13] However, those techniques have their limitations. Obtaining tissue biopsies is invasive and prone to sampling error, and systemic markers may not reflect molecular processes at the tissue or organ level. Notwithstanding the important insights derived from these studies, the limitations hinder the progress of our understanding of ICI-related toxicity in clinically relevant settings. Exploring complementary biomarker technologies may foster insights in the dynamics and interorgan networks involved in ICI-related toxicity.

Molecular imaging as biomarker technology

Molecular imaging with positron emission tomography (PET) meets the desired features of a complementary biomarker technology; it provides reproducible, quantitative data on a functional process at molecular level and with great sensitivity,[24] as compared with other clinical imaging techniques.[25] PET imaging encompasses a whole-body view, capturing interorgan-related processes in a non-invasive fashion. This allows longitudinal monitoring, which is a prerequisite for a dynamic process such as ICI-related toxicity. PET imaging therefore may add spatiotemporal information at the tissue or organ level to the complex data on molecular and cellular scale, derived from molecular assays and tissue analyses.[26]

Radiolabeled radiopharmaceuticals targeting cell surface markers or molecular pathways define the specificity of molecular imaging. In adjacent fields of research, novel radiopharmaceuticals with immune-relevant targets are increasingly explored to study therapy efficacy, (auto) immune processes and commensal bacteria.[27–29] As these molecular and cellular targets are overlapping with the proposed mechanisms of ICI-related toxicity, these advances may serve the need to investigate mechanisms of ICI-related toxicity in a clinical setting.

This review discusses molecular imaging techniques that may be relevant in the context of ICI-related toxicity and could contribute to understanding, predicting and monitoring ICI-related toxicity. We reflect on the potential of these techniques considering their biological relevance and applicability in the clinic.

MOLECULAR IMAGING APPROACHES

Data from translational studies describe T-cell infiltration in toxicity-affected organs as a critical early step in the development of ICI-related toxicity. Analysis of biopsies of colon, liver, and postmortem myocardium shows abnormal CD8 T cell abundance in affected tissues.[30–32] It has been hypothesized that cytotoxic (CD8) T cells in healthy tissue triggered by ICIs can be explained by two mechanisms, one being the activation of autoreactive T cells, which are either pre-existing,[33] or recognize shared epitopes between tumor and self-antigens. This mechanism has been suggested for ICI-related dermatitis, cardiomyopathy, and thyroiditis and is characterized by the coincidence of autoantibodies.[34–36] Second, the inflammatory effects of checkpoint inhibition in the gastrointestinal tract and lung can be attributed to a disturbance of the strictly regulated balance between pathogen clearance and tissue integrity by the mucosal immune system.[37] This results in a sharp increase in immune cell infiltration into tissues. The cells and mediators involved provide targets for molecular imaging which can be divided into T-cell targets and targets related to an inflammatory microenvironment, as depicted in figure 1 and online supplemental table S1.

Imaging T-cell involvement

T-cell activation and proliferation

On immune cell activation, two processes provide molecular imaging targets: (1) upregulated immune cell specific markers, and (2) changes in metabolic profiles. The interleukin-2 receptor (IL-2R) is such an activation-induced marker, and several SPECT (Single-photon Emission Computed Tomography) and PET probes are currently in the clinical phase to visualize activated T cells as a response to ICI.[38 39] Similarly, OX40 expression is enhanced on activated immune cells and has been explored as a PET imaging target in response analysis to cancer vaccines and ICI in murine models.[40 41] Molecular imaging of a third immune cell activation marker, CD69 is preclinically investigated in the context of immunotherapy and inflammatory arthritis.[42 43] Visualization of these markers in organs normally devoid of immune cells could identify activated immune cell infiltration during ICI treatment.

The Warburg effect, switching to glucose as the primary energy source, requires enhanced uptake by the cells' glucose transporters, including GLUT-1.[44 45] This concept of differentiating between healthy and malignant or inflamed tissue by abnormal glucose uptake is the hallmark of the routinely used [^{18}F]FDG ([^{18}F]Fluoro-2-deoxyglucose) PET and has been studied in the field of ICI treatment as well. Data from multiple studies showed that [^{18}F]FDG PET/CT was able to detect ICI-related toxicity in several organs including thyroiditis and pneumonitis.[46] In addition, distinct [^{18}F]FDG uptake patterns have been described for other organs affected by ICI-related toxicity, including arthritis-like affected joints and colitis.[47 48] Here, [^{18}F]FDG is not specific for uptake by T

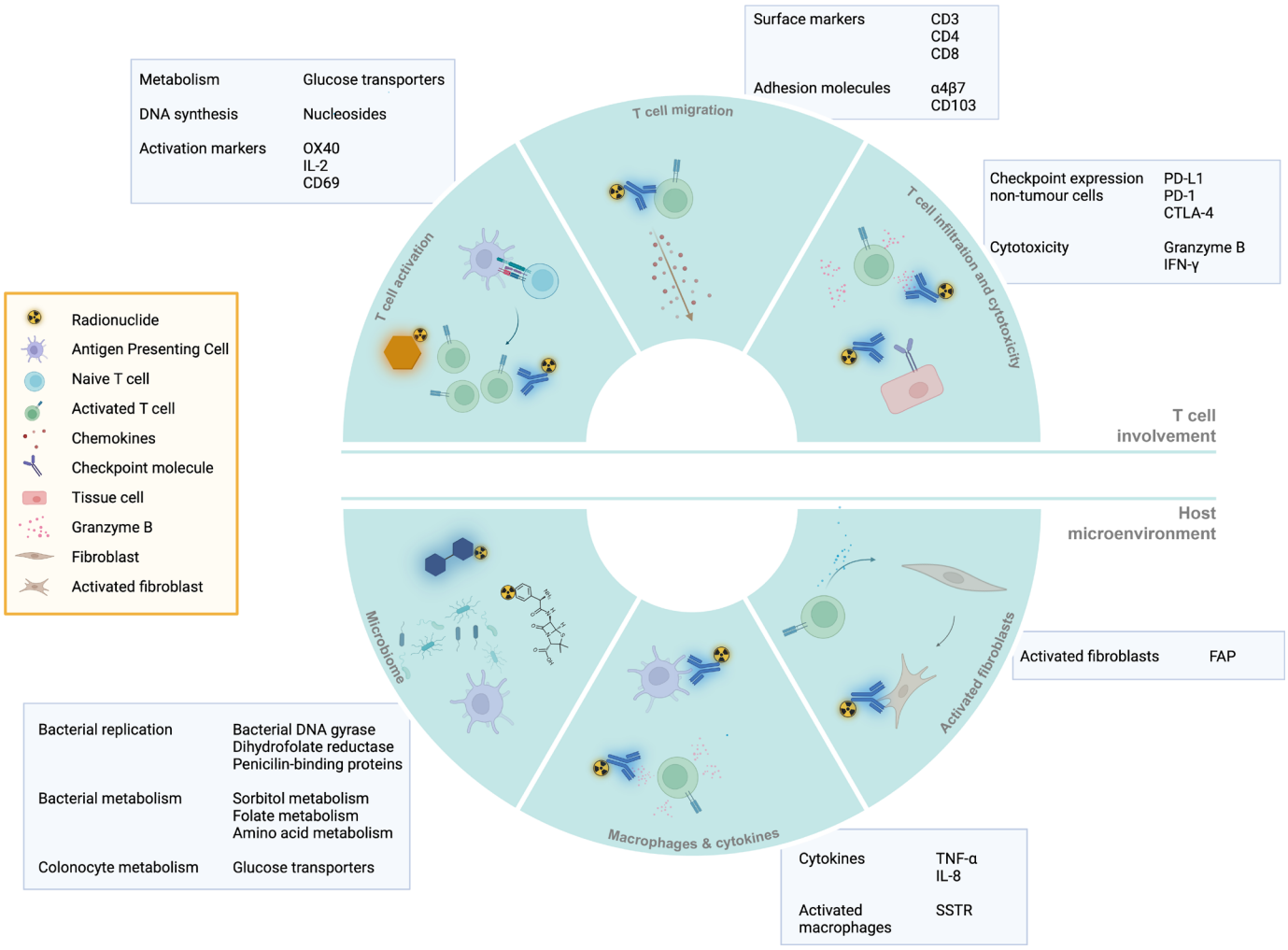

Figure 1 Targets for molecular imaging in the context of immune checkpoint inhibitor-related toxicity. Metabolic substrates, cell surface receptors and soluble factors can be targeted to investigate the processes involved in ICI-related toxicity and can be divided into T cell-related targets and targets contributing to an inflammatory host microenvironment. CTLA-4, cytotoxic T lymphocyte associated protein-4; FAP, fibroblast activation protein; IFN-γ, interferon-γ; IL-2, interleukin-2; IL-8, interleukin-8; PD-1, programmed death-1; PD-L1, programmed death ligand-1; SSTR, somatostatin receptor; TNF-α, tumor necrosis factor-α.

cells and most likely represents an increase of activated neutrophils and monocytes,[49] indicating a more advanced stage in the development of inflammation, involving the recruitment of these heavily glycolytically active innate immune cell populations. For this reason, [^{18}F]FDG is likely not suitable for assessment of activation of T cells specifically.

Uptake of nucleosides is increased in activated T cells. 9-β-D-arabinofuranosylguanine (AraG) is a T cell-selective nucleoside analog, which is radiolabeled to visualize metabolically active T cells.[50 51] [^{18}F]F-AraG PET/CT is explored to assess immunotherapy effects in several disease settings, with multiple trials currently recruiting.[52–54] This includes one study which showed increased myocardial [^{18}F]F-AraG uptake which could indicate subclinical cardiotoxicity in ICI-treated patients.[55] Furthermore, [^{18}F]Fluorothymidine has been applied as a proliferation marker for lymphocytes in oncological settings.[56 57] Radiopharmaceuticals based on other nucleotides are in development as well, but these are either not metabolized by T cells or not yet used clinically.

T-cell density and migration

The presence of T cells can be visualized by radiolabeled radiopharmaceuticals targeting T cell-specific surface markers. Anti-CD3 and anti-CD4 radiopharmaceuticals have been developed but are not yet investigated clinically.[58 59] The potential of anti-CD8 PET radiopharmaceuticals is currently being explored in the context of immunotherapy efficacy, using antibody fragments labeled with ^{68}Ga, ^{18}F, or ^{89}Zr.[60–62] These radiopharmaceuticals are predominantly tested for their ability to visualize CD8 T cell infiltration in solid tumors and inflammatory diseases and show potential to monitor response to treatment.[60 62–64] However, increased radiopharmaceutical uptake in normal tissues has also been observed. Particularly in tissues with low CD8 lymphocyte presence in healthy conditions, and therefore low background signal

such as muscle and endocrine organs, ICI-related toxicity related to T-cell presence may be easily visualized.[64] Investigating CD8+T cell distribution by PET imaging can non-invasively demonstrate the presence of cytotoxic T cells in toxicity-susceptible organs. In a recent case report, we describe increased uptake of an anti-CD8 PET radiopharmaceutical in the pituitary gland of a patient diagnosed with ICI-induced hypophysitis, already days before clinical symptoms occurred.[65] Likewise, increased anti-CD8 PET radiopharmaceutical uptake was reported in a case of ICI-induced recurrence of Hashimoto's thyroiditis.[62] Endocrine organs are normally devoid of T cells which maximizes the ability to detect the presence of T cells. The detection limits for increased T cell numbers in organs that are normally densely populated by patrolling T cells, remain to be investigated.

Different radionuclides enable distinctive approaches for CD8 T-cell visualization. Using a long-lived radionuclide like ^{89}Zr, could allow for cell tracking by repeated acquisitions, but in general suffers from noise signal. ^{18}F has favorable signal-to-noise ratios and therefore may be more optimal for accurate quantification of signal. However, the short half-life of ^{18}F requires repeated injections when serial snapshots of CD8 T cell distributions are of interest. Further studies should settle the correlation between quantitative PET parameters and number of CD8 target cells in the tissue, with regards to the characteristics of the different radionuclides used. However, quantification of CD8 expression does not imply cytotoxic activity. A small number of cells could potentially cause significant cytotoxicity, while a positive PET signal may also indicate the presence of rather inactive cells.

Immune cell migration is guided by trafficking signals including chemokines and integrins, providing targets for imaging the direction of immune cells. The integrin CD103 is involved in tissue migration and retention of leukocytes. It is expressed on T cells, including tumor-resident and lamina propria resident CD8 T cells.[66 67] Anti-CD103 PET probes have been developed originally to visualize tumor resident T cells but might be employed to visualize tissue resident T cells in ICI-related toxicity-affected organs as well. CD103 antibody fragments labeled with ^{89}Zr or ^{68}Ga showed high selectivity and sensitivity for their targets in preclinical models.[68 69] Likewise, the gut-homing integrin α4β7 has been targeted with radiopharmaceuticals in preclinical inflammatory bowel disease models, but thus far without clinical translation.[70 71] Radiopharmaceuticals targeting integrins could visualize migrating T cells in ICI-treated patients in an early stage of ICI-related toxicity.

T-cell infiltration and cytotoxicity

Checkpoint expression on APCs, T regs, and endothelium ensures T-cell tolerance to healthy organs, including the pituitary gland and vasculature.[72 73] Checkpoints modulate inflammatory processes, including T-cell infiltration, and can be monitored with molecular imaging.[74] During ICI therapy, this protective tolerogenic state is abolished. Whole-body checkpoint expression, presence of APCs and T regs, as well as ICI antibody distribution can be visualized by radiolabeling ICI, which could potentially be informative for studying ICI-related toxicity.

Tumor uptake of different radiolabeled anti-PD-L1 antibodies has been investigated in patients with non-small cell lung carcinoma, renal cell carcinoma, and head and neck cancer.[75–77] In two of those studies the uptake of labeled anti-PD-L1 in non-tumor organs was also analyzed during inflammatory events. The observation of radiolabeled ICI uptake at inflammatory sites can be a first indication of the applicability of this imaging technique to investigate the expression of PD-L1 on affected tissues or the presence of PD-L1 positive immune cells. This information may be of value in the prediction of ICI-related toxicity. Increased ^{89}Zr-labeled atezolizumab uptake was reported in a case of sinusitis and of bursitis, however, these events were regarded as pre-existing and not ICI-related.[75] In another study, three cases of ICI-related toxicity did not show increased uptake on a pretreatment ^{89}Zr-labeled durvalumab PET/CT at the concerned sites.[76]

Anti-PD-1 therapies target PD-1 on T cells, other lymphocytes, and myeloid cells. In patients with NSCLC, both ^{89}Zr-labeled pembrolizumab and nivolumab have been employed to predict treatment response to both respective ICIs by PET imaging.[78 79] As a secondary outcome, ^{89}Zr-labeled nivolumab and pembrolizumab signal was reported in non-malignant lymph nodes and spleen, which the authors attributed to the presence of PD-1 positive lymphocytes and dendritic cells. Furthermore, uptake of ^{89}Zr-labeled pembrolizumab was reported in sites of inflammation after surgery, viral infection, and pre-existing Hashimoto thyroiditis.[78] These results are a first indication of the potential of labeled ICI to visualize PD-1 positive immune cells at the site of inflammation. In the context of ICI-related toxicity, visualizing PD-1 positive immune cells at the site of toxicity can enable early detection or confirmation of PD-1 positive immune cell infiltration.

Similar to PD-1 and PD-L1, radiolabeled anti-CTLA-4 is clinically available and could provide insight into CTLA-4 expression in non-tumor tissue.[80 81] Visualization of CTLA-4 expression on tissues can indicate susceptibility to ICI-related toxicity as blocking of the checkpoints will disable immune tolerance at those sites. Systematic analyses of the radiopharmaceuticals targeting immune checkpoint distribution in vivo, as a determinant of target expression and tissue accumulation of therapeutic antibodies targeting ICI, have yet not been reported.

T-cell cytotoxicity can be visualized by targeting markers of T-cell cytotoxic products, indicative of T cell effector function. Radiopharmaceuticals targeting excreted Granzyme B, a cytotoxic molecule, have been applied in preclinical models of ICI-treated mice,[82 83] and a clinical study is currently recruiting (NCT04169321). By visualization of released cytotoxic products, T-cell cytotoxicity can be non-invasively detected in organs suspected of ICI-related toxicity.

Imaging the host microenvironment

Host microbiome

The community of commensal bacteria that harbor the human intestine, referred to as the gut microbiome, is essential for the training of the immune system and maintaining immunological balance.[84] Previous research in preclinical models and ICI patients has established an association between specific gut microbial signatures and response to ICI therapy.[85 86] The presence of *Bacteroides* strains correlates with a better treatment response or a lower incidence of ICI-related colitis in patients with melanoma.[87 88] Recent interest in this phenomenon has resulted in studies investigating microbiota signatures as predictive biomarkers and therapeutic agents for ICI-related colitis and other toxicities.[89–91] Non-invasive evaluation of the presence and localization of specific dominant bacterial strains could give complementary information by adding spatial information as to which segments of the large mucosal surfaces of the intestines are involved in addition to metagenome sequencing. However, limiting to bacterial imaging of the microbiome is the vast outnumbering of commensal bacteria in the intestines in both numbers and cellular diversity. Among these hundreds to thousands of bacterial species, many strains will overlap in favorite metabolic substrates.[92] This may impair the ability to detect increases in the dominance of certain strains among many other smaller strains sharing similar metabolic pathways, thus impairing both sensitivity and specificity of PET imaging. In addition, whether venous or oral administration of the radiopharmaceutical is more effective for the detection of colonic bacteria remains to be investigated.

Radiopharmaceuticals that have been developed to target bacteria include antimicrobial agents ciprofloxacin,[93] ertapenem[94] and trimethoprim.[95] However, as all three antimicrobial agents have a broad spectrum of action, specificity is lacking to identify specific bacterial species important in ICI-related toxicity.

Radiopharmaceuticals based on bacteria-specific substrates para-aminobenzoic acid (PABA) and ^{18}F-labeled sorbitol ([^{18}F]fluorodeoxysorbitol) are being investigated to distinguish bacterial infection from sterile inflammation. Both sorbitol and PABA are not specific enough to visualize specific bacterial species of interest in ICI-related toxicity but could be indicative of total bacterial metabolic activity.

[^{18}F]FDG PET/CT signatures of the colon could be a surrogate biomarker for colonic bacterial load and bacterial diversity, as a higher glucose uptake in colonocytes presumably indicates lower availability of bacterial-derived butyrate.[96] Indeed, a higher colonic [^{18}F]FDG uptake has been associated with lower bacterial load after antibiotic treatment, a lower microbiome diversity, and a worse response to ICI therapy or chemotherapy.[97 98] Whether colonic [^{18}F]FDG uptake correlates with ICI-related toxicity has yet to be confirmed.

Cytokines

The significance of cytokines in ICI-related toxicity came forward in multiple translational studies.[99 100] Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine produced in ICI-related toxicity. Treatment with infliximab or other TNF-blocking antibodies is advised for serious or steroid-refractory cases of irAEs.[101] Imaging with labeled TNF antibodies has been investigated across various TNF-driven diseases. In patients with pulmonary sarcoidosis, which is a known ICI-related irAE,[102] quantitative analyses of SPECT imaging with ^{99m}Tc-labeled infliximab correlated better with disease outcomes than [^{18}F]FDG PET or serum TNF.[103] ^{99m}Tc-labeled infliximab was employed to assess local TNF expression in patients with arthritis, where uptake was higher in affected versus non-affected joints[104] and Crohn's disease, where bowel uptake was low.[105] A PET radiopharmaceutical targeting TNF-α is currently in preclinical use and could be employed to assess local TNF-α excretion at sites of ICI-related toxicity.[106]

IL-8 is a cytokine involved in the recruitment of neutrophils to the site of inflammation. To visualize neutrophil infiltration during disease activity, radiolabeled IL-8 has been applied in patients with IBD.[107] Uptake of the radiolabeled IL-8 correlated with histology of inflammatory lesions. These results indicate the ability to visualize cytokines in active colitis, which is possibly applicable in ICI-related colitis as well. Similarly, for visualization of interferon-γ (IFN-γ), a proinflammatory cytokine secreted by CD8 T cells, several probes have been developed which are currently in the preclinical phase.[108 109] Challenging the imaging of these soluble targets is the short window in which target and radiopharmaceutical can interact because of fast receptor binding and internalization of cytokines. Furthermore, the chance of side effects is significant as radiolabeled cytokines are biologically active and high serum concentrations of cytokines might cause background signal.

Myeloid cells

Involvement of activated macrophages in the development of ICI-related toxicity has been indicated for myocarditis and other immune-related events.[110 111] Activated macrophages express the somatostatin receptor types 1 and 2 (SSTR1 and 2) which are targeted by [^{68}Ga]Ga-DOTA(0)-Phe(1)-Tyr(3)-octreotide.[112] SSTR-based PET/CT has successfully been applied to visualize activated macrophages in a handful of clinical cases of acute myocardial inflammation and ICI-related myocarditis.[113–115] Multiple SSTR-targeting radiopharmaceuticals are under development. These radiopharmaceuticals are not suitable to detect changes in organs including thyroid, spleen, liver, and adrenal glands due to natural expression of SSTR or macrophage residency. The myocardium, normally not inhabited by immune cells, is therefore an ideal subject for these radiopharmaceuticals to investigate ICI-related toxicity.

Activated fibroblasts

Besides their supportive function, stromal cells including fibroblasts activate and guide immune cells by, for example, production of cytokines.[116 117] In different inflammatory diseases, a role for activated fibroblasts becomes increasingly apparent. These cells overexpress fibroblast activation protein (FAP), which can be visualized using radiolabeled FAP inhibitors (FAPI) tagged with positron-emitting radionuclides. Although the initial development of FAP-targeted PET radiopharmaceuticals was mainly aimed at visualizing cancer-associated fibroblasts, FAPI PET/CT has made an entry in the field of inflammation imaging.[118 119] Studies in small groups of patients reveal increased radiopharmaceutical uptake of radiolabeled FAPI in primary arthritis and intestinal inflammation.[120–122] Recently, inflammatory processes induced by ICI therapy have been visualized by FAPI PET/CT in multiple clinical manifestations of ICI-related toxicity. In a case of anti-PD-1 induced arthritis, inflammatory activity at the affected joint was detected on FAPI PET/CT.[123] Furthermore, cardiac toxicity induced by ICI therapy, a relatively rare but potentially fatal adverse event, was detected by FAPI PET/CT.[124–126] Furthermore, some first results indicate that ICI-induced thyroiditis can be identified on FAPI PET/CT.[127] FAPI PET/CT could help the diagnosis of ICI-related toxicity events because it provides the ability to identify tissue remodeling due to ICI-related inflammation on a whole-body level. This is an advantage over biopsies which might not completely capture the heterogeneity of inflammatory processes.

DISCUSSION

Linking imaging technology to clinical needs

Implications of PET imaging technology

To have clinical impact, imaging techniques must fit criteria specific for the molecular process and tissues studied. The radiopharmaceutical of choice implicates the pharmacokinetic characteristics in vivo and therefore the matching radionuclide. Generally, the size of radiopharmaceuticals is associated with clearance time and target-to-background ratios of the obtained images. Of importance in ICI-related toxicity imaging is the excretion route of radiopharmaceuticals. Larger radiopharmaceuticals such as antibodies and antibody fragments are excreted mainly via the hepatic route, complicating differentiation of specific uptake from excreted radiopharmaceuticals in liver and bowel, which are organs affected by ICI toxicity. Non-specific uptake of [^{18}F]FDG in brain and cardiac tissue implies the incompatibility of this technique to identify metabolic changes due to ICI-related toxicity in these organs. Larger radiopharmaceuticals labeled with long-lived radioisotopes lead to a higher total radiation exposure for patients. This complicates the ability to perform repeated PET imaging while longitudinal monitoring is relevant in ICI-related toxicity. Radionuclides with longer half-lives allow for delayed and repeated imaging after injection, although the simultaneous clearance of the radiopharmaceutical from its target might hamper studying dynamics of the targeted cell types. Logistically, longer-lived radioisotopes are less widely available but can be produced centrally and transported over longer distances, which enables application at locations without production facilities. On the other hand, as larger molecules have a slower clearance, the time between injection and optimal image acquisition is longer, leading to more challenging logistics such as patient planning. This is especially critical for monitoring toxicity in an acute setting, more demanding for patients due to multiple visits. This contrasts with the same-day protocols of shorter-lived radioisotopes, which might be favored in cases of more acute clinical importance. In addition to safety related to ionizing radiation exposure, biological and allergic reactions to radiopharmaceuticals must be considered. Examples include radiolabeled cytokines and allergenic epitopes of antibodies which might trigger additional immunological reactions, for which ICI-treated patients might be at higher risk. The choice of target and radiopharmaceutical also requires consideration of the detection limit. A highly specific radiopharmaceutical will be able to discriminate the specific target of interest even at low expression but requires a highly sensitive method of detection. Ongoing development of imaging and processing techniques is resulting in more sensitive scanners, enabling imaging of lowly expressed targets and with lower doses. This is relevant for imaging ICI-related toxicity, as these developments allow for the visualization of even a small number of immune cells capable of causing significant damage.

Challenges of imaging host responses in ICI related toxicity

Imaging host response is highly relevant in immune oncology clinics but can be challenging due to the characteristics of the immune system. First, most markers of immune function are soluble and dynamic, requiring different quantification approaches compared with static targets. Single time point imaging might be less suitable for imaging cells and mediators of the immune system. Second, the physiological process of inflammation aims to eradicate pathogenic and tumor cells while preserving tissue integrity. Therefore, it is challenging to determine what level of baseline inflammation can be deemed relevant to predict the development of toxicity during ICI treatment. In addition, these parameters will likely differ between organs and between patients. A focus on organ-specific thresholds during the validation of imaging methods might contribute to more accurate interpretation of imaging signatures. Furthermore, inflammation is associated with increased perfusion and permeability in tissues, complicating the assessment of the radiopharmaceutical signal to be specific or due to these processes.

A downside of molecular imaging is the inability to multiplex different markers. Visualizing immune cell infiltration using a single surface marker does not provide detailed information about the cell's phenotype or functional state. For instance, macrophages share

common receptors, but their M1 and M2 subtypes have very distinctive functions in inflammation, the first being proinflammatory and the second active in tissue repair. This pluralism of many immune cells complicates the selection of a single marker that accurately reflects the process to be visualized.

Of importance is the timing of imaging; within three stages of ICI treatment, imaging can be performed to investigate ICI-related toxicity. Characterization of host factors using imaging before treatment could investigate a predictive value for the occurrence of ICI-related toxicity. Examples include imaging of the host microbiome and non-tumor checkpoint expression. Second, imaging early during treatment could detect toxicity in a subclinical stage. Here, the optimal timing is challenging as some ICI-related toxicities develop days after the first cycle, while others arise months later. However, the majority of the toxic events is expected between 6 and 9 weeks after therapy initiation.[17] Performing imaging in this period could detect toxicity in an early stage, before symptoms arise. Whether imaging at these early time points might give insight into the development of toxicity, remains to be investigated. To meet these challenging requirements, we would propose to tailor the imaging protocol to the expected toxicity profile of the specific ICI treatment regimen. Lastly, imaging could be applied during the management of ICI-related toxicity to give insights into the active processes and pathways involved, which could help guide treatment decisions in cases with a clinical dilemma. Here, standard of care diagnostic tools might be complemented with information from imaging techniques. Consequently, molecular imaging might not be the preferred research tool for all irAEs. Organ-specific challenges may hamper imaging interpretation as well as timing and duration of processes involved. Detecting immune cell activation and presence is possibly most valuable in the active phase of the ICI-related inflammation, while fibroblasts are likely involved in a later stage, resolving the toxicity-related inflammation. However, molecular imaging may be of added clinical importance in cases when a tissue biopsy cannot be taken safely. Moreover it may provide functional organ-specific information on systemic processes, such as immune cell activation and trafficking.

Opportunities and suggestions for future studies

Based on the clinical experience to date, [^{18}F]FDG, anti-CD8 and FAPI PET/CT may hold the most potential to contribute to research into ICI-related toxicity. [^{18}F]FDG is widely available and a robust marker of increased glucose uptake, but a pitfall is its low specificity and therefore inability to discriminate between tumor, immune or metabolically active parenchymal cells. Furthermore, ICI-related toxicity processes might be low metabolically active, especially when only lymphocytes and no innate immune cells are involved. In some cases, ICI-related toxicity was successfully detected by FAPI PET, but to a lesser extent by [^{18}F]FDG PET.[123] This could demonstrate an advantage of FAPI PET over [^{18}F]FDG PET. Visualizing cytotoxic T-cell infiltration by targeting CD8 has shown potential in two clinical cases of ICI-related toxicity, and prospective trials are ongoing.

Since many receptors and pathways involved in ICI response and toxicity overlap, combined studies investigating both effects can serve as a crucial first step in evaluating the potential of molecular imaging radiopharmaceuticals for understanding ICI-related toxicity. For future clinical implementation, the difference between the optimal timing of imaging for detecting toxicity and to assess treatment response must be considered. A combined assessment could be preferred in terms of logistics and radiation dose exposure but might not be accurate due to the spatiotemporal differences between antitumor response and toxicity.

Integrating PET imaging with clinical trials on novel immune checkpoint therapies could yield valuable insights into the effects of these treatments. However, time points at which response and toxicity effects are expected might not coincide, requiring a flexible protocol. Acquiring additional translational data, including data on circulating immune cell profiles and data on cell infiltrates and bacterial composition in toxicity-involved organs, enables further characterization of the mechanisms of ICI-related toxicity and can validate imaging results. In addition, novel analysis tools based on artificial intelligence (AI) are increasingly applied and hold great promise for denoising, segmentation and pattern recognition of PET images. Furthermore, AI can facilitate prediction modeling using PET imaging datasets, taking into account the multiorgan view.

CONCLUSIONS

Molecular imaging is a promising field to contribute to insights into the mechanisms of ICI-related toxicity including T-cell behavior, involvement of supportive cell populations, and the role of commensal bacteria. Currently, radiopharmaceuticals to visualize CD8+T cell localization, activated fibroblasts, and elevated metabolic activity have the most potential to predict and monitor ICI-related toxicity, as indicated by anecdotal reports. Prospective studies using these radiopharmaceuticals should validate their diagnostic and predictive value in the clinic. Furthermore, other promising targets like Granzyme B and IFN-γ as markers of cytotoxic activity are under development for clinical use, extending the molecular imaging toolbox for the investigation of ICI-related toxicity.

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