Cancer Immunoimaging with Smart Nanoparticles

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Dynamic immunoimaging in vivo is crucial in patient-tailored immunotherapies to identify patients who will benefit from immunotherapies, monitor therapeutic efficacy post treatment, and determine alternative strategies for nonresponders. Nanoparticles have played a major role in the immunotherapy landscape. In this review, we summarize recent findings in immunoimaging where smart nanoparticles target, detect, stimulate, and deliver therapeutic dose in vivo. Nanoparticles interfaced with an immunoimaging toolbox enable the use of multiple modalities and achieve depth-resolved whole-body tracking of immunomarkers with high accuracy both before and after treatment. We highlight how functional nanoparticles track T cells, dendritic cells (DCs), tumor-associated macrophages (TAMs), and immune checkpoint receptors (ICRs), and facilitate image-guided interventions.

Keywords
Immunoimaging, image-guided immunotherapy, gold nanoparticles, SPIO, organic nanoparticles, T cell tracking, dendritic cell tracking

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Cancer Immunoimaging with Smart Nanoparticles

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Abstract

Dynamic immunoimaging in vivo is critical in patient-tailored immunotherapies to identify the patients who will benefit from immunotherapies, to monitor therapeutic efficacy post-treatment, and to determine alternative strategies for nonresponders. Nanoparticles have played a major role in the immunotherapy landscape. In this review we summarize the recent findings in immunoimaging where smart nanoparticles target, detect, stimulate, and deliver therapeutic dose in vivo. Nanoparticles interfaced with immunoimaging toolbox enable the use of multiple modalities and achieve depth-resolved whole body tracking of immunomarkers with high accuracy before and after treatment. Distinct from other reviews, we highlight for the first time how functional nanoparticles track T cells, dendritic cells, tumor-associated macrophages, and immune checkpoint receptors, and facilitate image-guided interventions.

Keywords: Immunoimaging, image-guided immunotherapy, gold nanoparticles, SPIO, organic nanoparticles, T cell tracking, dendritic cell tracking

Glossary

- Enhanced permeability and retention (EPR) effect: EPR effect allows macromolecules and nanoparticles to accumulate in the tumor interstitial spaces due to the leaky tumor vasculatures and the lack of lymphatic drainage system.

- Tumor microenvironment (TME): TME describes the mass surrounding cancer cells, including extracellular matrix, stromal cells, immune cells and the tumor vasculatures.

- Mononuclear phagocyte system (MPS): MPS system refers to the phagocytic cells, primarily splenic macrophages, Kupffer cells in the liver and the monocytes that are located in reticular connected tissues.

- Dendritic cells (DCs): DCs are professional antigen presenting cells that capture, process and present neoantigens to T cells. DCs play an important role in bridging adaptive and innate immune system.
T cells: T cells are lymphocytes that play a central role in adaptive immune system in defending against both intracellular and extracellular pathogens. For example, in adoptive T cell therapy, healthy T cells are systemically delivered in cancer patients to build a stronger immune system and fight against cancer.

Tumor associated macrophages (TAMs): TAMs are macrophages that promote tumor progression and immunosuppressive TME by producing cytokine and chemokines.

Immune checkpoint receptors (ICRs): ICRs are regulatory receptors in the immune system that maintain self-tolerance. Upregulation of ICRs in cancer allows tumors to evade immune surveillance. Blockades of ICRs with therapeutic antibodies have recently shown to significantly improve clinical outcome in cancer patients.

Positron Emission Tomography (PET) imaging: PET imaging is a nuclear imaging technique that detects the annihilations of photons from the positron emitting radionuclide. PET provides sensitive three-dimensional results and is often used to monitor metabolic changes in TME.

Raman Spectroscopy: Raman spectroscopy measures the characteristic vibrational states, including bending, stretching and rotational modes of molecules. Raman spectroscopy is highly specific to the composition of the molecule being probed, and therefore useful for high resolution dynamic imaging in vivo.

Computed Tomography (CT): CT tomography provides more detailed information of soft tissue, bones and vasculatures than conventional x-ray images by combining multiple cross-sectional images of the body acquiring from different angles.

Magnetic Resonance Imaging (MRI): MRI imaging is a non-invasive clinical diagnostic and treatment monitoring technique that utilizes magnetic field and radio waves to produce three-dimensional images and great anatomical details.

Fluorescence Imaging: Fluorescence imaging visualizes fluorescent dyes that are attached to proteins or imaging probes to understand biological processes and to track dynamic changes.

Emerging Need for Immunoimaging

Cancer immunotherapies including blockade of immune checkpoint receptors (ICRs), adoptive T cell therapy (ACT), and vaccine delivery have recently driven a paradigm shift in patient survival. Dynamic molecular imaging of immune cells and markers has transformed our ability to understand innate and adaptive immune responses in the pathogenesis of cancer [1, 2].
This transformation is in part motivated by the failure of conventional imaging and histopathology to accurately predict response to immunoagents in a clinical setting. For traditional cancer therapies, evaluation of treatment response is based on the reduction of tumor size, and the absence of new tumors in accordance with the Response Evaluation Criteria in Solid Tumors (RECIST) [3]. However, in immunotherapies, pseudoprogression has emerged as a distinct response pattern, where activated immune cells infiltrating the tumor milieu elicit an increase in tumor volume and a delayed treatment response [4, 5]. Therefore, immunoimaging is indispensable to assess changes in tumor burden, allow early therapeutic intervention, reflect dynamic changes in immunomarkers during immunotherapies, and avoid premature termination of effective treatment based on RECIST criteria.

Immune cells including T cells, dendritic cells (DCs) and tumor associated macrophages (TAMs) and ICRs are critical in maintaining immune surveillance and in cancer immunotherapies. T cells are an important effector of the immune system and are critical for inhibition of tumor growth, invasion, and metastasis. In the past decade, ACT have demonstrated remarkable success in remission-free survival of patients necessitating accurate imaging of T cells in vivo for both planning of ACT and for monitoring treatment response. DCs are professional antigen presenting cells that link the innate and adaptive responses of the immune system. However, DCs-based therapeutic vaccines lack efficient migration into targeted peripheral lymph nodes (LN). Noninvasive tracking of DC migration in vivo is therefore imperative for targeted delivery of vaccines, and has been enabled by DCs labeled with smart nanoparticles (SNPs). TAMs are immune cells that drive an immunosuppressive tumor microenvironment (TME) and promote metastasis. Immunotherapy strategies that either suppress pro-tumor M2 phenotype TAMs or reprogram them to an antitumor M1 phenotype have gained tremendous momentum [6, 7].
However, success of these therapies relies on quantifying the distribution of TAMs in the TME with high-resolution imaging facilitated with SNPs. In addition to immune cells, ICRs such as programmed cell death 1 (PD-1), which are negative costimulatory molecules, are overexpressed on cytotoxic T lymphocytes (CTLs). Upregulation of ICRs impedes T cell activation and enables tumors to evade immune surveillance [8-10]. Immunotherapies blocking ICRs have shown tremendous promise in clinical trials among many tumor types, but ~75% cancer patients currently do not respond to this regimen in part due to poor prediction of ICRs in tumors [11-13]. Real-time tracking of ICR distribution before, during, and after ICR blockade therapies is highly desirable to ultimately improve patient-tailored immunotherapies.

![Figure 1. Schematic of design considerations for smart nanoparticles including size, shape, surface charge, material composition, and stiffness integrated with appropriate labels to enable imaging of immune cells and immunomarkers for image-guided immunotherapies. Glossary: polyethylene glycol (PEG), gold nanoparticles (Au NPs), superparamagnetic iron oxide (SPIO), dendritic cells (DCs), tumor associated macrophages (TAMs), immune checkpoint receptors (ICRs).](image)
**Design Considerations of Smart Nanoparticles for Immunoimaging**

The emerging need to accurately image the immune TME has led to an unmet clinical need for safe, biocompatible, and multifunctional SNPs that enable us to diagnose patients who will respond to immunotherapies even before treatment begins. Further, SNPs also provide an early response to therapy improving the treatment outcome of responders, accelerate clinical decisions for those requiring alternative therapies, and minimize toxicities and high costs of unsuccessful treatment for those identified as nonresponders. An expansive list of NP-based detection of various immune cells and the corresponding imaging toolbox employed is shown in Table 1. However, there are key design considerations of SNPs including their size, shape, surface properties, and composition that need to be evaluated for their utility in the TME (Fig.1). These design parameters of SNPs govern their biodistribution and clearance *in vivo* and ability to track immunomarkers. Size of SNPs is of foremost importance as NPs <10 nm undergo rapid renal clearance, whereas those >200 nm have heterogeneous distribution in the TME due to poor enhanced permeability and retention (EPR) effect [14]. This size-dependent glomerular filtration cut-off was established by quantifying the glomerular sieving coefficient (θ) (the ratio of urine amount to plasma amount) of NPs, where θ approaches 1 when Stokes-Einstein radius (r) is ~1 nm and θ decreases to ~0 if r >5 nm [15]. However, this cut-off is smaller for rigid NPs that do not deform easily. SNP size is also crucial for penetration into solid tumors, where SNPs >10 nm only enter via interendothelial cell gaps across the blood vessel walls. But SNPs <10 nm extravasate from normal capillaries that also have intercellular gaps resulting in deep tumor penetration [16]. A recent study showed 2 nm and 6 nm gold nanoparticles (AuNPs) penetrate necrotic regions of the tumor that are defined by poor vasculature and oxygen levels in both tumor spheroids and *in vivo*. However, 15 nm AuNPs remain in the outer layers of the TME with sufficient blood and
nutrient supply localizing in the angiogenic areas of tumor microvasculars [17]. Whereas most of these studies emphasize that SNPs uptake in TME relies on the EPR effect, Leong and co-workers have shown SNPs could induce endothelial cell (EC) leakiness (NanoEL) which is correlated to the size and surface charge of SNPs [18, 19]. NanoEL is caused by disruption of EC interactions by binding to critical adherens junction proteins which exert a disruptive force causing vasculature leakiness. The authors showed 10-30 nm AuNPs induce NanoEL in ECs and improve transport of SNPs to the targeted tissue through opening of the paracellular route, a finding that is essential for SNPs accumulation in the immune TME [19].

Shape of SNPs is also pivotal for imaging immune cells as transport of flowing SNPs is controlled by their margination (i.e. radial drift) towards the blood vessel walls and is controlled by SNPs translational and rotational motion. Multiple forces dictate NP motion, including buoyancy, gravity, drag, van der Waals interactions, and steric repulsive interactions. Under a balance of these forces spherical NPs follow a streamlined straight flow, whereas rod-shaped NPs experience lateral drift that varies depending on the angle of their orientation. In addition to these forces, SNPs uptake in the TME is also determined by their endocytosis pathway, which is directed by the SNPs shape. Xie and coworkers compared nanostars, nanorods, and nanotriangles and showed nanotriangles had the highest uptake in cells driven by uptake via the Dynamin pathway [20]. A later study then showed spherical AuNPs were largely membrane-bound with rapid cellular excretion via exocytosis, but Au nanostars (AuNSs) were translocated to cytoplasm with restricted mobility and suppressed exocytosis rate [21]. We have observed AuNSs support these findings, where high uptake was observed in vitro and long circulation times in vivo [22, 23]. SNPs shape also determines their uptake by macrophages of the mononuclear phagocyte system (MPS), where uptake of spherical SNPs is favored over nanorods and nanochains [24, 25]. These
findings were supported by a recent work where Oh and colleagues showed high aspect-ratio nanorods escape from TAMs enabling their localization in tumors, and are rapidly cleared by the liver through efficient Kupffer cell-hepatocyte transfer [26]. Therefore, morphological modulation of SNPs is critical to their in vivo fate and their ability to hone in immune cells.

The surface charge of SNPs also governs their ability to reach the TME and subsequent clearance via the MPS. Studies show positively charged SNPs accumulate in liver hepatocytes while negatively charged SNPs have nonspecific distribution in liver [27]. While cationic SNPs demonstrate high cellular uptake since cancer cells have negative surface charge [28], immunogenicity and toxicity of charged SNPs have given rise to significant concerns. The simplest approach to minimize SNPs toxicity and uptake by the MPS has been achieved by coating polyethylene glycol (PEG), a nontoxic food and drug administration (FDA) approved hydrophilic polymer. PEG coating increases SNPs solubility, overall stability, protease resistance, and in vivo half-life, and decreases adsorption of opsonin, a serum protein known to form protein corona, which collectively reduces their uptake by macrophages [29]. In an early work, Chan and co-workers showed that the PEG grafting density controls the adsorption of >70 different serum proteins on the surface of AuNPs, where a higher PEG density reduced the total serum protein adsorption [30]. A recent work corroborated these findings by showing that PEG grafting density and corresponding PEG conformation (mushroom to brush) control the plasma circulation of SNPs in vivo [31]. SNPs with a dense inner (primary) PEG layer thermodynamically prevent serum proteins from accessing NPs via steric repulsion, and outer PEG layer (mushroom regime) enables topographical stability to kinetically interfere with protein corona formation resulting in long circulating SNPs. These physicochemical properties are not individually responsible for the fate
of SNPs in vivo, but entanglement of these design parameters often complicates the prediction of the functionality of SNPs [32].

The material composition of SNPs has been the driving factor in our ability to track immune cells migration with an expansive immunoimaging toolbox (Table 1) and provide mechanistic understanding of immunotherapies. Therefore, this review focuses on three major classes of SNPs: AuNPs, superparamagnetic iron oxide nanoparticles (SPIONs), and organic NPs categorized by composition. These SNPs were chosen due to abundant literature findings on these classes of material, and each of these material has a unique advantage in terms of shape and surface tunability to achieve higher cellular uptake (AuNPs), small size with natural magnetic properties for magnetic resonance imaging (MRI) contrast (SPIONs), and ability to combine imaging and therapy for image-guided approaches (organic NPs). We found that the cancer type or immune cell type does not dictate success in immunoimaging with SNPs, but how the material property allows to control other physicochemical properties and also enable their interaction with the immune TME. Each SNP section discusses the merits of the material and reviews the recent progress in immunoimaging correlating to the design consideration discussed here.

**Gold Nanoparticles**

AuNPs have been used for two decades as imaging contrast agents and as photothermal actuators for light-based therapies and have been successful in humans [33]. The rapid surge of AuNP based SNPs for immunoimaging is primarily attributed to their high biocompatibility, minimal toxicity, and ease of synthesis that allows to tune their design parameters and corresponding near-infrared (NIR) light absorption properties. Further, Au surface chemistry is amenable to straightforward bioconjugation through well-established approaches. These
properties of AuNPs have been leveraged for immunoimaging with contrast-enhanced computed tomography (CT), since AuNPs attenuate x-rays and delineate soft tissues. The X-ray attenuation capacity of AuNPs is size-dependent, where a study comparing 4-60 nm AuNPs found that since X-ray attenuation depends on the target area, smaller AuNPs with larger surface area have higher X-ray attenuation. Therefore, the 4 nm AuNPs served as the most efficient CT contrast agent in their study[34]. In this effort, Meir and coworkers demonstrated that T cells labeled with 20 nm glucose-coated AuNPs can be tracked *in vivo* with CT imaging (Fig. 2a) [35]. Note that T cell tracking *in vivo* can only be achieved if the cells are labeled with AuNPs *ex vivo* and then introduced in mouse models via ACT. The glucose coating was critical to high stability of AuNPs and for enhanced uptake by T cells, since glucose metabolism in lymphocytes drives their growth, proliferation, and effector function [36]. Longitudinal CT imaging demonstrated their robust approach resulted in migration and honing of T cells in tumors for up to 48 hours post ACT (Fig.2a). In another work, the same group also detected programmed cell death-ligand 1 (PD-L1) immunomarker via antiPD-L1 conjugated AuNPs which showed higher accumulation in tumors relative to control AuNPs conjugated with isotype-matched IgG (Fig.2b) [37]. Depth-resolved CT images showed PD-L1 targeted AuNPs achieved better tumor penetration and higher infiltration than the control (Fig.2c). These findings suggest that relying solely on the EPR effect to initiate passive accumulation in tumors is ineffective in tumor targeting, and modulating the surface properties of SNPs with appropriate ligands is crucial to enable targeted detection of immune cells.
Figure 2. Immunoimaging with gold nanoparticles. (a) Glucose modification of AuNPs to enable uptake in T cells. (b) PD-L1 conjugated AuNPs targeted B16 tumors with CT imaging. PD-L1 labelled AuNPs resulted in (c) longitudinal tracking of AuNP labelled T cells with CT imaging 24-72h post-delivery. (d) Transmission electron micrograph of AuNSs showing their anisotropic morphology. (e) Schematic showing mice bearing mammary tumors were systemically administered with a mixture of antiEGFR-pMBA-AuNS and antiPD-L1-DTNB-AuNS to detect both markers. (f) Ex vivo SERS map of whole tumor lesions shows multiplexed detection of both EGFR (green, pMBA 1580 cm$^{-1}$) and PD-L1 (red, DTNB 1325 cm$^{-1}$). (g) Zoomed in ex vivo Raman maps and corresponding Raman spectra showing areas with (1) no SERS signal (2) PD-L1-rich tissue, (3) EGFR-rich tissue, and (4) both receptors. (h) Schematic of synthesis of tannic acid-coated core shell AuNP were radiolabeled (${}^{124}$I-TA-Au@AuNPs) for (i) tracking bone marrow-derived DCs with PET/CT imaging. (a) was adapted with permission from American Chemical Society ref. [35], (b) – (c) were adapted with permission from American Chemical Society ref. [37], (d) – (g) were adapted with permission from Royal Society of Chemistry ref. [22], (i) was adapted with permission from Springer Nature ref. [38], and (h) was adopted with permission from American Chemical Society ref. [39].

The importance of active targeting moieties was also supported by Ou and colleagues, where AuNSs conjugated with receptor specific antibodies were used for multiplexed detection (Fig.2d-e) of both PD-L1 and epidermal growth factor receptor (EGFR) simultaneously via surface enhanced Raman spectroscopy (SERS) in breast cancer in vitro [23] and in vivo [22]. One of the
advantages of AuNPs not achievable by other classes of SNPs, is the ability to modulate the shape based on simple modifications in synthetic chemistry. AuNSs are of significant interest for imaging due to their unique architecture consisting of a spherical core which acts as the receiver antenna and protrusions that concentrate the light on the tips via the nanoantenna effect [40]. This phenomenon generates strong electromagnetic fields at the tips of AuNSs resulting in $10^9$-$10^{12}$ enhancements in SERS [41, 42]. In this study, longitudinal SERS in vivo probed the signal of Raman reporter molecules, DTNB (1325 cm$^{-1}$) and pMBA (1580 cm$^{-1}$), conjugated to AuNSs surface corresponding to detection of PD-L1 and EGFR, respectively. Interestingly, we found maximum accumulation of AuNSs in the TME was at 6h post IV delivery in mice, which is distinct from spherical NPs >50 nm that accumulate in tumors in 12-24 h indicating shape control is important for rapid tumor delivery [21]. Cellular-level resolution ex vivo SERS maps of whole tumor lesions indicated heterogeneous distribution of PD-L1 and EGFR (Fig. 2f-g) expected for breast tumors, and higher magnification SERS indicated areas within the tumor that were PD-L1-rich, EGFR-rich, and had both biomarkers. These latter results are particularly encouraging that the combination of shape-controlled SNPs and a powerful imaging modality enable strong correlation of in vivo signal with ex vivo analysis necessary to translate these technologies for patient stratification of immunotherapies.

In addition to active targeting, passive accumulation of SNPs can be utilized for quantitatively tracking of DCs by intelligent design that preserves their imaging functionality. Lee and colleagues decorated radionuclides ($^{124}$I/$^{125}$I) on 20 nm AuNPs through adenine-rich thiolated oligonucleotides which allowed a high number of radioisotopes per AuNPs. Next, they added an additional Au shell as a protective layer to provide in vivo stability to the radiolabels and enhance longitudinal tracking of DCs via multimodal positron emission tomography (PET) and Cerenkov
luminescence imaging (CLI) [38]. The high sensitivity of whole-body PET complemented the high resolution of CLI facilitated accurate molecular contrast in vivo. Time-course study with these SNPs showed DCs migration to draining popliteal lymph nodes (DPLNs) and a synergistic correlation of both imaging modalities in AuNPs distribution. In a follow-up work, Lee and the group showed labeling $^{124}$I radioisotopes with tannic acid modified AuNPs (Fig. 2h) was an important design consideration as that reduced the synthesis duration from 50h to ~40 minutes [39]. The addition of the protective gold shell, $^{124}$I-TA-Au@AuNPs, provided both serum stability of the SNPs and radiochemical stability, where most of $^{124}$I remained conjugated to the SNPs. $^{124}$I-TA-Au@AuNPs-labeled bone marrow-derived DCs demonstrated long-term reliable PET signal at the DPLNs (Fig. 2i), indicating the design of AuNPs catalyzes longitudinal tracking of DCs. The size of AuNPs is an important design consideration to accurately track immune cells in vivo as discussed in the previous section. Bhatnagar and coworkers showed with PET imaging a higher uptake in T cells of 7 nm PEG functionalized AuNPs conjugated with $^{64}$Cu radiolabels relative to 15 nm AuNPs suggesting careful optimization of design parameters for successful immune cell targeting [43].

**Superparamagnetic Iron Oxide Nanoparticles (SPIONs)**

SPIONs are FDA-approved T$_2$ contrast agents used in MRI and have been extensively applied to track immune cells such as DCs and T cells [44-46]. Tremendous efforts have been placed to optimize SPIONs design which include inhibiting oxidation of magnetic cores, reducing their aggregation in vivo, and optimizing surface charge for high labeling efficiency. For example, Wu and the group developed biocompatible anionic SPIONs by coating negatively charged block copolymer, poly(aspartic acid)-b-poly($\varepsilon$-caprolactone) (PAsp-PCL), used for DCs labeling.
(Fig.3a-c) [46]. This amphiphilic block polymer is nontoxic and biodegradable and already in clinical use. The size of the polymer coated SPIONs was controlled to ~125 nm and showed high T2 relaxivity, and labeled DCs were monitored under a clinical MR imager. Labeled DCs were unaffected in their viability, proliferation, and differentiation capacity demonstrating appropriate surface modification of SPIONs is key to accurate monitoring of immune cells. Xu and coworkers adopted a similar approach to improve the biocompatibility of SPIONs with a layer of alkylated polyetheleneimine and glycerol coating which reduced the positive surface charge of SPIONs [47]. Further, they labeled the DCs post maturation to prevent changes in their phenotype and observed image contrast in vivo and hypointense regions in the central area of draining LNs (Fig.3d). Whereas these studies did not use active targeting to direct SPIONs in DCs, an early work by Ahrens and colleagues showed CD11c conjugated SPIONs have enhanced uptake in DCs via receptor-mediated endocytosis giving rise to 6X higher T2 contrast than non-targeted SPIONs [48]. Similarly, Baumjohann and the group showed SPIONs conjugated with protamine sulfate, a cationic transfection agent that formed molecular complexes via electrostatic interactions, directed internalized SPIONs to endosomal compartments in DCs [49]. This resulted in in vivo lymph node homing and strong T2 contrast in MRI. These findings suggest surface modification of SPIONs will ultimately govern the translation of SPIONs in monitoring response to immunotherapies in patients. In this effort, Crisci and coworkers labeled porcine monocyte derived DC (MoDCs) with SPIONs since domestic pigs share similarities with humans and represent an ideal model for immunological studies (Fig.3e) [50]. The authors showed that after a single subcutaneous dose of pulsed MoDCs, pigs were able to elicit specific local and systemic immune responses.
Figure 3. Immunoimaging with superparamagnetic iron oxide nanoparticles and magnetic resonance imaging (MRI) (a) Schematic representation of dendritic cell labeling with SPIONs for MRI tracking in vivo. (b) Synthesis of block copolymer coated SPIONs and corresponding (c) TEM images. (d) MRI images in mouse models in vivo of draining lymph nodes showing enhanced contrast from alkylated polyethleneimine and glycerol coated SPIONs. (e) Schametic describing the utility of SPIONs in larger animals for labeling porcine monocyte-derived dendritic cells and corresponding MRI image of draining LNs where arrow shows SPIONs accumulation. (f) Schematic of 40 nm SPIONs conjugated with OVA and CpG via PEGylated phospholipid micelles for ligand free $^{67}$Ga labeling. (g) These SNPs provided effective lymphatic delivery of the vaccines and SPECT/CT imaging to track the SNPs and (h) biodistribution expressed as % injected dose per gram of tissue. aLN, axillary LN; inLN, inguinal LN; iLN, iliak LN; pLN, popliteal LN; bLN, braquial LN. (a) – (c) were adapted with permission from John Wiley and Sons ref. [46], (d) was adapted with permission from Elsevier ref. [47], (e) was adapted with permission from Elsevier ref. [50], and (f) – (h) were adapted with permission from American Chemical Society ref [51].

In addition to DCs, the versatility of SPIONs has also enabled their use in imaging T cells in vivo. The utility of SPIONs in immunology emerges from an early work, where SPIONs modified with NH$_2$ moieties demonstrated enhanced T-cell internalization bypassing the use of
transfection agents or electroporation [52]. These results are not surprising as naturally-existing amine molecules, such as histamine and dopamine among others, may directly interact with immune cells by modulating their activation [53]. Brewer and coworkers showed that three classes of immune cells including CD8$^+$ cytotoxic T cells, regulatory T cells, and myeloid-derived suppressor cells can be monitored after labeling with SPIONs and changes in their migration can be measured in vivo following treatment with vaccine-based immunotherapy [54]. These literature evidences suggest SPIONs are highly adaptable SNPs with size and surface tunability, where surface functionality drives their ability to hone in a number of immune cells for tracking in vivo. Whereas most of these studies have used SPIONs as an MRI contrast agent, a unique work by Ruiz-de-Angulo and colleagues indicated that strong MR contrast in vivo typically requires very high doses of SPIONs [51]. Therefore, they adopted a unique design consideration, where 40 nm iron oxide NPs were coated with PEGylated phospholipid (PEG–PLs) micelles to promote direct attachment of Ga$^{3+}$ ions for in vivo LN targeting (Fig.3f). The micelles enabled radiolabeling with $^{67}$Ga radioisotopes without macrocyclic chelators for SPECT imaging, and conjugation of the model antigen ovalbumin (OVA) or pathogen mimetic CpG without affecting the overall size of SPIONs. SPECT imaging tracked the SNPs in vivo correlating their migration via the lymphatic system to reach LNs (Fig.3g). Their findings suggest SPIONS are well-internalized by antigen presenting cells via the endocytotic pathway and reach the LNs draining the site of injection and more distal LNs from different anatomical regions of injection (Fig.3h). The versatility of SPIONs, however, allows both MR and nuclear imaging simultaneously, where $^{64}$Cu labeled SPIONs enabled multimodal PET-MRI for targeted detection of effector T cells in vivo [55]. While the authors did not adopt any surface modification, they still achieved rapid T cell labeling, where cationic SPIONs migrated to negatively charged cell membranes and transiently permeabilized the
membrane barriers with controlled concentrations of dimethyl sulphoxide (DMSO). These studies collectively show that composition of SPIONs controls their imaging functionality but the surface charge and chemistry control their biological functionality for immune cell targeting.

**Organic Nanoparticles**

In addition to inorganic SNPs, a number of work recently have used organic NPs composed of phospholipids, polyglucose, lipoproteins, polypeptide or polymers for labeling TAMs and ICRs. Many organic NPs have been FDA approved given their high biocompatibility, colloidal stability, ability to encapsulate both hydrophobic and hydrophilic cargos which can be delivered in response to external stimuli, and ease of intracellular degradation resulting in rapid in vivo clearance. Immunoimaging efforts with organic NPs was demonstrated by Perez-Medina and coworkers who incorporated $^{89}$Zr radiolabels into high density-lipoproteins (HDLs) either in the protein component of HDL ($^{89}$Zr-Al-HDL) or in the phospholipids ($^{89}$Zr-PL-HDL) (Fig.4a) to longitudinally track TAMs [56]. Both formulations showed sufficient serum stability, high radiolabel stability (Fig.4b), and tumor uptake in orthotropic 4T1 mammary tumors as observed in PET (Fig.4c). HDLs were preferably internalized by TAMs relative to monocytes, T cells, and cancer cells. However, the small size of these SNPs, <9 nm, did not allow time-course study of TAMs due to rapid renal clearance. Longer residence in TAMs can be achieved by designing larger organic NPs such as liposomes. Locke and colleagues designed liposomes terminated with lipids that targeted mannose receptors (CD206) overexpressed on TAMs (Fig.4d). Mannose plays a pivotal role in clearance of mannose-bearing serum glycoproteins released at sites of inflammation [57]. Mannosylated DOTA-encapsulated liposomes were radiolabeled with $^{64}$Cu and demonstrated high tumor uptake relative to MPS organs.
Figure 4. Immunoimaging of tumor associated macrophages (TAMs) with organic nanoparticles. (a) Structure and composition of $^{89}$Zr labelled HDLs and (b) their serum stability. Radiotracers were either labelled in the protein components or phospholipids to enable (c) PET/CT imaging in mouse models. (d) A schematic diagram of mannose modified liposomes. (e) $^{64}$Cu- and VT680-labeled macrin were administered to image TAMs at 24h post-injection. Macrin are polyglucose NPs with all biodegradable components and L-lysine cross linker. (f) Reconstructed
PET/CT showing segmented lung tumors (blue and cyan) and macrin (orange). Representative tumors with high macrin uptake (cyan tumors with arrows) were further highlighted in corresponding transverse sections. (g) Synthesis of pNPs: PLGA(OVA/ICG); antigen presentation (ovalbumin; OVA) and monitoring DCs (indocyanine green; ICG), PLGA(R837/STAT3 siRNA); combined immunomodulation with R837 (for activation of TLR7) and STAT3 siRNA (for silencing of immunosuppressive genes, STAT3). (h) Scanning electron microscopy (SEM) images of PLGA NPs (scale bar 500 nm). (i) Schematic of fluorescent polymeric reporter SNPs where both effector and reporter elements were conjugated within the SNPs core. (j) Quantification of immunotherapy response to tumor measured with fluorescence intensity ratio between tumor and normal tissues at different days after the treatment compared to those measured with IgG-reporter SNPs. (k) Schematic showing synthesis of PEI and genipin cross-linked functional OVA SNPs for image-guided immunotherapy. (l) Fluorescence imaging of these SNPs showed accumulation in the lymph nodes of mice. (m) Combined OVA SNPs showed therapeutic effects with higher MHC-1, CD86 and CD80 expression in the CD11c+ DCs. (a) – (c) were adapted with permission from Society of Nuclear Medicine and Imaging ref. [56], (d) was adapted with permission from Elsevier ref. [57], (e) – (f) were adapted with permission from American Chemical Society ref. [58], (g) – (h) were adapted with permission from Elsevier ref. [59], and (i) – (j) were adapted with permission from National Academy of Sciences of the United States of America ref. [60], (k) – (m) were adapted with permission from American Chemical Society ref. [61].

In addition to lipid based SNPs, Weissleder and Miller demonstrated polyglucose NPs (macrin) conjugated with $^{64}$Cu were also highly effective in tracking TAMs in vivo with PET imaging (Fig.4e) [58]. Macrin differs from previous cross-linked dextran NPs as all components are biodegradable, the cross-linker is L-lysine, and biochemical characteristics are optimized to avoid rapid renal clearance and maximize accumulation in TAMs. Macrin exhibited slightly negative surface charge (-12 mV), which provided just enough colloidal stability without compromising recognition by MPS in vivo. PET/CT imaging at 24 h post macrin delivery in vivo identified macroscopic tumor lesions (Fig.4f), with 10X higher uptake in TAMs relative to tumors. This study showed selective uptake in well-defined TAM populations can be achieved with a SNP designed to target TAMs and paired with a synergistic immunoimaging toolbox.

Besides imaging, unlike inorganic SNPs, organic NPs have a unique advantage, where by encapsulating a therapeutic entity within the organic core they combine both diagnostic and therapeutic components often coined as theranostic. Such multifunctional SNPs therefore enable
highly accurate screening of immune receptors and subsequent image-guided immunotherapies in a single clinical procedure. The most extensively studied theranostic organic NPs for immune modulation are composed of poly (lactic-co-glycolic acid) (PLGA), an FDA-approved polymer with high biocompatibility and serum stability. Heo and coworkers showed highly versatile PLGA allows to encapsulate model antigen OVA with near-infrared fluorophore indocyanine green (ICG) to enable fluorescence guided DC tracking (Fig.4g) [59]. Their unique design approach allowed to improve the in vivo targeting efficacy of small interfering RNA (siRNA) and bioavailability of immune response modifier (imiquimod, R837) for the activation of DCs by encapsulating both in PLGA SNPs. By using a simple emulsion evaporation method, the authors showed that ~150 nm PLGA SNPs (Fig.4h) are an excellent carrier for both hydrophilic and hydrophobic biomolecules ideal for immunoimaging and immunotherapies. Polymer-based SNPs have a distinct advantage with an expansive design space and plethora of polymers available for manipulating both the surface and core properties of these SNPs. Kulkarni and coworkers demonstrated this concept with a unique biology-inspired engineering of a reporter SNP adopting a two-staged stimuli-responsive polymer that delivered an immunotherapy payload to the tumor and subsequently provided a real time therapy response [60]. The reporter SNPs (Fig.4i) consisted of three components: (1) a polymeric backbone from low-molecular weight poly(isobutylene-alt-maleic anhydride) (PIMA), (2) an esterase-cleavable prodrug synthesized from an anticancer drug (effector element) conjugated to the polymer, and (3) an activatable reporter element which was cleavable by caspase-3 to which a FRET pair was conjugated such that cleavage of the amino-acid sequence resulted in revival of the fluorescence signal (without that stimuli fluorescence was quenched). Time-course imaging showed strong fluorescence signal for the PD-L1 conjugated SNPs relative to IgG control SNPs (Fig.4j). Flow cytometry confirmed activation of both CD8+
and CD4+ T cells with these SNPs, and significantly higher T cell infiltration in tumors that received PD-L1 conjugated SNPs. A recent work also used organic SNPs to develop a nanovaccine with OVA antigen crosslinked with genipin-polyethyleneimine (PEI) and an outer OVA layer (Fig.4k), which demonstrated self-fluorescence from the crosslinked polymers [61]. PEI improves the immune response as an adjuvant of nanovaccine and allows to absorb the outer antigen with electrostatic forces. Genipin does not have an immune function but facilitates accurate tracking of the inner antigen with self-fluorescence when cross-linked with PEI without the need of extrinsic labels for imaging. DCs labeled with these OVA SNPs were tracked in vivo with multispectral fluorescence imaging to examine their migration to lymph nodes (Fig.4l). The combined OVA nanovaccines induced a stronger DC maturation compared to free OVA and OVA SNPs, as demonstrated by significantly increased CD86, CD80, and MHC-I of the CD11c+ cells (Fig.4m). These work collectively demonstrate that organic SNPs are an excellent platform for image-guided vaccine delivery and elicit robust and persistent antigen-specific immune responses with real-time tracking capabilities.

Conclusions and Future Directions

In summary, this review summarizes the progress in immunoimaging with three classes of SNPs including AuNPs, SPIONs, and organic NPs that have enabled us to track immune cells and detect ICRs in vivo, and allowed image-guided immunotherapies in the TME. Early detection of immunomarkers with NP labels will ultimately enable us to identify those who are responders, and determine alternative treatment plan for those pre-identified as nonresponders. Further, we have discussed the role of size, shape, surface change, and surface coating of SNPs that determine their vascular transport and accumulation in the TME, and ability to induce endothelial leakiness. Whereas most of these traditional properties of NPs have been well studied, how NP stiffness
(elastic modulus) contributes to cellular uptake remains poorly understood specifically in the context of the immune microenvironment. The elasticity of NPs plays a critical role in NP wrapping in the cell membrane and be endocytosed into endosomal vesicles that bud off from the cell membrane. However, the mechanical properties of NPs alone do not direct their preferential uptake or evasion in cells; this is also dictated by the cells they interact with. For example, endothelial cells readily uptake soft NPs with low elasticity, whereas macrophages internalize hard NPs (>1 MPa) [62] suggesting an additional degree of freedom to tune SNPs uptake in different immune cells. But as discussed previously, these properties are entangled and a systematic study is necessary to understand how these parameters can be optimized to design a library of SNPs for utility in immunotherapies. Experimental results will need to be combined with theoretical investigations to understand NP wrapping in cell membrane and the corresponding role of NP properties and surface ligand density in these processes [63, 64]. Calculations will reveal how coupling between membrane bending and stretching in the wrapping process dictates the membrane deformation energy, and how the SNP design coupled with the type of immune cell will drive the rate, and number of SNPs uptake in the immune TME.

We envision the importance of SNPs in the immunotherapy landscape will ultimately be interfaced with emerging areas such as tracking circulating immune cells which are associated with tumor location and differentiation [65], and hybrids of cancer cells fused with immune cells that potentiate tumor heterogeneity and stage [66, 67]. Further, single cell tracking of NP-labeled immune cells will reveal crosstalk between the different immune cells, and highlight signaling pathways within individual immune cells that are suppressed in the TME [68-71]. These fundamental understanding will ultimately result in novel vaccines with higher clinical efficacy. Image-guided approaches with SNPs could be extended to other therapeutic modalities, where
immunotherapies are combined with photothermal [72-74], photodynamic [75], and chemotherapies [76, 77] to induce adaptive immune responses and stimulate immunological memory. Therefore, SNPs with multiple functionalities can ultimately advance the drug development pipeline and significantly reduce the cost involved in translating these immunoagents from laboratory to human applications.

**Outstanding Questions**

(1) What design parameters need to be considered to synthesize smart nanoparticles that accurately monitor dynamic changes in immunomarkers during the course of immunotherapy?

(2) What are the restrictions and challenges that are currently hindering clinical translation of smart nanoparticles?

(3) What physicochemical properties of smart nanoparticles are most important to direct them to tumor-associated macrophages relative to macrophages resident in liver and spleen?

(4) What fundamental studies can be pursued with smart nanoparticles-labeled immune cells to reveal crosstalk between different immune cells in cancer pathogenesis?

(5) Does nanoparticle induced endothelial cell leakiness have a role in immunotherapies reaching poorly vascularized tumors?
Table 1. Imaging immune cells and immunomarkers with smart nanoparticles utilizing a broad array of clinical and preclinical imaging modalities to guide immunotherapies in cancer and other diseases.

<table>
<thead>
<tr>
<th>Target</th>
<th>Agent</th>
<th>Imaging modality</th>
<th>Summary</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cells</td>
<td>Gold nanoparticle (AuNP)</td>
<td>CT</td>
<td>Engineered T-cells that were transduced to express melanoma-specific T-cell receptors were loaded with AuNPs as a CT contrast agent. Correlated CT imaging with live fluorescence imaging revealed the accuracy of CT cell tracking abilities.</td>
<td>[35]</td>
</tr>
<tr>
<td>Highly derivatized</td>
<td>Highly derivatized cross-linked iron oxide nanoparticle (CLIO-HD)</td>
<td>MRI</td>
<td>CLIO-HDs allowed to track T cells and MRI imaging showed the recruitment of CLIO-HD-labeled OVA-specific CD8+ T cells to intact tumors in vivo. Furthermore, these CD8+ T cells were recruited in a heterogeneous manner, both spatially and temporally.</td>
<td>[78]</td>
</tr>
<tr>
<td>gold nanoparticle</td>
<td>64Cu superparamagnetic iron oxide nanoparticle (64Cu-SPION)</td>
<td>PET, MRI</td>
<td>SPIIONs conjugated with 64Cu were used for multimodal imaging to detect accumulation of labeled-T cells. MRI provided high-resolution anatomically correlated images and PET enabled high sensitivity and specificity.</td>
<td>[55]</td>
</tr>
<tr>
<td>functionalized with</td>
<td>Gold nanoparticle functionalized with 64Cu and PEG</td>
<td>PET/CT</td>
<td>CAR+ T cells in vivo biodistribution was tracked through labeling T cells with 64Cu-labeled AuNPs and PET/CT imaging.</td>
<td>[43]</td>
</tr>
<tr>
<td>64Cu</td>
<td>Ultrasmall superparamagnetic iron oxide (USPIO)</td>
<td>MRI</td>
<td>USPIO SNPs conjugated to the HIV tat peptide were used to label both murine and human CD4+ T cells, which showed suppressive responses and were tracked with MRI in vivo.</td>
<td>[79]</td>
</tr>
<tr>
<td>Iron oxide nanoparticles (IOPC-NH2)</td>
<td>Iron oxide nanoparticles (IOPC-NH2)</td>
<td>MRI</td>
<td>Fluorescent labeled iron-oxide SNPs were used to label rat and human T-cells and were used to detect immune cells at the allograft heart and lung in vivo with MRI.</td>
<td>[80]</td>
</tr>
<tr>
<td>Poly(lactide-co-</td>
<td>Poly(lactide-co-hydroxymethylglycolic acid) nanoparticle (pLHMGHA NP)</td>
<td>NIR, FLI</td>
<td>OVA antigen encapsulated and fluorescently labeled pLHMGHA SNPs showed sustained release of antigen in vitro and antigen cross-presentation by DCs to antigen-specific T cells. Fluorescence imaging showed the relocation of both antigen and SNPs from the injection site to the draining lymph nodes.</td>
<td>[81]</td>
</tr>
<tr>
<td>Iron oxide nanoparticle</td>
<td>Iron oxide nanoparticle</td>
<td>FLI</td>
<td>Iron oxide SNPs were coated with polyaspartamide derivatives to enhance their biocompatibility, stability, and fluorescence detection. The polymer coating allowed efficient binding to the surface of CD4+ T cells without internalization.</td>
<td>[82]</td>
</tr>
<tr>
<td>Nanoparticle Type</td>
<td>Identification</td>
<td>Imaging Modality</td>
<td>Description</td>
<td></td>
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<td>-------------------------------------------</td>
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<tr>
<td>Dendritic cells Upconversion nanoparticle (UCNP)</td>
<td>UCL</td>
<td>Antigen-loaded UCSNPs were used to label DCs, which were accurately tracked by <em>in vivo</em> UCL imaging. Antigen-specific immune response was observed.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mannose-chitosan-tumor cell lysate nanoparticle</td>
<td>FLI</td>
<td>Chitosan nanoparticles with surface mannose were loaded with tumor cell lysate generated from B16 melanoma cells. Fluorescence imaging revealed DC within the draining lymph nodes.</td>
<td></td>
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<tr>
<td>Ovalbumin nanoparticle (OVA NP)</td>
<td>FLI</td>
<td>A combined nanovaccine with OVA and labeled with the rhodamine B was tracked <em>in vitro</em> and <em>in vivo</em> with multispectral fluorescence imaging. The nanovaccine induced antigen-specific CD4+ and CD8+ T cell responses, and FLI was used to track vaccine delivery.</td>
<td></td>
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</tr>
<tr>
<td>Iron oxide-zinc oxide (Fe3O4-ZnO)</td>
<td>MRI, PL</td>
<td>SPIO core/ZnO shell SNPs were used as antigen delivery vehicles. Tumor antigens (CEA) were immobilized onto core-shell nanoparticles. PL of the ZnO allowed the SNPs to be examined using confocal microscopy. <em>In vivo</em> MRI revealed that the nanoparticle-antigen complex enhanced T-cell responses.</td>
<td></td>
<td></td>
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<tr>
<td>Radionuclide-embedded gold nanoparticle (RIe-AuNP)</td>
<td>PET/CT, CLI</td>
<td>RHe-AuNPs showed high radiosensitivity and excellent <em>in vivo</em> stability, which enabled dual nuclear and optical imaging. PET/CT and CLI were utilized to track migration of RHe-AuNPs labeled-DC to draining lymph nodes.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superparamagnetic iron oxide (SPIO)</td>
<td>MRI</td>
<td>SPIO-labeled DCs were injected intranodally in melanoma patients under ultrasound guidance. MRI allowed verification of the accurate delivery and monitoring of inter- and intra-nodal cell migration patterns.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radioiodine-124-labeled tannic acid gold core-shell nanoparticle (124I-TA-Au@AuNP)</td>
<td>PET/CT</td>
<td>124I-TA-Au@AuNPs with excellent radiochemical stability and biocompatibility were used for DC labeling and <em>in vivo</em> tracking of migration to lymphoid organs using PET/CT.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superparamagnetic iron oxide (SPIO)</td>
<td>MRI</td>
<td>SPIO-labeled DCs were tracked <em>in vivo</em> using cellular MRI. Labeling DCs with SPIO had no significant effect on DC viability, phenotype, or function.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quantum dot (QD)</td>
<td>FLI</td>
<td>QDs were selectively taken up by DCs <em>in vivo</em>, and could be used as a fluorescent marker for tracking of DC migration. Moreover, antigen-conjugated QDs enhanced T cell proliferation and cytokine production.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Material/Technique</td>
<td>Methodology</td>
<td>Details</td>
<td>References</td>
<td></td>
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<td>----------------------------------------------------------------------------------</td>
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<tr>
<td>Iron oxide nanoparticle (IONP)</td>
<td>PET/SPECT</td>
<td>IONPs coated with PEGylated phospholipid (PEG-PLs) micelles enabled radiolabeling with Ga³⁺ ions suitable for SPECT(⁶⁷Ga)/PET(⁶⁷Ga) imaging, and conjugation of the model antigen ovalbumin (OVA) or pathogen mimetic CpG without affecting the overall size of IONPs. SPECT imaging tracked the IONPs in vivo correlating their migration from the injection site to LNs.</td>
<td>[51]</td>
<td></td>
</tr>
<tr>
<td>Poly(lactic-co-glycolic acid) nanoparticle (PLGA NP)</td>
<td>FLI</td>
<td>PLGA NPs containing model antigen OVA and near-infrared enabled fluorescence imaging for DC tracking. These nanoparticles were taken up efficiently by DCs.</td>
<td>[59]</td>
<td></td>
</tr>
<tr>
<td>Macrophages</td>
<td>MRI</td>
<td>SPIO nanoparticles-enhanced MRI imaging was used to detect TAMs. Folate-engrafted nanoparticles were shown to enhance TAMs uptake.</td>
<td>[90]</td>
<td></td>
</tr>
<tr>
<td>⁸⁹Zr-dextran (⁸⁹Zr-DNP)</td>
<td>PET/CT</td>
<td>⁸⁹Zr-labeled DNP were used as probe for PET/CT imaging in a colon carcinoma model. ⁸⁹Zr-labeled DNP were biocompatible and accumulated specifically in tissue resident macrophages upon systemic administration.</td>
<td>[91]</td>
<td></td>
</tr>
<tr>
<td>⁸⁹Zr-apolipoprotein A-I-high density lipoprotein (⁸⁹Zr-AI-HDL) and ⁸⁹Zr-phospholipid-high-density lipoprotein (⁸⁹Zr-PL-HDL)</td>
<td>PET</td>
<td>High-density lipoprotein loaded with ⁸⁹Zr was used to track TAMs in a breast cancer model with PET imaging.</td>
<td>[56]</td>
<td></td>
</tr>
<tr>
<td>Mannosylated Liposome (MAN-LIP)</td>
<td>PET</td>
<td>MAN-LIPs were loaded with ⁶⁴Cu to allow tracking by PET imaging, and exhibited high tumor uptake.</td>
<td>[57]</td>
<td></td>
</tr>
<tr>
<td>⁶⁴Cu-Macrin (⁶⁴Cu-Macrin)</td>
<td>PET</td>
<td>⁶⁴Cu-labeled polyglucose nanoparticles (macrin) enabled in vivo PET imaging of TAMs. PET studies were complemented with confocal fluorescence microscope and flow cytometry. The studies confirmed macrin was predominantly uptake by TAMs.</td>
<td>[58]</td>
<td></td>
</tr>
<tr>
<td>B cells</td>
<td>NIR FLI/MRI</td>
<td>Dextran-coated SPIOs were used for B cells labeling and tracking. SPIO-labeled B cells were injected into mice for in vivo MRI and NIR fluorescence imaging to study B cell trafficking and distribution.</td>
<td>[92]</td>
<td></td>
</tr>
<tr>
<td>PD-L1</td>
<td>SERS</td>
<td>AuNS conjugated with both antibodies and Raman tags were used to diagnose PD-L1 and EGFR in breast cancer tumors in vivo using longitudinal SERS and ex vivo with Raman mapping.</td>
<td>[22]</td>
<td></td>
</tr>
</tbody>
</table>
Gold nanoparticles (AuNP) & CT & AuNPs served as contrast agents for CT imaging, and enabled noninvasive, longitudinal tracking of immunomodulators in tumors. CT imaging with αPDL1-AuNPs allowed early prediction of checkpoint blockade therapy responses. & [37]

Reporter nanoparticle & FLI & Reporter nanoparticles allowed simultaneous delivery of therapeutic agents and real-time fluorescence imaging to monitor treatment efficacy. & [60]

Myeloid-derived suppressor cells (MDSCs) & CyAL5.5-feraheme (CyAL5.5-FH) & NIR FLI & The CyAL5.5-FH superparamagnetic and fluorescent nanoparticles were used to understand immune cell function and TME using fluorescence imaging by probing MDSCs. & [93]

CD44v6 & SPION coated with oleic acid-carboxymethyl dextran (CMD) and antiCD44v6 & MRI & SPIONs were coated with OA and CMD conjugated with antiCD44v6 antibody to improve saturation magnetization, and to enhance MRI detection of lung cancer metastasis. & [94]

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a. Acronyms for imaging modalities: CT = computed tomography; MRI = magnetic resonance imaging; PET = positron emission tomography; PL= Photoluminescence; NIR = near-infrared; FLI = fluorescence imaging; UCL = upconversion luminescence; CLI = Cerenkov luminescence imaging; SERS = surface enhanced Raman spectroscopy.
References