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REVIEW

Emerging insights into intravital imaging, unraveling its role in cancer immunotherapy

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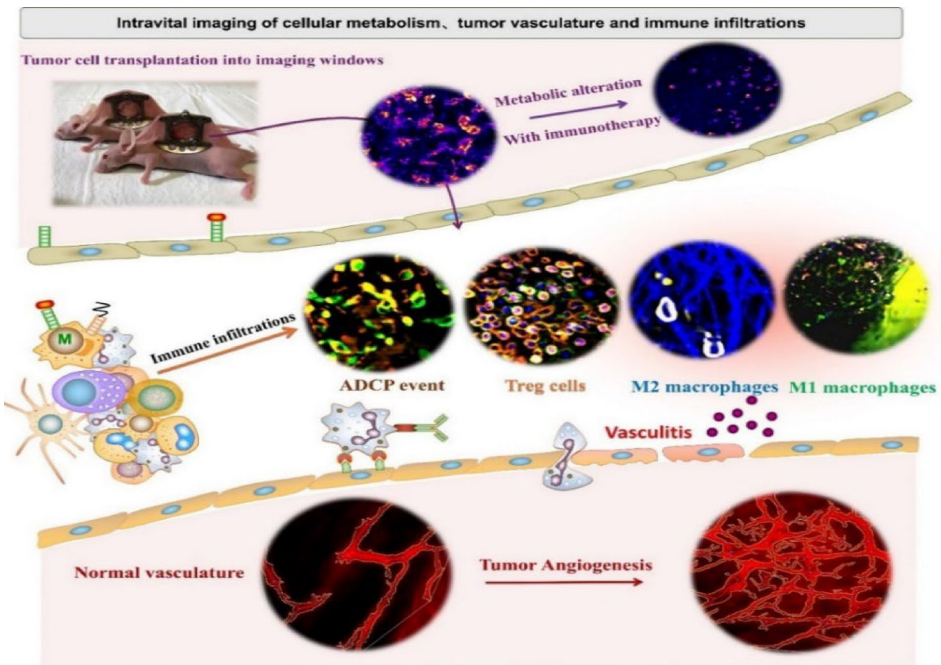
Abstract

Cancer immunotherapy has attracted great attention as a potential therapeutic approach for advanced malignancies due to its promising survival benefits. Comprehension of intricate interactions between the tumor microenvironment (TME) and immune checkpoint inhibitors (ICIs) is crucial for optimizing and improving immunotherapies. Currently, several experimental strategies are available to monitor this complexity but most of them fail to facilitate real-time monitoring of the immune response such as cellular phagocytosis and cytolysis. Consequently, the application of intravital imaging has been extensively studied in the domain of cancer immunotherapy. Intravital imaging has been proven to be a powerful real-time imaging modality that provides insights into intratumoral immune responses, cellular metabolic signatures, tumor vasculature, and cellular functions. This review aims to provide a comprehensive overview of the latest research on intravital imaging in cancer immunotherapy, especially addressing how intravital imaging sheds light on essential features of tumor immunity, immune infiltrations, tumor angiogenesis, and aids in the clarification of underlying immunotherapeutic mechanisms. Moreover, a variety of labeling tools, imaging windows and models for real-time visualizations of TME are also summarized. We will also investigate the full potential of using intravital imaging to circumvent the limitations of currently available imaging modalities, which hold promise to advent efficient immunotherapy for cancer patients.

Graphical abstract



Extended author information available on the last page of the article

Keywords Intravital imaging · Immunotherapy · Tumor angiogenesis · Tumor immunity · Imaging windows

Abbreviations

AIW	Abnormal imaging window
anti-TRP	Tyrosinase related protein
ADCP	Antibody-dependent cell-mediated phagocytosis
ADCC	Antibody-dependent cell-mediated cytotoxicity
Anti-HER2	Anti-human epidermal growth factor receptor 2
Anti-EGFR	Anti-epidermal growth factor receptor
Anti-TRP	Anti-tyrosinase-related protein
Anti-CSF1	Anti-colony stimulating factor 1
CIW	Cranial imaging window
CTLs	Cytotoxic T lymphocytes
CTLA-4	Anti-cytotoxic T lymphocyte-associated protein-4
CDC	Complement-dependent cytotoxicity
Ccr2	C-C chemokine receptor type 2
CNS	Central nervous system
CRISPR/Cas9	Clustered regularly interspaced short palindromic repeats
DSC	Dorsal skinfold window chamber
DsRed-2-RFP	Discosoma sp. coral-2-red fluorescent protein
DCs	Dendritic cells
DISC	Dynamic in situ cytometry
ECM	Extracellular matrix
EGFP	Enhanced green fluorescent protein
ECFP	Enhanced cyan fluorescent protein
EYFP	Enhanced yellow fluorescent protein
^{18}F-FDG PET	Fluorine-18 fluorodeoxyglucose positron emission tomography
FDC	Follicular dendritic cells
FITC	Fluorescein isothiocyanate
FAD	Flavin adenine dinucleotide
GFP	Green fluorescent protein
ICIs	Immune checkpoint inhibitors
IVM	Intravital microscopy
KCs	Kupffer cells
LMII	Label-free metabolic intravital imaging
LIPSTIC	Labeling immune partnership by sorting intercellular contacts
MRI	Magnetic resonance imaging
MIW	Mammary imaging window
Mo	Monocytes
MMTV-PyMT	Mammary tumor virus-mammary gland specific polyomavirus middle T antigen
mAb	Monoclonal antibodies
mCFP	Cyan fluorescent protein
mYFP	Membrane-targeted yellow fluorescent protein
N	Neutrophils
NIRF	Near-infrared fluorescent
NAD(P)H	Reduced nicotinamide adenine dinucleotide (phosphate) hydrogen
NIR	Near-infrared
OX40	Tumor necrosis factor receptor
OXPHOS	Oxidative phosphorylation
PET	Positron emission tomography
PD-1	Anti-programmed cell death 1
PD-L1	Programmed cell death ligand 1
PE	Phycoerythrin
RFP	Red fluorescent protein
SPECT	Single photon emission computed tomography
SHG	Second harmonic generation
SCCHN	Squamous cell cancer of head and neck
TME	Tumor microenvironment
TILs	Tumor-infiltrating lymphocytes
TAMs	Tumor-associated macrophages
Treg	Regulatory T lymphocytes
TAMs	Tumor-associated macrophages
US	Ultrasound
VEGF	Vascular endothelial growth factor

Introduction

While there have been notable advancements in tumor immunotherapy in recent years, we still have a poor understanding of how the immune system functions within the intricate TME and how effectively harness immune cells against different types of tumors. Current immunotherapies are only effective for certain patients and specific cancers, highlighting the need for a comprehensive mechanistic understanding to enhance treatment efficacy and develop new strategies. Various methods are available to investigate these mechanisms. Techniques like manipulating specific immune cell types (*e.g.,* using knockout models or antibody-based depletion) or genetically altering tumor cells are commonly employed in preclinical models. For human cases, advanced technologies such as high-dimensional flow cytometry or single-cell RNA sequencing are used to establish essential correlations and propose potential mechanisms for further exploration [1]. However, most of these strategies have not directly tracked the

immune reactions within tumors, such as cell–cell interactions, intratumoral cell migration and signaling pathways. Understanding how these dynamic events are influenced by immunotherapies could reveal in vivo mechanism of cancer immunotherapy and complement the inability of in vivo immune cell tracking using macroscopic imaging techniques such as positron emission tomography (PET), magnetic resonance imaging (MRI), ultrasound (US) imaging and single photon emission computed tomography (SPECT) [2–5].

Fluorine-18 fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET) has become a widely used technique for imaging immunotherapeutic efficiency in clinical oncology, enabling earlier assessment of treatment response and early detection of brain disorders [6]. Radiolabeled probes as a promising agent for targeting immune biomarkers may help address some of the limitations associated with tumor biopsies. These agents, imaged using single photon emission computed tomography (SPECT) or PET/CT, enable in vivo, real-time, and non-invasive imaging of tumor biomarker expression as well as immune responses to novel therapies. Notably, PET/CT is a highly sensitive and quantitative imaging modality that facilitates the evaluation of both primary tumors and their associated metastases. Furthermore, PET/CT imaging can be repeated at multiple time points, allowing for the longitudinal monitoring of tumor responses. However, nuclear imaging techniques are primarily constrained by the use of ionizing radiation, limited spatial resolution, and the capacity to detect only a single marker at a time [7]. In contrast, hyperpolarized MRI, utilizing ^{13}C-lactate or ^{13}C-pyruvate, has garnered considerable attention for monitoring cancer progression and immunotherapeutic response, owing to its enhanced sensitivity and multiplexing capabilities. Despite these advantages, its application remains limited by challenges in single-cell tracking due to insufficient spatial resolution [7]. Intravital microscopy (IVM) is a developed optical imaging technique that enables deep tissue imaging without the need for fixation or sectioning. This method facilitates the examination of the dynamic behavior of cells under both physiological and pathological conditions in living organisms. Moreover, this innovative technique offers significant potential for immunotherapy research by providing new platforms to investigate cellular responses to immune checkpoint inhibitors in vivo. As such, this study presents a comprehensive analysis of the currently available IVM, in vivo imaging window chambers and models. It also explores the potential of in vivo imaging to enhance our understanding of tumor heterogeneity, tumor angiogenesis, the response to immunotherapy, and immunosuppression. The application of intravital imaging is anticipated to increase in response to recent achievements in cancer immunotherapy.

Current available microscopes for in vivo imaging

Tissues are heterogeneous in terms of their cellular and non-cellular components, as well as the dynamic interactions between these elements. To investigate the behavior and fate of individual cells within these complex tissues, in vivo imaging has been developed to visualize intact, living tissues at cellular and subcellular resolution. The optical microscope is the fundamental tool used in in vivo imaging, with standard fluorescence microscopy commonly employed for imaging tissue sections and conducting immunohistochemical analyses to diagnose disease and determine cancer types. However, the laser scanning confocal microscope and two-photon microscope are the primary tools for in vivo imaging at high spatiotemporal resolution. Laser scanning confocal microscopes generate high-quality images using point and line scanning strategies and are primarily utilized to observe cellular interactions and their associated three-dimensional distributions. To meet the increased imaging speed required for visualizing living cells, spin-disk confocal microscopy was developed, significantly enhancing imaging speed while maintaining image quality. Unlike conventional single-point confocal scanning, spin-disk confocal microscopy employs a multi-point, simultaneous scanning mode, centered around a Nipkow turntable. This method is particularly effective for imaging organs influenced by respiratory motion and heartbeat-induced jitter. The two-photon microscope, on the other hand, produces high-quality images with minimal sample damage by reducing photon absorption, background fluorescence, and photobleaching, while enhancing longitudinal resolution. Consequently, it is highly valuable for deep tissue imaging in in vivo settings. Collectively, these three microscopy techniques enable the acquisition of real-time, longitudinal, and three-dimensional images, providing detailed insights into the dynamic behavior of immune cells within specific tissue regions, including information of morphology, movement, migration, and cell–cell interactions. Such high-resolution spatiotemporal dynamic information is crucial for understanding the participation of multiple cell types in key events during immune responses. Therefore, advancements in microscopy for in vivo imaging have significantly enhanced our understanding of the immune response.

Imaging windows and models

The main focus of cell and molecular biology research has been the examination of cells in laboratory dishes and the analysis of cellular constituents, such as genes and proteins. Direct ex vivo labeling is an effective method for

labeling immune cells and monitoring immune infiltrates in vivo, whereas the use of reporter gene systems (e.g., fluorescence based) is in fact a constantly growing field with application both in basic and preclinical research. Researchers worldwide are now isolating various fluorescent proteins with different spectral properties, particularly green fluorescent protein (GFP) and red fluorescent protein (RFP) [8]. These commercially available fluorescent protein genes (i.e., GFP, Discosoma red fluorescent protein (DsRed), YFP) can be used as reporters in the engineering of transgenic animals and cell lines. They are driven by the promoter of genes that are of interest. Tumor cells from patients can be orthotopically or ectopically implanted into immunodeficient mice, such as cell line-derived xenograft models or patient-derived xenograft models, for the microscopic study of antitumor immunity (Fig. 1). Transgenic mice that are designed to spontaneously develop tumors, such as B-cell lymphoma models in Em-myc mice [9] and breast cancer models in MMTV-PyMT mice [10], have been used for observing immune responses during tumor formation. Therefore, fluorescent tracking techniques have been integrated into targeted animal models to facilitate the development of innovative imaging methods for spontaneously growing tumors.

Intravital imaging has also been carried out in a variety of anatomical organs in tumor-bearing mice, including the lung, breast, brain, head and neck, skin, pancreases, liver, lymph nodes, spinal cord and kidney (Fig. 2) (Table 1). Imaging window chambers provide the opportunity to capture images of a single location at different times, thereby offering valuable insights into the progression of a particular immune response within the same organ [11–16]. Figure 2 presents several cancer models and anatomical imaging windows that can be used for intravital imaging research. Mice aged 8 to 10 weeks were implanted with a dorsal skinfold window chamber (DSC), abnormal imaging window (AIW), or cranial imaging window (CIW), mammary imaging window, heart imaging windows, lung imaging window, pancreases imaging window, spinal cord imaging window and head and neck imaging window (Fig. 2A–I). Cancer cells were injected under the fascia and allowed to grow for ~ 1 week. Tumor growth was monitored by intravital fluorescence microscopy and visualization of vasculature color. Table 1 provides a summary of different tumor models, anatomical sites, and types of immune cells that have been the subject of studies on intravital imaging.

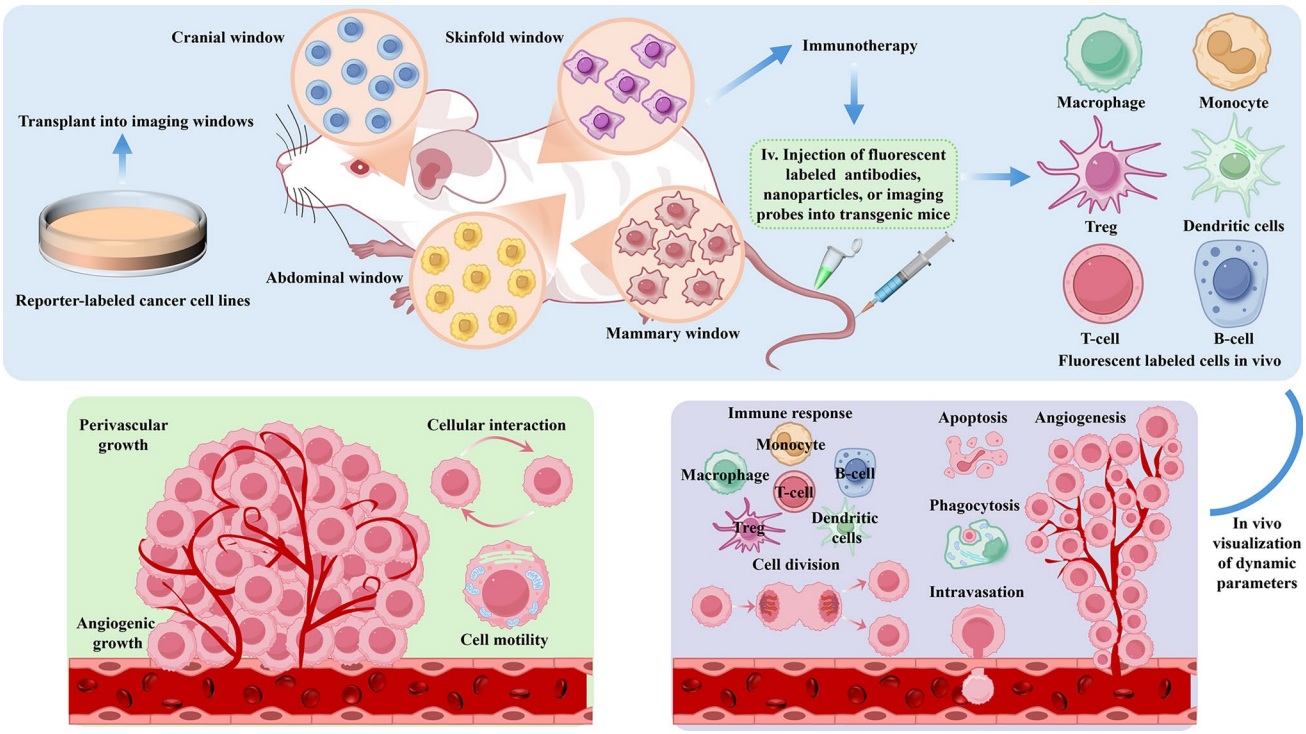


Fig. 1 The diverse dynamic parameters of the TME that can be collected utilizing intravital imaging. Superficial mammary and subcutaneous tumors can be observed using skinfold windows and mammary imaging windows. These techniques have accelerated research into the dynamics of angiogenesis during tumor growth; the immune response involving TAMs, CD8 + T cells, Tregs, and DCs against tumor cells; and the intravasation of tumor cells facilitated by cancer-associated fibroblasts. This imaging technique has also enabled the analysis and monitoring of cell division, cell motility, cellular interactions, and phagocytosis event

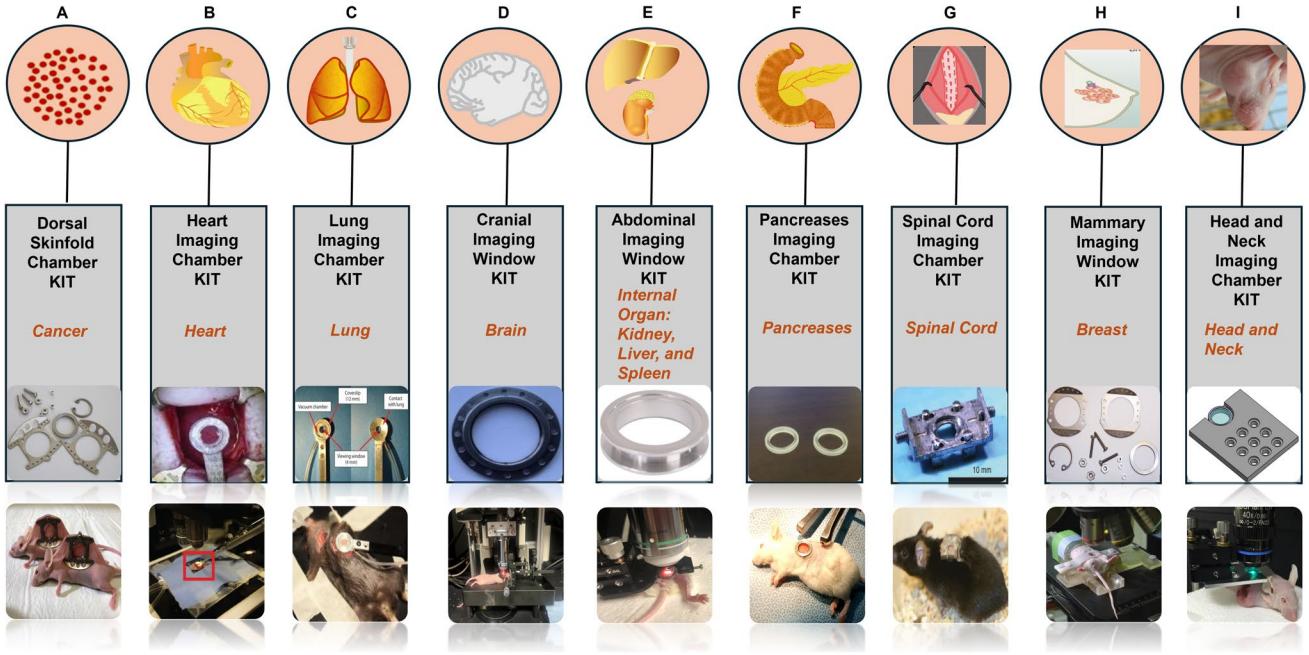


Fig. 2 Window design and implementation. **A** Dorsal skinfold chamber for the monitoring of cancer progression. **B** Heart imaging windows; **C** Lung imaging window; **D** Cranial imaging window; **E** Abdominal imaging window for intravital imaging of internal organs including kidney, liver and spleen; **F** pancreases imaging window; **G** spinal imaging window; **H** mammary imaging window; **I** head and neck imaging window

Intravital imaging of dynamic parameters within the TME

Reporter-labeled cancer cell lines are frequently transplanted into various imaging windows, such as AIW, DSC, MIW, or CIW, to facilitate simultaneous multicolor "rainbow imaging" of dynamic processes in living cells both in vitro and in vivo (Fig. 1). An exemplary instance of recent cell biology research involves the use of dual-color fluorescence imaging. This technique entails transplanting tumors that express red RFP into transgenic mice that express GFP [8]. Likewise, Yang.et.al transplanted 4T1_ PB3R-RFP cancer cells into the skinfold window of female BALB/c mice, which was subjected to intravital imaging 1 week after tumor inoculation. The researchers successfully visualized 4T1_ PB3R-RFP cancer cells (red) and second harmonic generation (SHG) for up to one month (Fig. 5B). These models vividly reveal the details of tumor-stroma interactions and immune responses, including immune infiltration, tumor angiogenesis, intravasation, cell motility, apoptosis, and division (Fig. 1). There are substantial ongoing investigations to visualize these dynamic parameters via intravital imaging. For example, Hoffman et al. developed a dual-color PC-3 human prostate cancer cell line that stably expresses histone H2B-GFP in the nucleus and Discosoma sp. Coral (DsRed-2-RFP) in the cytoplasm [8]. Simultaneous dual-color real-time fluorescence imaging enables the identification of cells in different phases of the cell cycle by analyzing their nuclear-cytoplasmic ratios. Live apoptotic cells have been identified by their nuclear morphology. This dual-color cell line facilitates the establishment of a mouse imaging window for imaging apoptosis and the cell cycle in vivo in real time [8]. Similarly, Jeffrey et al. utilized multiphoton microscopy to demonstrate that tumor cell intravasation is associated with perivascular macrophages in mammary tumors. Additionally, they reported that perivascular macrophages in mammary tumors are related to tumor cell intravasation even without local angiogenesis [17]. These preclinical studies support intravital imaging as a robust tool for real-time visualization of dynamic parameters within the TME.

Intravital imaging of immune components

The clinical success of cancer immunotherapy highlights the significance of immune cell imaging, particularly non-invasive techniques to evaluate the function and density of T cells and TAMs to monitor the therapeutic responses [48, 49]. Therefore, there is an urgent need for the development of new technologies for the in vivo visualization of immune components during immunotherapy.

Table 1 Anatomical sites, tumor models, tumor vasculature, immune and cancer cells utilized in intravital imaging studies

Window chambers Anatomical sites	Tumor model	Cancer cells, immune cells and angiogenesis	Window chamber	References
Cranial	CT2A and GL261 murine glioma	TAMs and tumor-associated vasculature	CIW	[16]
	Murine calvaria bone marrow models	Mo		[18]
	Murine encephalitis model, EIA	T		[19]
Heart	Beating heart of ubiquitin-GFP mice	leukocytes		[20]
Lung	Athymic NCR nude mice (nu/nu)	Dual-color HT1080 and dual-color MMT cells		[21]
	MCA-205 murine fibrosarcoma	T		[22]
	4T1 murine breast cancer orthotopically	N		[23]
	MacBlue mice B6	Tumor-associated vasculature	Miniature vacuum-stabilized imaging window	[24]
Skin	4T1 murine breast cancer	CD4 + T cells, DC, TAMs	DSC	[25]
	4T1 murine breast cancer	TILs		[26]
Mammary fat pad	Orthotopic breast cancer	Angiogenesis	MIW	[27]
	4T1 murine breast cancer	TAMs and tumor-associated vasculature		[28]
	Female MMTV-PyMT/c-fms-EGFP mice	TAMs and DC		[29]
Pancreas	Pancreatic cancer	Tumor-associated vasculature	DSC	[30]
	GFP-Kras^{G12D}-PDECs orthotopically	DCs, CTLs, Tregs		[31]
Colorectal cancer	SL4 or CT26 murine colorectal cancer	Mo	AIW	[15]
Ear	Dorsal ear of the BALB/c mouse	Vasculature of the ear dermis	DSC	[32]
	C57BL/6 mouse ear dermal vasculature	Vasculature of the ear dermis		[33]
Liver	Em-myc murine B cell lymphoma	B, KCs		[34]
	C26 cell-injected spleens of BALB/c mice	C26 cells	AIW	[35]
Lymph nodes	Type I diabetic NOD mice	Tregs		[36]
	B16.F0 murine melanoma	TAM, FDC, B		[37]
	Inguinal lymph node in the BALB/c mouse	T and B	AIW	[38]
Kidney	EL4 murine thymoma	DC and CTL		[39]
Spinal cord	Transgenic mice expressing enhanced YFP (EYFP) in afferent axons	Axons and microglia	Spinal cord imaging window	[40]

	Type	Application	References
Transgenic mice	Actb-EGFP C57BL/6	Visualization of immunomodulatory and inhibitory functions of hepatic sinusoidal endothelial cells	[41]
	CD11c-YFP OTI-DsRed/CFP/YFP	Monitoring of the interaction between DCs and T cells	[42]
	DPE-GFP	TAMs-mediated resistance pathway in anti-PD-1 immunotherapy	[43]
	Ccr2 − / − mice	Immunosuppressive behavior of Ly6Clo monocytes	[15]
	C57BL/6-Tg (CAG-EGFP) 1Osb/J	Observation of antigen-containing microparticles	[44]
	Hu-Mouse	The utility of humanized mice for testing naturally derived biomaterials	[45]
	WAP-Myc	Monitoring of changes in the TME in a breast cancer model with spontaneous metastasis	[46]
	Cxcr6 + /GFP	Analysis of the infiltration process of lymphocytes	[47]

Abbreviations: TAMs, tumor associated macrophages; Mo, monocytes; CIW, Cranial imaging window; N, Neutrophils; DSC, dorsal skin fold chamber; TILs, tumor-infiltrating lymphocytes; AIW, abdominal imaging wind; DCs, dendritic cells; CTLs, cytotoxic T lymphocytes; KCs, Kupffer cells; FDC, follicular dendritic cells; Treg, regulatory T lymphocytes; MMTV-PyMT: mammary tumor virus-mammary gland specific polyomavirus middle T antigen; EYFP, enhanced yellow fluorescent protein; EGFP, enhanced green fluorescent protein; CFP, cyan fluorescent protein; Ccr2, C-C chemokine receptor type 2

Immune checkpoint inhibitors (ICIs)

Targets of ICIs include anti-cytotoxic T lymphocyte-associated protein-4 (CTLA-4), CD40, anti-programmed cell death 1(PD1), programmed cell death ligand 1 (PD-L1), lymphocyte-activation gene 3 (LAG-3), ecto-5'-nucleotidase (CD73), CD47, CD24, tumor necrosis factor receptor superfamily member4 (OX40), and CD137 [46]. The 2018 Nobel Prize in Physiology or Medicine was awarded to the scientists who discovered CTLA-4 and PD-1. Inhibitors targeting PD-1, PD-L1, and CTLA-4 are among the most successful clinical immunosuppressive

therapies [50]. Yet, a major obstacle to immunotherapy is that only a small fraction of cancer patients may respond to ICIs. The highest response rate can be as high as 50% in MSI-H tumors and melanoma, but it is less than 10% in gliomas [51]. Moreover, there is no complete understanding of the dynamics of immunotherapies. Intravital imaging has demonstrated that ICIs can greatly affect the intratumor dynamics of TAMs and T cells. For example, Yang.et.al demonstrated that the engulfment of NAD(P)H^{hi} CD47-AF488 + cancer cells by F4/80-PE macrophages were imaged in a skinfold triple-negative breast cancer model with time-course anti-CD47 mAb immunotherapies. Such intravital imaging provides the first in vivo evidence of CD47 blockade-mediated antibody-dependent cellular phagocytosis (ADCP) (Fig. 3 and 5C–D). Lau et al. conducted a study using intravital imaging in a mice melanoma model to observe changes in the morphology and migration of CD8 + T cells before and after treatment with anti-PD-L1. The results showed that CD8 + T cells at the tumor edge exhibited Lévy-like movement with a significantly higher movement rate compared to those near the tumor tissue, optimizing their chances of encountering tumor cells. After the anti-PD-L1 injection, the amount of T cells in the tumor tissue increased while their movement rate decreased sharply. Thus, this study underscores the value of intravital imaging in understanding intratumor T cell dynamics during anti-PD-L1 immunotherapy [52]. Another study of anti-CTLA-4 mAb immunotherapy in

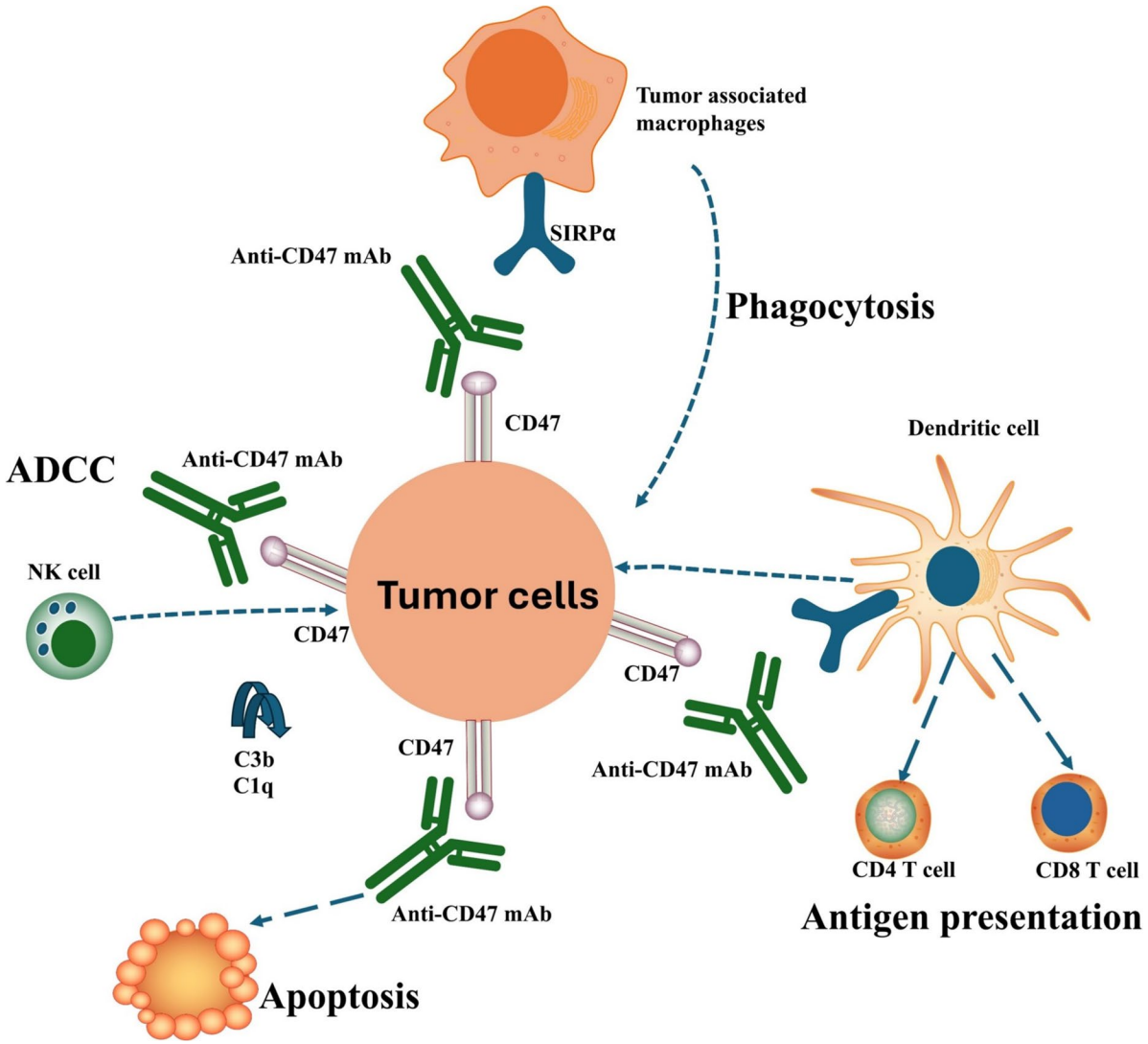


Fig. 3 CD47 mAb binds to their CD47 receptor on tumor cells and thereby induce the killing of these cells by complement-dependent cytotoxicity as well as via macrophage medicated ADCP and NK cells medicated ADCC, both of which require the interaction of Fc receptors on the effector cells with the Fc domain of the bound mAbs. Many studies have shown that the interaction of CD47 on cancer cells with SIRPα on macrophages serves to inhibit ADCP and ADCC events

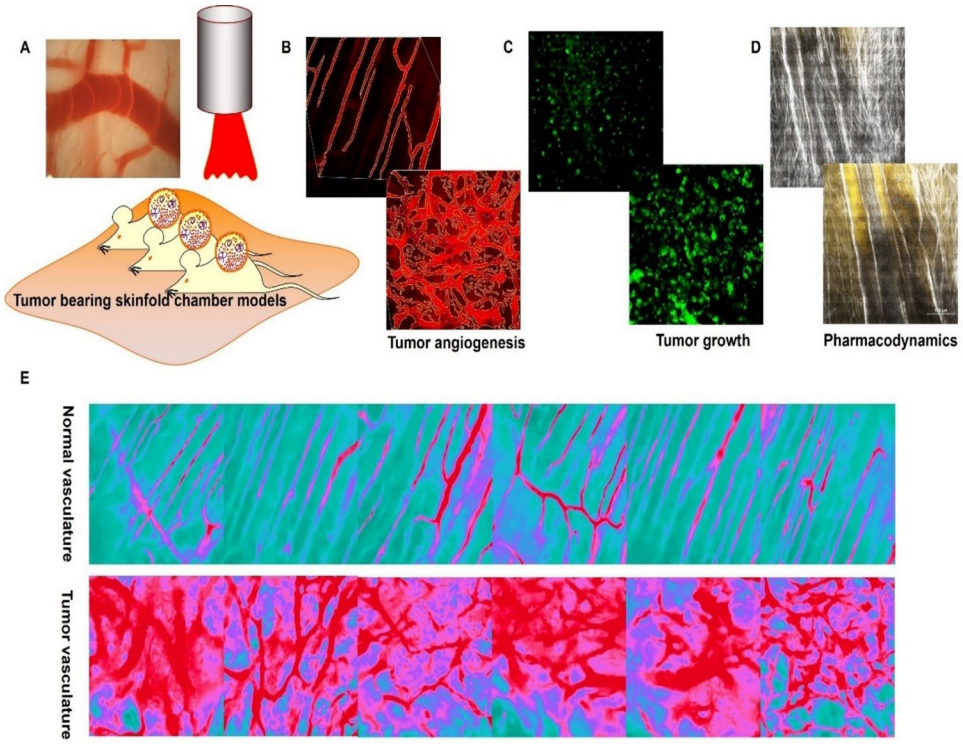


Fig. 4 The normal vasculature, tumor vasculature, tumor growth, and tumor angiogenesis in the TME. **A** Tumor-bearing skinfold chamber models; **B** Tumor angiogenesis in tumor-bearing skinfold models with tumor cell transplantation; **C** Cancer cells (green); **D** Pharmacodynamics of imaging probe in tumor vessels (yellow). **E** Normal and tumor vasculature in the skinfold window models (red)

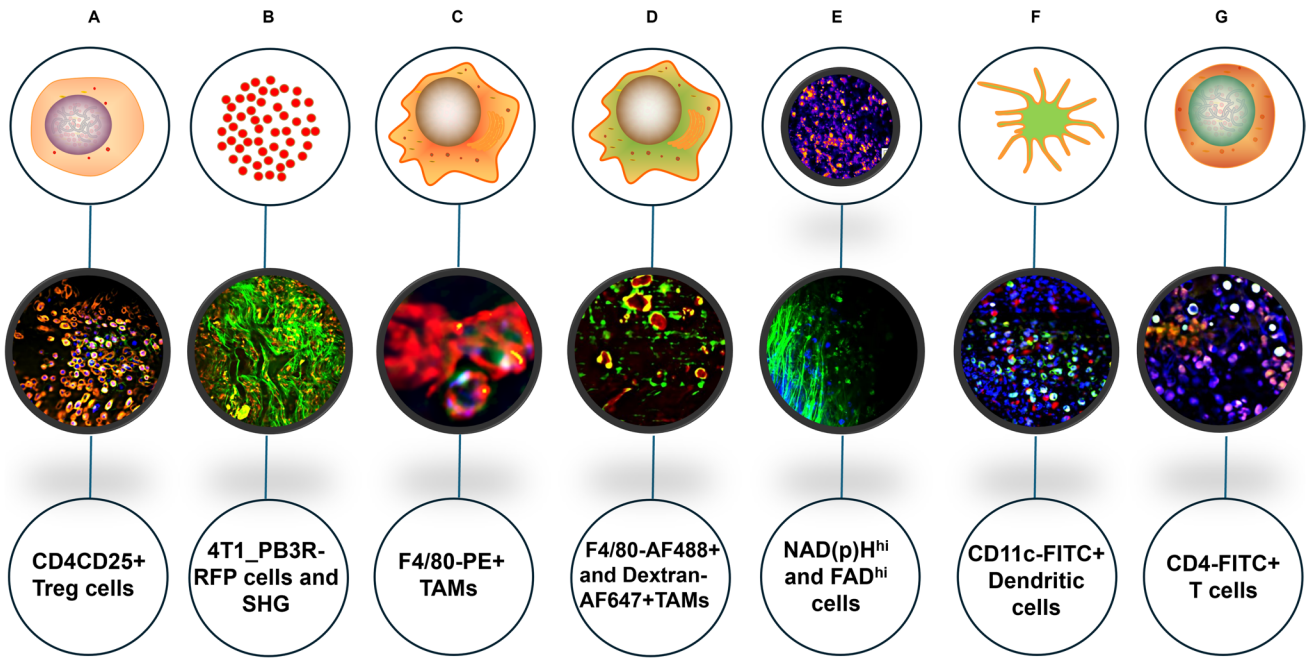


Fig. 5 Intravital imaging of immune components. **A** Intravital imaging of CD4 + CD25 + regulatory T cells in triple-negative breast cancer models. **B** Overlap between 4T1_PB3R-RFP cells (red) and NAD(P)H^{hi} cells (green). **C** Engulfment of NAD(P)H^{hi} (blue) and CD47-AF488 + cancer cells (green) by F4/80-PE + macrophages (red) were clearly observed, indicating macrophage-medicated ADCP events. **D** Co-localizations between F4/80-AF488 + TAMs (green) and Dextran AF647 targeted TAMs were clearly visualized. **E** Label-free metabolic intravital imaging of NAD(P)H and FAD in TME provides endogenous stromal and cancer cell contrast. Collagen was visualized as fibrillar structures through second harmonic generation (SHG). **F** Intravital imaging of CD11c-FITC + cells (green), NAD(P)H^{hi} cells (blue), and 4T1_PB3R-RFP + cells (red). **G** Intravital imaging of CD4-FITC + cells in skinfold triple-negative breast cancer model

4T1 and B16 cancer models seemed to enhance the overall motility of CD8 + T cells, though the underlying mechanisms remain unclear [53]. Arlauckas et al. performed in vivo imaging to reveal that the Fc receptor on myeloid cells significantly influences the efficacy of PD-1 inhibition [54]. They found that PD-1 antibodies transferred from T cells to PD-1-negative tumor-associated macrophages shortly after binding. This interaction occurs between the glycosylated Fc region of the PD-1 antibody and the Fc receptor on myeloid cells. Blocking the Fc receptor significantly improved the therapeutic effects of ICIs, suggesting that the Fc receptor is a critical factor in the low response rate of PD-1 inhibitor treatments [54].

Tumor-targeting antibodies

Monoclonal antibodies (mAb) treatments have been widely applied in clinical setting with better safety profiles and robust successes in reducing tumor burden [55]. Tumor-targeting mAb may pursue in several ways, including antibody-dependent cell-mediated cytotoxicity (ADCC) (Fig. 3), complement activation, and inhibition of signaling pathways [56]. Studies have reported the clinical use of anti-human epidermal growth factor receptor 2 (anti-HER2) mAb, anti-CD20 mAb and anti-epidermal growth factor receptor (anti-EGFR) for targeting certain breast cancers, B lymphomas, squamous cell cancer of head and neck (SCCHN), and colorectal cancers. Several in vivo mechanisms of action have been proposed for mAbs, such as ADCP (Fig. 3 and 5C–D), complement-dependent cytotoxicity (CDC), and ADCC [57]. Nevertheless, the specific mechanisms by which mAb eliminates tumor cells in living organisms and the specific anatomical areas where these mechanisms take place are still unknown. Intravital imaging is a robust tool for addressing these issues. A recent study showed that three distinct anti-EGFR mAbs, i.e., panitumumab, zalutumumab, and cetuximab, were equally effective in opsonizing tumor cell lines in vitro. In addition, all three mAbs promoted ADCP in cancer cells via liver macrophages (KCs), as observed through intravital imaging [58]. Another promising study also identified the key mechanism of anti-CD20 treatment as KCs medicated ADCP events in a spontaneous lymphoma mouse model and established an experimental framework for enhancing the therapeutic efficacy of mAbs [34]. Furthermore, the imaging of KCs revealed their ability to engulf C26 colon carcinoma cells and B16 melanoma cells after being treated with anti-EGFR and tyrosinase-related protein (anti-TRP). This process effectively decreases the risk of liver metastasis in animals [59]. Although numerous studies have addressed circulating tumors, the specific effects of mAbs on tumors originating from lymphoid tissues have not yet been investigated using intravital imaging.

Applications of intravital imaging in studies on adoptive cell therapy

Adoptive cell therapy (ACT) has witnessed a rapid expansion of novel therapeutic approaches in its immune-oncology pipeline in recent years [60]. This growth is largely attributed to the recent approval of chimeric antigen receptor (CAR)-T cell therapies and their remarkable efficacy in treating certain hematologic tumors. A central focus of ACT is the genetic modification of T cells to enable them to specifically target and eliminate tumor cells. Currently, genetically modified T cells employed in ACT are either engineered with a chimeric antigen receptor (CAR) or a T cell receptor (TCR) for this purpose. While CAR-T cell therapies have already been successfully applied into clinical practice, their application to the treatment of solid tumors remains challenging.

The integration of intravital microscope into CAR-T immunotherapy for real-time monitoring of cellular dynamic and treatment efficiency holds promise for overcoming these obstacles. For example, Weigelin et al. visualized efficacious CTL-mediated tumor cell killing by intravital live-cell two-photon microscopy and demonstrated that the synergy of adoptive T cell therapy and agonist anti-CD137 mAb consistently achieved tumor eradication [61]. Mulazzani et al. visualized the movement of anti-CD19 CAR-T cells and non-targeted CAR-T cells within solid tumors in vivo using the cranial window chamber model. Their findings revealed that the infiltration of different CAR-T cell types into intracranial tumors was comparable following tail vein injection. However, when directly injected into the brain, anti-CD19 CAR-T cells penetrated more deeply into the tumor parenchyma, whereas non-targeted CAR-T cells predominantly localized at the tumor periphery [62]. Additionally, intravital imaging was employed by Mastereo et al. to demonstrate that effector T cells were more abundant around blood vessels than in hypoxic tumor regions, implying that the normalization of tumor vasculature may represent an effective strategy to enhance the efficacy of CAR-T cell therapy [63]. In conclusion, intravital imaging serves as a critical technological tool for analyzing the processes and mechanisms associated with the implementation of adoptive cell therapies. The comprehensive, longitudinal data obtained through this technique can reveal crucial insights into immune cell functionality and offer valuable guidance for advancing the understanding of immunotherapy mechanisms.

Targeting T cells

T cells are important effector cells for targeting tumors and foreign pathogens and are responsible for peripheral tolerance to non-harmful antigens. T cell immunology has received great attention due to the considerable

effectiveness of cancer immunotherapy [64]. Many studies have indicated that the accumulation of TILs is a powerful prognostic factor for different types of malignancies and responses to immunotherapy [48, 49]. It is hence crucial to explain the in vivo behavior of T cells to comprehend the pathologic underpinnings of diseases. For example, Yang.et.al exploited the use of a mixture with CD4-FITC and CD25-PE antibodies to target regulatory T cells (Treg) with a high FAD (flavin adenine dinucleotide) signature in a skinfold triple-negative breast cancer model, they found that the main metabolic phenotype of Treg cells is oxidative phosphorylation (OXPHOS) (Fig. 5A). Breart.et.al discovered that tumor regression following the transfer of in vitro-activated CTLs was primarily due to the direct action of CTLs on individual tumor cells, with minimal bystander effects. Unexpectedly, it took an average of 6 h for a single CTL to kill one target cell, suggesting that the slow killing rate inherently limits the efficiency of antitumor T cell responses [65] (Table 2). Likewise, Mrass et al. reported that CD44 plays a crucial role in stabilizing the polarity of migrating cytotoxic T lymphocytes by anchoring cytoskeletal proteins to the cell membrane at their uropods [66] (Table 2). A melanoma animal model with resident memory CD8 + T cells in the epidermis showed that T cell behavior is essential for sustaining cancer-immune balance [67](Table 2). Therefore, the ability of intravital imaging to visualize the timing, location, and mechanism of tumor cell killing in vivo provides new insights into how T immune effectors target cancer cells and how local TMEs may evade immune responses.

Targeting TAMs

Tumor-associated macrophages, originating mainly from blood monocytes, represent the most abundant innate immune population in the TME [68, 69]. TAMs can constitute more than 50% of the tumor tissue mass, with their presence being positively correlated with poor prognosis in most cancers [70]. Macrophage migration is associated with the advancement of various pathologies, such as cancer and inflammation and is crucial for preserving tissue homeostasis, preventing infections, and promoting tissue repair [68]. TAMs release inflammatory cytokines, creating a chronic inflammatory environment that promotes tumor growth, angiogenesis, lymphangiogenesis, immunosuppression, tumor cell invasiveness, therapy resistance, and metastasis [71]. Both preclinical and clinical studies demonstrate that TAMs are polarized by the local tumor environment into a protumoral phenotype [72]. TAMs promote tumor cell invasiveness and aid in metastasis by escorting tumor cells and establishing the premetastatic niche through extracellular matrix (ECM) remodeling. Consequently, TAMs, like T cells, have drawn great attention due to their predominant abundance in solid tumors and crucial functions in tumor progression [73, 74]. Longitudinal intravital imaging has been extensively applied to track the dynamic activities of macrophages. For example, Gerhardt et.al applied intravital imaging to verify that macrophage depletion by anti-colony stimulating factor 1 (anti-CSF1) mAb restores the function and patterning of the tumor vasculature in mice glioma models (GL261 and CT2A) [16]. Similarly, intravital imaging

Table 2 Intravital imaging of immune components

Animal model	Application (Target cells)	Contrast agents	References
A model of spontaneous lymphoma	B	RFP	[34]
4T1 and B16 cancer models	CD8 + T cells	GFP	[53]
EL4/EG7 subcutaneous tumor model	CTLs	A membrane-targeted mCFP or mYFP	[65]
A mouse model in which the adoptive transfer of in vitro-primed, ovalbumin-specific CTLs	CTLs	/	[66]
A mouse model of transplantable cutaneous melanoma	Memory CD8 + T cells	mCherry-	[67]
Transgenic DPE-GFP reporter mice	TAMs	AF647	[54]
GL261 and CT2A mouse glioma models	TAMs	FITC and Tomato	[16]
FcγRI-deficient mice	KCs	DiI, PE	[58]
MMTV-PyMT mouse breast cancer model	DCs and TAMs	EGFP	[29]
A subcutaneous fibrosarcoma MacBlue mice	TAMs	ECFP	[11]
CD11c-EYFP transgenic mice	DCs	EYFP	[80]
Human breast cancer xenograft mouse	NK	NIRF ESNF13	[82]
EL4 thymoma mouse models	NK	CFP	[55]

Abbreviations: Treg, regulatory T cells; RFP, red fluorescent protein; PE, phycoerythrin; CTLs, cytotoxic T lymphocytes; mCFP, cyan fluorescent protein; mYFP, membrane-targeted yellow fluorescent protein; AF647, Alexa Fluor 647; TAMs, tumor-associated macrophages; FITC, fluorescein isothiocyanate; KCs, Kupffer cells; DiI, a long-chain carbocyanine dye; PE, phycoerythrin; DCs, dendritic cells; EGFP, enhanced green fluorescent protein; ECFP; enhanced cyan fluorescent protein; EYFP: yellow fluorescent protein; NIRF, Near-infrared fluorescent

has also been applied in a MMTV-PyMT mice breast cancer model to thoroughly characterize the impact of the myeloid cells depletion medicated by anti-CSF1R mAb, revealing a decrease of cells containing markers for DCs and TAMs, resulting in delayed tumor progression [29]. Gui.et.al demonstrated that inhibiting mesenchymal migration in a subcutaneous fibrosarcoma model was associated with reduced TAM recruitment and tumor progression [11]. Thus, real-time visualization of TAMs function and dynamic during tumor progression is essential to predict immunotherapeutic efficiency.

Targeting dendritic cells (DCs)

Dendritic cells (DCs) are pivotal in bridging the innate and adaptive immune systems, playing a crucial role in eliciting both humoral and cellular responses against specific antigens [75]. Upon uptake of tumor antigens, DCs migrate to draining lymph nodes and present these antigens to T cells, thereby activating specific T cells that recognize and eliminate tumor cells. Intravital imaging has proven valuable for studying the in vivo dynamics and function of DCs, providing insights into DC biology across various contexts such as immunotherapy, autoimmune disease, inflammatory, allergy, transplantation, and vaccination [54].

Recent advancements in tumor vaccine research have led to the proposal that enhancing the migratory capabilities of DCs could further augment the efficacy of anti-tumor therapies [54]. In vivo imaging has been instrumental in supporting these advancements. For instance, Kim et al. identified an inhibitory role for transgelin-2 in the migration and antigen presentation of DCs within a cancer model. The knockout of the transgelin-2 gene in DCs did not affect their maturation or differentiation. However, intriguingly, the ability of DCs to migrate to lymph nodes and establish immune synapses was significantly impaired, leading to a diminished capacity for immune recognition of tumor antigens. Moreover, the direct incubation of deubiquitinated recombinant transgelin-2 with DCs in vitro further enhanced both the antigen presentation and migratory abilities of DCs, thereby improving the efficacy of anti-tumor therapies [76]. Consequently, these findings suggest that transgelin-2 may serve as a critical target for the development of DC-targeting vaccines in future therapeutic strategies.

Besides, intravital imaging can provide insights into the interaction between DCs and T cells. DCs efficiently screen large portions of the T cell repertoire before engaging in long-lasting interactions with specific T cells [77, 78]. A single DC can interact with several antigen-specific T cells simultaneously, often up to ten, with the number of antigens on the DCs determining the duration of interactions and the number of T cells per DC [77]. Intravital imaging has also provided evidence of the presence of DCs and CD4 + T cells after vaccination [79]. Studies have shown that by combining ovalbumin with an antibody against the DC surface lectin DEC-205 (anti-DEC-OVA) and targeting it to endogenous DCs, it was possible to observe long-term interactions between antigen-specific CD4 T cells and DCs. These interactions were discovered to be indicative of both immunogenic and tolerogenic T cell priming and were observed in both inflammatory and steady-state settings [80].

Targeting natural killer (NK) cells

NK cells have attracted attention as a possible candidate for future immunotherapeutic treatment due to their crucial function in immune surveillance against malignancy and viral infection [81]. Intravital imaging has been investigated for NK cells as a robust imaging tool to monitor their in *vivo* functions and dynamics. Similar to DCs, ex vivo staining is a commonly used technique for NK cells. For example, Uong et al. applied the near-infrared (NIR) fluorescence ESNF13 for the ex vivo staining of NK cells, which were then intravenously injected into human breast cancer xenograft mouse and visualized using fluorescence intravital imaging [82]. The strong fluorescent signal of the ESNF13-stained NK cells made it possible to track tumor-invading cells for up to 7 days. Similarly, Tavri et al. intravenously implanted NK-92-scFv (MOC31)-zeta cells modified with a chimeric antigen receptor into a prostate cancer model and stained the NK cells with the near-infrared fluorophore DiD. Intravital imaging was then used to track the accumulation of DiD labeled cells for up to 24 h following the injection [82]. Recent NK cell initiatives have focused on ADCC, which targets cancer cells and causes them to undergo apoptosis by utilizing the innate immune system's antitumor cytotoxicity (Fig. 3). Despite being extensively demonstrated by in vitro studies, a few studies have addressed the imaging of in vivo ADCC events mediated by NK cells in animal models with immunotherapies or tumor-targeting antibody therapies based on intravital imaging. In this regard, a study tested the subcutaneous injection of EL4 thymoma cells into mouse models and revealed that the combination of Fc-mediated recognition of mAbs by NK cells and recognition of NKG2D ligands may strongly affect NK cell behavior and promote sustainable interactions with targets *in v*ivo [55]. Therefore, the rational design for promoting NK cell arrest and cooperation between activating receptors is highly beneficial for enhancing NK cell-mediated ADCC events.

Intravital imaging of tumor angiogenesis

Tumor-associated angiogenesis plays a crucial role in the expansion and multiplication of solid tumors. Intravital imaging has offered unprecedented insights into the visualization

and assessment of tumor size, structure, and vasculature (Fig. 4A–C). To temporarily outline blood vessels and target tumors under fluorescence microscopy, high molecular weight fluorescent tracers, such as AF680-conjugated 2MDa dextran or Mn_3O_4 radiotracers, are injected until the tracers diffuse out and contrast fades [33]. Zhan et al. synthesized Mn_3O_4 nanoparticles in a recent study, conjugating them with the radioisotope copper-64 and the anti-CD105 antibody TRC105 for targeted imaging of tumor vasculature in mice. The Mn-conjugated nanoparticles (64Cu–Mn@PEG-TRC105) exhibited excellent radio-stability and demonstrated high specificity for tumor targeting [83]. These methods allow for the dynamic measurement of vessel length, diameter, volume, and surface area, as well as the analysis of branching patterns and intercapillary distance in developing or regressing tumors (Fig. 4E). Intravital imaging has characterized normal vascular networks, which are organized into distinct units, including arterioles, capillaries, and venules, exhibiting well-structured architecture with dichotomous branching and hierarchical order (Fig. 4E). In contrast, the tumor vessels displayed dilated, tortuous, saccular, and irregularly distributed characteristics. The tumor vasculature appeared disorganized, with trifurcations and branches of uneven diameters (Fig. 4E). Fractal analysis of in vivo images of normal and tumor vascular networks suggested that the former are optimized for efficient nutrient delivery through diffusion to all cells, whereas the latter are constrained by matrix mechanical properties. Additionally, mechanical stress generated by proliferating tumor cells may compress blood vessels and lymphatics, further influencing tumor vascular structure. In addition to providing mechanistic insights, intravital imaging could also offer novel prospects for improving therapy. For instance, tumor angiogenesis leads to the formation of immature, dysfunctional blood vessels, primarily due to an imbalance in the production of pro- and anti-angiogenic factors by tumors. Intravital imaging studies in cancer patients and animal models have verified that restoring the equilibrium of these signaling molecules within tumors can result in the "normalization" of the tumor vasculature, thereby improving its function. Administration of cytotoxic agents, targeted agents, or immunotherapies during periods of vascular normalization may increase treatment effectiveness. These findings have generally contributed to the development of novel approaches for the treatment of malignancies. In the long run, these insights will provide crucial information for stimulating or dampening the immune system to treat cancerous, autoimmune, infectious, or allergic diseases.

Uncovering cellular metabolism to predict immunotherapeutic efficacy

Extensive studies on cancer metabolism have shown that the TME changes are a direct result of oncogenic transformations, which cause cancer cells to take on a new metabolic phenotype. The TME consists of various cell populations within a complex matrix, often characterized by poorly differentiated vasculature. This results in inefficient nutrient and waste removal as well as oxygen delivery, causing nutrient scarcity within the TME. The rapid growth of cancer cells and immune cells leads to a competition for limited resources, which puts a strain on the body's ability to defend against tumors. The resulting harsh metabolic environment can suppress immune responses, forcing infiltrating immune cells to adapt metabolically and become more tolerant, which diminishes their antitumor efficacy. Strategies to manipulate cell metabolism offer promising avenues for cancer treatments. Specifically, targeting cancer metabolism to improve nutrient availability in the TME or modulating immune metabolism to enhance inflammation can increase the effectiveness of cancer treatments.

Intravital microscopy enables real-time, label-free, and single-cell imaging of metabolic alterations in vivo for the prediction of immunotherapeutic efficiency. These metabolic changes can be measured by autofluorescent metabolic coenzymes, *i.e.*, NAD(P)H (reduced nicotinamide adenine dinucleotide (phosphate) hydrogen) and FAD, which are naturally present in all cells (Fig. 5E). The metabolic reprogramming of tumor and immune cells is closely related to the cellular phenotype and cancer progression. For example, Wenxuan Liang's team designed a two-photon fluorescence microscope that uses the natural fluorescence of NADH for imaging and is capable of tracking the life dynamics of cultured tumor cells in apoptosis-inducing mouse subcutaneous tumor models [54]. This system provides metabolic information and complementary structural information, facilitating the functional histological imaging of unlabeled organs in situ, which could be useful for clinical diagnosis and therapy. Skala et al. investigated metabolic changes during the administration of a potent triple-combination immunotherapy in a murine melanoma cancer model [84]. This treatment involved the administration of an intratumoral anti-GD2 mAb fused to an IL2 immunocytokine, along with external beam radiation and intraperitoneal anti-CTLA-4. This combination leads to in situ vaccination and complete recovery from GD2 + murine cancers [29]. Moreover, they developed a mCherry-labeled T cell mouse model to observe concurrent metabolic trends in both tumor and CD8 + T cells during immunotherapy. Similarly, Yang et al. developed a

label-free metabolic intravital imaging (LMII) technique to detect two-photon excited autofluorescence signals from NAD(P)H and FAD (Fig. 5E). These signals serve as reliable markers for monitoring metabolic responses to innate immunotherapy, such as anti-CD47 immunotherapy [25]. Meanwhile, to identify the metabolic phenotype of DCs, DC-specific primary FITC-conjugated CD11c antibody could be applied to target the DC populations and verified the colocalization between DCs and NAD(P)H^{hi} cells (Fig. 5F). These findings demonstrated that intravital metabolic imaging allows for the single-cell quantification of metabolic alterations in immune and tumor cells during immunotherapy. In combination with traditional assays, this technique can help identify key immune cell populations and the critical times during immunotherapy when metabolic changes occur.

Current limitations of intravital imaging

Despite its innovative nature and high resolution in deep tissue, intravital imaging faces several challenges that limit its use in certain research areas. Currently, this technique is mostly applied to immune-related organs, such as the lymph nodes, spleen, and bone marrow, as well as other internal organs, such as the central nervous system (CNS), pancreas, and liver [85, 86]. However, imaging intrathoracic organs remains difficult due to their anatomical inaccessibility, significant tissue movement during breathing, and lung collapse when the thoracic cavity is opened [87]. To address these challenges, new surgical models are needed to minimize organ movement while maintaining physiological conditions. Recently, vacuum suction devices that apply light negative pressure to press the tissue of interest against the coverslip have been developed. These devices have partially overcome this issue, allowing imaging of metastasis initiation events in the mouse lung [14].

Meanwhile, compared to other in vivo imaging modalities, intravital microscopy has relatively small field of view, limited penetration depth and relatively bulk size, which can be a hindrance to diverse IVM applications in the clinical. Also, the necessity of fluorescence would be an obstacle to clinical applications. On the other hand, whole-body imaging systems, which has been widely used in the clinic, are suitable for viewing large parts of tissues and analyzing the function of whole tissues, although these methods in general have low spatial resolution, unlike IVM [7]. Thus, the use of IVM and whole-body imaging systems can be well combined to obtain more comprehensive biological information that cannot be obtained by a single method. IVM and whole-body imaging modalities compensate for the weakness of each other's imaging system. Also, the constant development of IVM will surely broaden our knowledges on the field of biological and biomedical sciences by providing novel insights, which cannot be achieved by conventional experimental techniques [7].

Lastly, the lack of a novel and standardized computational analysis of cell-pathogen and cell–cell interactions has hindered experimental progress in this field. Currently, analyzing multidimensional data sets primarily relies on manual cell identification due to some technical challenges in the acquisition process. Although there have been recent attempts to develop automated protocols for the quantitative analysis of intravital imaging data, cumulative errors from automatic segmentation remain a significant problem [88]. The consistent lack of reliable automated protocols can cause bias in the analysis. Thus, new strategies are needed to effectively address the challenges associated with in vivo imaging experiments.

Conclusion and future perspectives

Predicting and monitoring treatment response is a critical function of oncologic imaging. Due to the unique biological mechanisms of immune checkpoint inhibitors (ICIs), the patterns of tumor response and progression associated with immunotherapy differ from those observed with conventional cytotoxic chemotherapies. Radiological responses to immunotherapy can be delayed or may occur after an initial transient enlargement of lesions or the emergence of new lesions, a phenomenon known as pseudoprogression.

To address this limitation, multiphoton or two-photon excitation microscopy has introduced a new era in biological imaging. This advanced technique utilizes near-infrared excitation lasers, which can penetrate thicker specimens, allowing researchers to observe living cell behavior deep within tissues without any thin sectioning for days to months [89]. Reduced photobleaching and toxicity enable the visualization of live and intact specimens over extended periods. Recent studies using intravital two-photon imaging have revealed insights into the immune system, which is characterized by intricate networks with a mixture of lymphocytes, TAMs, and DCs that constantly move throughout the body and engage in specific interactions [89]. However, multiphoton or two-photon excitation microscopy has several limitations. First, fluorescence labeling and second-harmonic generation allow for the detection of target cells and organs, but not all visual fields containing structural components and cellular information can be detected by multiphoton microscopy [18]. Therefore, observations must be interpreted cautiously to avoid misinterpretation. Second, although multiphoton microscopy can penetrate tissue at greater depths compared to conventional confocal microscopy, it is constrained by a maximum depth of 800–1,000 μm in soft tissues (e.g., cerebral cortex) and

200 µm in hard tissues (e.g., bone) [18]. Consequently, it is primarily applicable to small animals such as mice and rats. Additionally, due to the significant scattering of light by the skin, internal organs need to be exteriorized. This requires performing surgical procedures and changing oxygen and humidity levels, which can potentially affect cellular activity. Innovations in fluorochrome and optical systems, such as enhanced light emission and resolution, are necessary to solve these problems.

In addition to the optimization of fluorochrome and optical systems, novel approaches should be incorporated into intravital imaging to enhance cancer diagnosis and targeted therapy. Therefore, intravital imaging should be considered an important part of the preclinical design of immunotherapies. For example, this imaging technique can be combined with other techniques, such as dynamic in situ cytometry (DISC), which relies on cell surface labeling to link motility parameters and phenotypic markers [90]. Meanwhile, intravital imaging can be coupled with antibody-based contrast agents or radiopharmaceuticals to enable sensitive monitoring of disease progression. The stability, affinity, and specificity of antibodies are enhanced when conjugated with nanoparticles, radioisotopes, or therapeutic agents [83]. Furthermore, imaging can also be integrated with methods such as the Labeling immune partnership by sorting intercellular contacts (LIPSTIC), Clustered regularly interspaced short palindromic repeats (CRISPR/Cas9), spatial transcriptomics, single-cell sequencing, and mass spectrometry technology, which will further establish intravital imaging as a critical and unique tool, deepening our understanding of immune cell behaviors in the TME and guiding immunotherapeutic interventions for malignancies.

In conclusion, determining tumor immune phenotypes, as well as predicting and evaluating responses to immunotherapy and the development of treatment-related toxicities, remains a significant challenge in medical imaging. Conventional imaging methods for assessing tumor response and and targeted diagnosis have clear limitations. The integration of functional and molecular imaging techniques, particularly intravital imaging and PET, alongside the development of artificial intelligence (AI) technologies for treatment response assessment, holds promise for providing more accurate and specific information regarding immunotherapy outcomes. These techniques, combined with significant advancements in data processing, are creating new opportunities for patient management and the advancement of precision oncology.

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Declarations

Conflict of interest The authors declare no competing interests.

References

1. Boulch M, Grandjean CL, Cazaux M, Bousso P (2019) Tumor immunosurveillance and immunotherapies: a fresh look from intravital imaging. Trends Immunol 40(11):1022–1034. https://doi.org/10.1016/j.it.2019.09.002
2. Krekorian M, Fruhwirth GO, Srinivas M, Figdor CG, Heskamp S, Witney TH, Aarntzen EH (2019) Imaging of T-cells and their responses during anti-cancer immunotherapy. Theranostics 9(25):7924–7947. https://doi.org/10.7150/thno.37924
3. Fruhwirth GO, Kneilling M, De Vries IJM, Weigelin B, Srinivas M, Aarntzen EH (2018) The potential of in vivo imaging for optimization of molecular and cellular anti-cancer immunotherapies. Mol Imag Biol 20:696–704. https://doi.org/10.1007/s11307-018-1254-3
4. Volpe A, Kurtys E, Fruhwirth GO (2018) Cousins at work: how combining medical with optical imaging enhances in vivo cell tracking. Int J Biochem Cell Biol 102:40–50. https://doi.org/10.1016/j.biocel.2018.06.008
5. Tarighatnia A, Fouladi MR, Nader ND, Aghanejad A, Ghadiri H (2022) Recent trends of contrast agents in ultrasound imaging: a review of the classifications and applications. Mater Adv 3(9):3726–3741. https://doi.org/10.1039/D1MA00969A
6. Aghahosseini F, Salehi Y, Farzanefar S, Kashi MB, Eppard E, Ahmadzadehfar H, Mirzaei S, Vahidfar N, Aghanejad A (2024) Recent advances in the diagnosis of Alzheimer's disease: a brief overview of tau PET tracers in nuclear medicine. Curr Radiopharm. https://doi.org/10.2174/0118744710314668240718070109
7. Choo YW, Jeong J, Jung K (2020) Recent advances in intravital microscopy for investigation of dynamic cellular behavior in vivo.

BMB Rep 53(7):357–366. https://doi.org/10.5483/BMBRep.2020.53.7.069
8. Hoffman RM (2004) In vivo imaging with fluorescent proteins: the new cell biology. Acta Histochem 106(2):77–87. https://doi.org/10.1016/j.acthis.2004.02.001
9. Grandjean CL, Garcia Z, Lemaître F, Bréart B, Bousso P (2021) Imaging the mechanisms of anti-CD20 therapy in vivo uncovers spatiotemporal bottlenecks in antibody-dependent phagocytosis. Sci Adv. https://doi.org/10.1126/sciadv.abd6167
10. Guy CT, Cardiff RD, Muller WJ (1992) Induction of mammary tumors by expression of polyomavirus middle T oncogene: a transgenic mouse model for metastatic disease. Mol Cell Biol 12(3):954–961. https://doi.org/10.1128/mcb.12.3.954-961.1992
11. Gui P, Ben-Neji M, Belozertseva E, Dalenc F, Franchet C, Gilhodes J, Labrousse A, Bellard E, Golzio M, Poincloux R, Maridonneau-Parini I, Le Cabec V (2018) The protease-dependent mesenchymal migration of tumor-associated macrophages as a target in cancer immunotherapy. Cancer Immunol Res 6(11):1337–1351. https://doi.org/10.1158/2326-6066.CIR-17-0746
12. Garris CS, Arlauckas SP, Kohler RH et al (2018) Successful anti-PD-1 cancer immunotherapy requires T cell-dendritic cell crosstalk involving the cytokines IFN-γ and IL-12. Immunity 49(6):1148–1161. https://doi.org/10.1016/j.immuni.2018.09.024
13. Lehmann S, Perera R, Grimm HP et al (2016) In vivo fluorescence imaging of the activity of CEA TCB, a novel T-cell bispecific antibody, reveals highly specific tumor targeting and fast induction of T-cell-mediated tumor killing. Clin Cancer Res 22(17):4417–4427. https://doi.org/10.1158/1078-0432.CCR-15-2622
14. Headley MB, Bins A, Nip A, Roberts EW, Looney MR, Gerard A, Krummel MF (2016) Visualization of immediate immune responses to pioneer metastatic cells in the lung. Nature 531(7595):513–517. https://doi.org/10.1038/nature16985
15. Jung K, Heishi T, Khan OF et al (2017) Ly6C lo monocytes drive immunosuppression and confer resistance to anti-VEGFR2 cancer therapy. J Clin Invest 127(8):3039–3051. https://doi.org/10.1172/JCI93182
16. Mathivet T, Bouleti C, Van Woensel M et al (2017) Dynamic stroma reorganization drives blood vessel dysmorphia during glioma growth. EMBO Mol Med 9(12):1629–1645. https://doi.org/10.15252/emmm.201607445
17. Wyckoff JB, Wang Y, Lin EY, Li JF, Goswami S, Stanley ER, Segall JE, Pollard JW, Condeelis J (2007) Direct visualization of macrophage-assisted tumor cell intravasation in mammary tumors. Cancer Res 67(6):2649–2656. https://doi.org/10.1158/0008-5472.CAN-06-1823
18. Kikuta J, Ishii M (2012) Recent advances in intravital imaging of dynamic biological systems. J Pharmacol Sci 119(3):193–197. https://doi.org/10.1254/jphs.12R03CP
19. Kawakami N, Nägerl UV, Odoardi F, Bonhoeffer T, Wekerle H, Flügel A (2005) Live imaging of effector cell trafficking and autoantigen recognition within the unfolding autoimmune encephalomyelitis lesion. J Exp Med 201(11):1805–1814. https://doi.org/10.1084/jem.20050011
20. Lee S, Vinegoni C, Feruglio PF, Fexon L, Gorbatov R, Pivoravov M, Sbarbati A, Nahrendorf M, Weissleder R (2012) Real-time in vivo imaging of the beating mouse heart at microscopic resolution. Nat Commun 3(1):1054. https://doi.org/10.1038/ncomms2060
21. Kimura H, Hayashi K, Yamauchi K, Yamamoto N, Tsuchiya H, Tomita K, Kishimoto H, Bouvet M, Hoffman RM (2010) Real-time imaging of single cancer-cell dynamics of lung metastasis. J Cell Biochem 109(1):58–64. https://doi.org/10.1002/jcb.22379
22. Rytelewski M, Haryutyunan K, Nwajei F, Shanmugasundaram M, Wspanialy P, Zal MA, Chen CH, El Khatib M, Plunkett S, Vinogradov SA, Konopleva M, Zal T (2019) Merger of dynamic two-photon and phosphorescence lifetime microscopy reveals dependence of lymphocyte motility on oxygen in solid and hematological tumors. J Immunother Cancer 7(1):78. https://doi.org/10.1186/s40425-019-0543-y
23. Park J, Wysocki RW, Amoozgar Z et al (2016) Cancer cells induce metastasis-supporting neutrophil extracellular DNA traps. Sci Transl Med. https://doi.org/10.1126/scitranslmed.aag1711
24. Entenberg D, Rodriguez-Tirado C, Kato Y, Kitamura T, Pollard JW, Condeelis J (2015) In vivo subcellular resolution optical imaging in the lung reveals early metastatic proliferation and motility. Intravital 4(3):1–11. https://doi.org/10.1080/21659087.2015.1086613
25. Yang M, Mahanty A, Jin C, Wong ANN, Yoo JS (2022) Label-free metabolic imaging for sensitive and robust monitoring of anti-CD47 immunotherapy response in triple-negative breast cancer. J Immunother Cancer 10(9):e005199. https://doi.org/10.1136/jitc-2022-005199
26. Ruocco MG, Pilones KA, Kawashima N, Cammer M, Huang J, Babb JS, Liu M, Formenti SC, Dustin ML, Demaria S (2012) Suppressing T cell motility induced by anti-CTLA-4 monotherapy improves antitumor effects. J Clin Invest 122(10):3718–3730. https://doi.org/10.1172/jci61931
27. Shan S, Sorg B, Dewhirst MW (2003) A novel rodent mammary window of orthotopic breast cancer for intravital microscopy. Microvasc Res 65(2):109–117. https://doi.org/10.1016/S0026-2862(02)00017-1
28. Junankar S, Shay G, Jurczyluk J et al (2015) Real-time intravital imaging establishes tumor-associated macrophages as the extraskeletal target of bisphosphonate action in cancer. Cancer Discov 5(1):35–42. https://doi.org/10.1158/2159-8290.CD-14-0621
29. Lohela M, Casbon AJ, Olow A, Bonham L, Branstetter D, Weng N, Smith J, Werb Z (2014) Intravital imaging reveals distinct responses of depleting dynamic tumor-associated macrophage and dendritic cell subpopulations. Proc Natl Acad Sci USA 111(47):E5086–E5095. https://doi.org/10.1073/pnas.1419899111
30. Maeda A, Dacosta RS (2014) Optimization of the dorsal skinfold window chamber model and multi-parametric characterization of tumor-associated vasculature. Intravital 3(1):e27935. https://doi.org/10.4161/intv.27935
31. Jang JE, Hajdu CH, Liot C, Miller G, Dustin ML, Bar-Sagi D (2017) Crosstalk between regulatory T cells and tumor-associated dendritic cells negates anti-tumor immunity in pancreatic cancer. Cell Rep 20(3):558–571. https://doi.org/10.1016/j.celrep.2017.06.062
32. Kilarski WW, Güç E, Teo JC, Oliver SR, Lund AW, Swartz MA (2013) Intravital immunofluorescence for visualizing the microcirculatory and immune microenvironments in the mouse ear dermis. PLoS ONE 8(2):e57135. https://doi.org/10.1371/journal.pone.0057135
33. Honkura N, Richards M, Laviña B, Sáinz-Jaspeado M, Betsholtz C, Claesson-Welsh L (2018) Intravital imaging-based analysis tools for vessel identification and assessment of concurrent dynamic vascular events. Nat Commun 9(1):2746. https://doi.org/10.1038/s41467-018-04929-8
34. Montalvao F, Garcia Z, Celli S, Breart B, Deguine J, Van Rooijen N, Bousso P (2013) The mechanism of anti-CD20-mediated B cell depletion revealed by intravital imaging. J Clin Invest 123(12):5098–5103. https://doi.org/10.1172/JCI70972
35. Ritsma L, Steller EJ, Beerling E et al (2012) Intravital microscopy through an abdominal imaging window reveals a pre-micrometastasis stage during liver metastasis. Sci Transl Med. https://doi.org/10.1126/scitranslmed.3004394
36. Tang Q, Adams JY, Tooley AJ, Bi M, Fife BT, Serra P, Santamaria P, Locksley RM, Krummel MF, Bluestone JA (2006) Visualizing regulatory T cell control of autoimmune responses in nonobese

diabetic mice. Nat Immunol 7(1):83–92. https://doi.org/10.1038/ni1289
37. Moalli F, Proulx ST, Schwendener R, Detmar M, Schlapbach C, Stein JV (2015) Intravital and whole-organ imaging reveals capture of melanoma-derived antigen by lymph node subcapsular macrophages leading to widespread deposition on follicular dendritic cells. Front Immunol 6:114. https://doi.org/10.3389/fimmu.2015.00114
38. Cahalan MD, Parker I, Wei SH, Miller MJ (2003) Real-time imaging of lymphocytes in vivo. Curr Opin Immunol 15(4):372–377. https://doi.org/10.1016/S0952-7915(03)00079-7
39. Boissonnas A, Licata F, Poupel L, Jacquelin S, Fetler L, Krumeich S, Théry C, Amigorena S, Combadière C (2013) $CD8^+$ tumor-infiltrating T cells are trapped in the tumor-dendritic cell network. Neoplasia 15(1):85–94. https://doi.org/10.1593/neo.121572
40. Farrar MJ, Bernstein IM, Schlafer DH, Cleland TA, Fetcho JR, Schaffer CB (2012) Chronic in vivo imaging in the mouse spinal cord using an implanted chamber. Nat Methods 9(3):297–302. https://doi.org/10.1038/nmeth.1856
41. Yu X, Chen L, Liu J, Dai B, Xu G, Shen G, Luo Q, Zhang Z (2019) Immune modulation of liver sinusoidal endothelial cells by melittin nanoparticles suppresses liver metastasis. Nat Commun 10(1):574. https://doi.org/10.1038/s41467-019-08538-x
42. Boissonnas A, Licata F, Poupel L, Jacquelin S, Fetler L, Krumeich S, Thèry C, Amigorena S, Combadière C (2013) $CD8^+$ tumor-infiltrating T cells are trapped in the tumor-dendritic cell network. Neoplasia 15(1):85–94. https://doi.org/10.1593/neo.121572
43. Arlauckas SP, Garris CS, Kohler RH, Kitaoka M, Cuccarese MF, Yang KS, Miller MA, Carlson JC, Freeman GJ, Anthony RM, Weissleder R, Pittet MJ (2017) In vivo imaging reveals a tumor-associated macrophage-mediated resistance pathway in anti-PD-1 therapy. Sci Transl Med. https://doi.org/10.1126/scitranslmed.aal3604
44. Yang F, Liu S, Liu X, Liu L, Luo M, Qi S, Xu G, Qiao S, Lv X, Li X, Fu L, Luo Q, Zhang Z (2016) In vivo visualization of tumor antigen-containing microparticles generated in fluorescent-protein-elicited immunity. Theranostics 6(9):1453–1466. https://doi.org/10.7150/thno.14145
45. Wang RM, Johnson TD, He J, Rong Z, Wong M, Nigam V, Behfar A, Xu Y, Christman KL (2017) Humanized mouse model for assessing the human immune response to xenogeneic and allogeneic decellularized biomaterials. Biomaterials 129:98–110. https://doi.org/10.1016/j.biomaterials.2017.03.016
46. Utz B, Turpin R, Lampe J, Pouwels J, Klefström J (2020) Assessment of the WAP-Myc mouse mammary tumor model for spontaneous metastasis. Sci Rep 10(1):18733. https://doi.org/10.1038/s41598-020-75411-z
47. Qi S, Lu L, Zhou F, Chen Y, Xu M, Chen L, Yu X, Chen WR, Zhang Z (2020) Neutrophil infiltration and whole-cell vaccine elicited by N-dihydrogalactochitosan combined with NIR phototherapy to enhance antitumor immune response and T cell immune memory. Theranostics 10(4):1814–1832. https://doi.org/10.7150/thno.38515
48. Trujillo JA, Sweis RF, Bao R, Luke JJ (2018) T cell-inflamed versus non-T cell-inflamed tumors: a conceptual framework for cancer immunotherapy drug development and combination therapy selection. Cancer Immunol Res 6(9):990–1000. https://doi.org/10.1158/2326-6066.CIR-18-0277
49. Havel JJ, Chowell D, Chan TA (2019) The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. Nat Rev Cancer 19(3):133–150. https://doi.org/10.1038/s41568-019-0116-x
50. Shiravand Y, Khodadadi F, Kashani SMA, Hosseini-Fard SR, Hosseini S, Sadeghirad H, Ladwa R, O'Byrne K, Kulasinghe A (2022) Immune checkpoint inhibitors in cancer therapy. Curr Oncol 29(5):3044–3060. https://doi.org/10.3390/curroncol29050247
51. Cloughesy TF, Mochizuki AY, Orpilla JR et al (2019) Neoadjuvant anti-PD-1 immunotherapy promotes a survival benefit with intratumoral and systemic immune responses in recurrent glioblastoma. Nat Med 25(3):477–486. https://doi.org/10.1038/s41591-018-0337-7
52. Lau D, Garçon F, Chandra A, Lechermann LM, Aloj L, Chilvers ER, Corrie PG, Okkenhaug K, Gallagher FA (2020) Intravital imaging of adoptive T-cell morphology, mobility and trafficking following immune checkpoint inhibition in a mouse melanoma model. Front Immunol 11:1514. https://doi.org/10.3389/fimmu.2020.01514
53. Pentcheva-Hoang T, Simpson TR, Montalvo-Ortiz W, Allison JP (2014) Cytotoxic T lymphocyte antigen-4 blockade enhances antitumor immunity by stimulating melanoma-specific T-cell motility. Cancer Immunol Res 2(10):970–980. https://doi.org/10.1158/2326-6066.CIR-14-0104
54. Deng D, Hao T, Lu L, Yang M, Zeng Z, Lovell JF, Liu Y, Jin H (2024) Applications of intravital imaging in cancer immunotherapy. Bioengineering 11(3):264. https://doi.org/10.3390/bioengineering11030264
55. Deguine J, Breart B, Lemaître F, Bousso P (2012) Cutting edge: tumor-targeting antibodies enhance NKG2D-mediated NK cell cytotoxicity by stabilizing NK cell-tumor cell interactions. J Immunol 189(12):5493–5497. https://doi.org/10.4049/jimmunol.1202065
56. Reichert JM, Dhimolea E (2012) The future of antibodies as cancer drugs. Drug Discov Today 17(17–18):954–963. https://doi.org/10.1016/j.drudis.2012.04.006
57. Weiner LM, Surana R, Wang S (2010) Antibodies and cancer therapy: versatile platforms for cancer immunotherapy. Nat Rev Immunol 10(5):317–327. https://doi.org/10.1038/nri2744
58. Gruijs M, Braster R, Overdijk MB, Hellingman T, Verploegen S, Korthouwer R, van der Wilk BJ, Parren PWHI, van der Vliet HJ, Bögels M (2022) van Egmond M (2022) Epidermal growth factor receptor as target for perioperative elimination of circulating colorectal cancer cells. J Oncol 1:3577928. https://doi.org/10.1155/2022/3577928
59. Gül N, Babes L, Siegmund K, Korthouwer R, Bögels M, Braster R, Vidarsson G, Ten Hagen TL, Kubes P, Van Egmond M (2014) Macrophages eliminate circulating tumor cells after monoclonal antibody therapy. J Clin Invest 124(2):812–823. https://doi.org/10.1172/JCI66776
60. Weigelin B, Den Boer AT, Wagena E, Broen K, Dolstra H, De Boer RJ, Figdor CG, Textor J, Friedl P (2021) Cytotoxic T cells are able to efficiently eliminate cancer cells by additive cytotoxicity. Nat Commun 12(1):5217. https://doi.org/10.1038/s41467-021-25282-3
61. Weigelin B, Bolaños E, Teijeira A, Martinez-Forero I, Labiano S, Azpilikueta A, Morales-Kastresana A, Quetglas JI, Wagena E, Sánchez-Paulete AR, Chen L, Friedl P, Melero I (2015) Focusing and sustaining the antitumor CTL effector killer response by agonist anti-CD137 mAb. Proc Natl Acad Sci USA 112(24):7551–7556. https://doi.org/10.1073/pnas.1506357112
62. Mulazzani M, Fräßle SP, von Mücke-Heim I et al (2019) Long-term in vivo microscopy of CAR T cell dynamics during eradication of CNS lymphoma in mice. Proc Natl Acad Sci USA 116(48):24275–24284. https://doi.org/10.1073/pnas.1903854116
63. Manaster Y, Shipony Z, Hutzler A, Kolesnikov M, Avivi C, Shalmon B, Barshack I, Besser MJ, Feferman T, Shakhar G (2019) Reduced CTL motility and activity in avascular tumor areas. Cancer Immunol Immunother 68:1287–1301. https://doi.org/10.1007/s00262-019-02361-5
64. Kumar BV, Connors TJ, Farber DL (2018) Human T cell development, localization, and function throughout life. Immunity 48(2):202–213. https://doi.org/10.1016/j.immuni.2018.01.007

65. Breart B, Lemaître F, Celli S, Bousso P (2008) Two-photon imaging of intratumoral CD8$^+$ T cell cytotoxic activity during adoptive T cell therapy in mice. J Clin Invest 118(4):1390–1397. https://doi.org/10.1172/JCI34388
66. Mempel TR, Marangoni F (2008) CD44 keeps tumor killers polarized. Immunity 29(6):843–845. https://doi.org/10.1016/j.immuni.2008.12.004
67. Park SL, Buzzai A, Rautela J et al (2019) Tissue-resident memory CD8$^+$ T cells promote melanoma-immune equilibrium in skin. Nature 565(7739):366–371. https://doi.org/10.1038/s41586-018-0812-9
68. Lahmar Q, Keirsse J, Laoui D, Movahedi K, Van Overmeire E (1865) Van Ginderachter JA (2016) Tissue-resident versus monocyte-derived macrophages in the tumor microenvironment. Biochim Biophys Acta 1:23–34. https://doi.org/10.1016/j.bbcan.2015.06.009
69. Allavena P, Sica A, Solinas G, Porta C, Mantovani A (2008) The inflammatory micro-environment in tumor progression: the role of tumor-associated macrophages. Crit Rev Oncol Hematol 66(1):1–9. https://doi.org/10.1016/j.critrevonc.2007.07.004
70. Noy R, Pollard JW (2014) Tumor-associated macrophages: from mechanisms to therapy. Immunity 41(1):49–61. https://doi.org/10.1016/j.immuni.2014.06.010
71. Movahedi K, Laoui D, Gysemans C, Baeten M, Stangé G, Van den Bossche J, Mack M, Pipeleers D, Veld P, De Baetselier P, Van Ginderachter JA (2010) Different tumor microenvironments contain functionally distinct subsets of macrophages derived from Ly6C (high) monocytes. Cancer Res 70(14):5728–5739. https://doi.org/10.1158/0008-5472.CAN-09-4672
72. Biswas SK, Allavena P, Mantovani A (2013) Tumor-associated macrophages: functional diversity, clinical significance, and open questions. Semin Immunopathol 35:585–600. https://doi.org/10.1007/s00281-013-0367-7
73. Thorsson V, Gibbs DL, Brown SD et al (2018) The immune landscape of cancer. Immunity 48(4):812–830. https://doi.org/10.1016/j.immuni.2018.03.023
74. Engblom C, Pfirschke C, Pittet MJ (2016) The role of myeloid cells in cancer therapies. Nat Rev Cancer 16(7):447–462. https://doi.org/10.1038/nrc.2016.54
75. Santos PM, Butterfield LH (2018) Dendritic cell-based cancer vaccines. J Immunol 200(2):443–449. https://doi.org/10.4049/jimmunol.1701024
76. Kim HR, Park JS, Park JH, Yasmin F, Kim CH, Oh SK, Chung IJ, Jun CD (2021) Cell-permeable transgelin-2 as a potent therapeutic for dendritic cell-based cancer immunotherapy. J Hematol Oncol 14:1–23. https://doi.org/10.1186/s13045-021-01058-6
77. Bousso P, Robey E (2003) Dynamics of CD8$^+$ T cell priming by dendritic cells in intact lymph nodes. Nat Immunol 4(6):579–585. https://doi.org/10.1038/ni928
78. Miller MJ, Hejazi AS, Wei SH, Cahalan MD, Parker I (2004) T cell repertoire scanning is promoted by dynamic dendritic cell behavior and random T cell motility in the lymph node. Proc Natl Acad Sci USA 101(4):998–1003. https://doi.org/10.1073/pnas.0306407101
79. Miller MJ, Safrina O, Parker I, Cahalan MD (2004) Imaging the single cell dynamics of CD4$^+$ T cell activation by dendritic cells in lymph nodes. J Exp Med 200(7):847–856. https://doi.org/10.1084/jem.20041236
80. Shakhar G, Lindquist RL, Skokos D, Dudziak D, Huang JH, Nussenzweig MC, Dustin ML (2005) Stable T cell-dendritic cell interactions precede the development of both tolerance and immunity in vivo. Nat Immunol 6(7):707–714. https://doi.org/10.1038/ni1210
81. Hammer Q, Rückert T, Romagnani C (2018) Natural killer cell specificity for viral infections. Nat Immunol 19(8):800–808. https://doi.org/10.1038/s41590-018-0163-6
82. Uong TNT, Lee KH, Ahn SJ, Kim KW, Min JJ, Hyun H, Yoon MS (2018) Real-time tracking of ex vivo-expanded natural killer cells toward human triple-negative breast cancers. Front Immunol 9:825. https://doi.org/10.3389/fimmu.2018.00825
83. Abaei S, Tarighatnia A, Mesbahi A, Aghanejad A (2024) Antibody conjugates as CT/MRI theranostics for diagnosis of cancers: a review of recent trends and advances. Sens Diagn 3:1428–1441. https://doi.org/10.1039/D4SD00132J
84. Heaton A, Hoefges A, Rehani P, Burkard N, Feils A, Spiegelman D, Tsarovsky N, Hampton A, Erbe A, Rakhmilevich AL, Sondel P, Skala M (2022) 72 Intravital multiphoton autofluorescence imaging is sensitive to changes in CD8 T cell and tumor cell metabolism during immunotherapy in a murine melanoma model. BMJ 10(2):A77–A78. https://doi.org/10.1136/jitc-2022-SITC2022.0072
85. Mempel TR, Henrickson SE, Von Andrian UH (2004) T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. Nature 427(6970):154–159. https://doi.org/10.1038/nature02238
86. Mandl JN, Torabi-Parizi P, Germain RN (2014) Visualization and dynamic analysis of host-pathogen interactions. Curr Opin Immunol 29:8–15. https://doi.org/10.1016/j.coi.2014.03.002
87. Sumen C, Mempel TR, Mazo IB, Von Andrian UH (2004) Intravital microscopy: visualizing immunity in context. Immunity 21(3):315–329. https://doi.org/10.1016/j.immuni.2004.08.006
88. Klauschen F, Ishii M, Qi H, Bajénoff M, Egen JG, Germain RN, Meier-Schellersheim M (2009) Quantifying cellular interaction dynamics in 3D fluorescence microscopy data. Nat Protoc 4(9):1305–1311. https://doi.org/10.1038/nprot.2009.129
89. Ishii T, Ishii M (2011) Intravital two-photon imaging: a versatile tool for dissecting the immune system. Ann Rheum Dis 70(Suppl 1):i113–i115. https://doi.org/10.1136/ard.2010.138156
90. Moreau HD, Lemaître F, Terriac E, Azar G, Piel M, Lennon-Dumenil AM, Bousso P (2012) Dynamic in situ cytometry uncovers T cell receptor signaling during immunological synapses and kinapses in vivo. Immunity 37(2):351–363. https://doi.org/10.1016/j.immuni.2012.05.014

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