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Behavior of immune players in the tumor microenvironment

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Abstract

Purpose of review—Tumors recruit various immune cells with seemingly contrasting functions. Yet, the precise role of these cells *in situ* remains vastly unknown. This review presents a new discovery effort that employs intravital imaging to study immune players directly in tissues.

Recent findings—Cytotoxic T lymphocytes (CTLs) that recognize cognate antigenic peptide can infiltrate tumors from the periphery to the center, and physically engage and eliminate antigen-presenting tumor cells. Nevertheless, the reported kinetics for tumor cell killing by CTLs *in vivo* is surprisingly low as it takes several hours for one CTL to eliminate one tumor cell. Also, T regulatory (Treg) cells can create a suppressive milieu that restricts the release of CTL cytotoxic granules, which protects tumor cells from being killed. CTLs may be further subverted during lengthy interactions with tumor-associated macrophages (TAMs). Finally, TAMs can directly facilitate tumor invasion by recruiting tumor cells nearby vessels and promoting their intravasation.

Summary—Intravital imaging has started to uncover tumor-related immune events as they unfold *in vivo*. The technology should be exploited in the coming years to dissect further the tumor microenvironment and to define therapeutics that augment antitumor immunity.

Keywords

imaging; immunotherapy; *in vivo*; macrophages; T cells

Introduction

Cytotoxic T lymphocytes (CTLs) can kill tumor cells that express cognate tumor antigens. Thus, CTLs can, in principle, reject malignant tumors while preserving neighboring normal tissue. The biology of CTLs has been under scrutiny over the past decades, and has benefited from several technical advances: In the 1960s, an assay was developed for quantitative analysis of cytotoxic activity *in vitro* [1]. In the 1990s, soluble agents were developed for labeling and isolation of antigen-specific CTLs *ex vivo* [2]. More recently, with the advent of molecular imaging, we can study CTLs directly *in vivo*. This review discusses new in-vivo observations on CTLs in the context of cancer.

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Antitumor immunity

Ex-vivo monitoring of immune cells in patients with cancer has identified that tumor-specific CTLs frequently accumulate in tumors, recognize short antigenic peptides presented on the surface of tumor cells, and can exhibit potent antitumor functions [3–7]. However, CTLs that infiltrate tumor sites often fail to counter tumor progression. Experiments conducted *in vitro* suggest that CTLs are impaired to release cytotoxic materials or inflammatory cytokines that would otherwise trigger tumor cell death [8,9]. In addition, the finding that antitumor CTLs are generally fully functional in circulation and in tumor-free tissues in the same individual [8] suggests that the tumor microenvironment has a unique ability to compromise antitumor immunity. At least two immunosuppressive cell types – $FoxP3^+$ regulatory T (Treg) cells and tumor-associated macrophages (TAMs) – are found in high numbers in various human tumors [10–15] and suppress antitumor CTL immunity in experimental mouse models [16–18]. Other hematopoeitic (e.g. mast cells, neutrophils, NK cells, dendritic cells) and nonhematopoeitic cells (e.g. fibroblasts, endothelial cells) can also infiltrate tumors and at least some of them have been shown to promote cancer invasion and metastasis in tumor-bearing mice [19–21]. However, the in-vivo contribution of all the cells mentioned above, as well as of structural components, such as the extracellular matrix, remains speculative to a large extent. Only recently, technological advances have allowed researchers to track cells *in vivo* and to derive some quantitative information on their behavior and interplay with the environment.

In-vivo imaging technologies

Several imaging technologies have been developed for tracking cells *in vivo*; typically they include systems that derive information on the basis of the detection of photons [22] (optical imaging), radioactivity [23,24] (nuclear imaging), or magnetism [25] (magnetic resonance imaging). The techniques can track labeled cells or molecules at different depths in tissues and spatial resolutions. This review focuses on the analysis of the tumor microenvironment using fluorescence-based optical imaging technologies. These include multiphoton or confocal intravital microscopy that has been particularly useful to define the behavior of some immune cells that control tumor growth [26,27]. The techniques can track fluorescently labeled cells in three dimensions: at subcellular resolution and at depths up to 800 μm for multiphoton microscopy. Time-lapse recordings permit derivation of parameters of cell migration and interaction, although the recordings are typically limited to relatively small fields of view (e.g. 500 μm × 500 μm). Fluorescence-mediated tomography (FMT) technologies complement microscopic systems because they can reconstruct quantitatively three-dimensional maps of fluorescently labeled cells in large fields of view (e.g. the whole body of a mouse) and at ~1 mm resolution [28]. The combination of microscopic and macroscopic imaging offers the possibility to evaluate cellular activity and biodistribution quantitatively, for example, at strategic sites such as primary tumors, draining lymph nodes, or metastases, and can be repeated over time to track immune responses longitudinally [29,30•] (Fig. 1).

The optical imaging systems can detect cells labeled with different categories of agents, such as genetic fluorescent reporters [31], fluorescent chemical dyes [30•], or injectable targeting agents [32]. The genetic reporter approach is useful for microscopic imaging; however, it has limitations for analysis in deep tissue (e.g. >600 μm), because fluorescent proteins available today typically either fluoresce at unfavorable short wavelengths within the visible spectrum or show low quantum efficiency. Near-infrared fluorescent dyes allow efficient detection in deep tissues, because tissue absorbance and autofluorescence are minimal at these wavelengths, but are diluted out in labeled cells along with cellular division. Finally, injectable imaging agents that are specific for molecular targets offer the advantage of being usable in both experimental animals and humans, target endogenous cell populations directly, can carry multiple reporters for imaging at different resolutions and depths, and can combine diagnostic

and therapeutic interventional capabilities. Such agents, however, exist for a small minority of targets of interest in cancer and thus need further development. Optical systems that are not based on fluorescence also exist, such as bioluminescence imaging [33].

With the imaging systems and agents mentioned above, the current objectives are: to study cellular players directly *in situ*, to quantify and model information obtained by bioimaging, and to develop comprehensive approaches for the simultaneous investigation of various cell types in defined microenvironments (Fig. 2).

Tumor-specific cytotoxic T lymphocytes

A multiphoton microscopy study by the Weninger lab [34] analyzed the behavior of tumor-infiltrating CTLs in an experimental context in which the CTLs efficiently reject tumor cells. The authors investigated endogenous CTLs in mice with E7-expressing tumors after vaccination with an adenovirus that induces E7-specific CTLs, or adoptively transferred chicken ovalbumin (OVA) specific CTLs in mice with OVA-expressing tumors. In both cases, tumor cells and CTLs expressed different fluorescent proteins and thus could be tracked simultaneously. Time-lapse imaging revealed that tumor-specific CTLs migrate randomly throughout the tumor microenvironment, and that sustained migration requires recognition of cognate antigen by CTLs. Upon antigen recognition on the surface of tumor cells, some CTLs physically engage long-lasting interactions (>30 min), whereas others establish short-term and sequential contacts. The reasons for these different behaviors are unknown, but it is likely that the delivery of cytotoxic granules is possible only during long-lasting interactions [35••]. In some occasions, contact with CTLs directly preceded initiation of apoptosis in tumor cells, thus confirming that CTLs can trigger tumor cell killing as it was previously observed *in vitro* [36].

The Amigorena lab recently used an experimental approach [37••] similar to the one described above. The study shows that CTLs that accumulate in tumors initially locate in the tumor periphery and then infiltrate in the direction of the center. Peripheral CTLs typically show high velocities until they arrest on tumor cells; such arrest requires recognition by the CTLs of cognate antigen presented by the tumor cell. The outcome of CTL–tumor cell interactions was not studied here; however, the authors observed that arrested CTLs can eventually resume migration, typically in regions where neighboring tumor cells have been killed; these CTLs are presumably in search for intact tumor cells. Migrating CTLs sometimes follow collagen fibers or blood vessels, in which case they adopt an elongated morphology. The authors also noted that the presence of cognate antigen is required for CTL infiltration in the center of the tumor. This is in line with previous studies in patients with melanoma that used MHC/peptide tetramers to quantify CTLs specific for single antigens and identified that tumor antigen-specific CTLs account for a substantial fraction of total CD8 T cells present in the tumor site [38]. Another recent imaging study using ^{111}In-oxine-labeled CTLs and in-vivo nuclear imaging also revealed that CTLs reach the center of the tumor (and control tumor growth) only when tumor cells express the cognate antigen [39•].

A new multiphoton microscopy study by the Bousso lab further investigated the kinetics of tumor cell killing by CTLs, using a real-time single-cell killing assay [35••]. This involved the construction of genetically modified tumor cell lines that express cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) molecules linked by a DEVD motif. Tumor cells entering apoptosis activate caspase 3, which cleaves DEVD and results in disruption of Forster resonance energy transfer (FRET) between the two fluorescent moieties. A measure of FRET can be obtained by multiphoton-intravital microscopy (MP-IVM) in real-time and in individual cells. The approach revealed that tumor cells remain alive if they are not associated with CTLs, whereas nearly all the tumor cells that die during the imaging period have been stably engaged

with at least one CTL. By tracking a large number of CTL–tumor cell interactions, the authors could estimate that it takes on average 6 h for one CTL to kill one tumor cell. In addition, CTLs often remain attached to apoptotic tumor cells for several hours; thus the average time that occurs before one CTL is ready to attack a second tumor cell is above 6 h. It remains to be addressed why killing of tumor cells by CTLs takes such a long time. In fact, CTLs in lymph nodes can kill antigen-pulsed target cells and subsequently detach from them in only 15 min, and typically in <1 h [29]. The relatively low pace at which CTLs kill tumor cells may have consequences in patients with tumors that continue to grow despite the presence of relatively high numbers of infiltrating CTLs. If tumor cells divide faster than they are being killed, the balance between tumor progression and immune attack would eventually be tilted in the favor of the tumor.

In the context where CTLs efficiently reject tumors cells, an outstanding question is whether CTLs directly drive tumor rejection or if they recruit other cells, such as myeloid cells, that are responsible for tumor elimination. This question was addressed in the study mentioned above by co-injecting in the same animal a mixture of two populations of tumor cells, one expressing OVA and the other one not [35••]. Because injection of OVA-specific CTLs only rejected OVA-expressing tumor cells, the authors could conclude that CTLs, but not myeloid cells, are largely involved in tumor destruction. In fact, there is strong evidence that myeloid and other cells recruited in the tumor environment display potent tumorigenic functions. These cells are discussed in the following sections.

Tumor-specific T regulatory cells

Although in-vivo investigations of tumor-infiltrating Treg cells have not been reported yet, a multiphoton microscopy study in tumor-draining lymph nodes has shown that these suppressor cells control the function of neighboring CTLs [29]. Suppression does not require long-lasting interactions between CTLs and Treg cells, but is dependent on TGF-b receptor signaling in CTLs. This suggests that Treg cells create a suppressive milieu, for example, rich in TGF-b, allowing local control of CTL function [27]. The regulated CTLs exhibit no defect in proliferation, induction of cytotoxic effector molecules, and secretory granules, in-situ motility or ability to form antigen-dependent conjugates with target cells; however, they cannot release their cytotoxic granules while bound to their targets, and thus have a severely compromised ability to kill. The inhibition mediated by Treg cells is reversible since the suppressed CTLs quickly regain full killing capacity upon selective removal of Treg cells, indicating that antitumor functions of suppressed CTLs may be harnessed for therapy. It will be important to define whether Treg cells behave similarly in the tumor microenvironment, and to which extent they contribute to suppression in comparison with other cell types. For example, tumor cells and TAMs can produce copious amounts of TGF-b, and thus may personally participate in the suppression of CTL effector functions in the tumor microenvironment. In fact, the reduced kinetics of CTL-mediated tumor cell killing [35••], when compared to B cell killing [29], may be explained, at least in part, by tumor-associated factors that impede CTL effector functions. Future studies should also investigate how tumor-infiltrating Treg cells communicate with other cells in the tumor stroma. Interesting candidates are TAMs because these cells have the capacity to acquire either inflammatory or immunosuppressive functions [40], and recent in-vitro experiments indicate that Treg cells selectively polarize TAMs towards an immunosuppressive `M2-like' phenotype [41].

Tumor-associated macrophages

Cells of myeloid origin, notably TAMs or myeloid-derived suppressor cells (MDSCs), efficiently suppress CTL immunity *in vitro*, and even promote tumor growth, angiogenesis, and metastasis [13–15,42,43]. The accumulation of TAMs is therefore generally associated

with a poor prognosis [44], whereas their removal in animal models can lead to tumor regression [45,46]. An elegant in-vitro study showed recently that TAMs release reactive oxygen species and peroxynitrite, which results in the nitration of tyrosines in the TCR/CD8 complex on CTLs [47•]. These de-sensitized CTLs are less able to bind peptide/MHC molecules presented by tumor cells, and thus cannot efficiently mediate their effector functions. In-vitro studies have also shown that TAMs can produce an array of `M2-like' cytokines, particularly when tumors begin to invade and vascularize [15]. Because TAMs undergo prolonged physical interactions with CTLs *in vivo* [34], it is tempting to speculate that TAMs can feed CTLs with immunosuppressive signals and promote immune tolerance. However, TAMs sometimes release `M1-like' proinflammatory cytokines, for example, at sites of chronic inflammation or early during tumor development [15]. In this case TAMs interacting with CTLs could possibly enhance CTL effector functions. It is hoped that in-vivo molecular imaging approaches will help resolve these issues.

Although the outcome of TAM–CTL interactions has not been investigated *in vivo*, some intravital imaging studies have started to investigate the impact of TAMs on the behavior of tumor cells. The Condeelis lab has developed a technique for visualization of implanted GFP + carcinoma mammary cells and endogenous TAMs [48••]. The latter cells can be visualized either after injection of Texas-red dextran, or in genetically modified mice that express GFP under the control of a macrophage/neutrophil promoter. From these experiments it could be identified that TAMs accumulate in large numbers in the periphery of the tumor, and are found at decreasing density deeper inside the tumor, as this was previously reported using invasive approaches. TAMs in the center of the tumor associate with blood vessels, either as single cells or small clusters. Time-lapse imaging indicates that tumor cells close to TAMs (i.e. <20 μm) are more often motile than the ones not near TAMs. The motile tumor cells usually migrate at a relatively low speed (~4 μm/min) but toward perivascular TAMs, where they become tightly associated with the vessel surface and can eventually enter the blood flow. This process of TAM-associated tumor cell intravasation can occur in the absence of local angiogenesis. The authors further propose that a paracrine loop exists between TAMs and tumor cells, which involves EGF receptors on tumor cells that bind EGF produced by TAMs, and CSF-1 receptors on TAMs that bind CSF produced by tumor cells. This hypothesis is substantiated by the fact that administration of an inhibitor of either the EGF or CSF-1 receptor substantially decreases the number of tumor cells entering the circulation [48••], and corroborates with previous studies from the same group [42].

Other cells

The tumor stroma includes other hematopoietic and nonhematopoietic cells that have been suggested to shape tumor immunity, but the activity of these cells *in vivo* remains poorly defined. Carcinoma-associated fibroblasts, when mixed with tumor cells before injection into host animals, promote angiogenesis and cancer invasion [49], and a recent imaging study has substantiated that administered fibroblasts selectively accumulate around the angiogenic vasculature, but not in the nodules, of ovarian carcinomas [50]. Endogenous fibroblasts recruited to tumors can also be visualized in transgenic mice expressing a fluorescent reporter under the control of the promoter for vascular endothelial growth factor [51]. Diverse subsets of dendritic cells also accumulate in the tumor stroma [52] and likely regulate T cell immunity locally. Imaging techniques, including intravital videomicroscopy and multiphoton microscopy, have allowed researchers to visualize dendritic cell migration and interactions in various tissues [53]; however, the behavior of tumor-infiltrated dendritic cells has not yet been analyzed. Conversely, dendritic cell immunotherapy is being used in the clinic to promote antitumor CTL immunity [54,55]. This therapeutic approach could benefit from recent imaging tools that allow one to define if the injected cells are accurately delivered to their desired target site in cancer patients [56]. Other imaging tools have been developed recently to visualize

endothelial cells or tumor vessels, both at microscopic [57] and mesoscopic resolutions [58]. Some of them have potential clinical translatability and may facilitate the study of tumor pathophysiology and response to treatment. Finally, mast cells [19], neutrophils [20], and mesenchymal stem cells [21] likely also play crucial roles in cancer but have not been studied in detail with in-vivo molecular imaging techniques.

Perspectives and needs

From a technical standpoint, it is expected that increasingly refined methods of investigations will be developed shortly. Below are listed four improvements that would further advance the field of in-vivo molecular imaging (see also Table 1). The in-vivo studies used so far successfully document the behavior of cells in mice with genetically modified tumor cell lines implanted subcutaneously. Future studies could employ perhaps more relevant biological systems that use spontaneously growing tumors [59,60•] and antitumor CTLs recognizing true tumor antigens [61]. Immune cells are frequently adoptively transferred before imaging. The development of new mouse models with imaging reporters in specific cell types, or new injectable imaging agents that target endogenous cell types [22,32], represent interesting alternatives that would not require adoptive transfer and would have increased clinical translatability. Third, the ability to track the location of molecules (e.g. cytokines, proteases) within cells would have a tremendous impact on our understanding of how cells communicate with their environment and mediate their functions. This will require the generation of sophisticated molecular reporters and imaging systems that can monitor genes that are expressed at physiological levels. The new experimental strategies may involve fusion of genes of interest with fluorescent proteins [62], covalent protein labeling with quantum dots [63], or activatable optical sensors [64]. Imaging studies usually inform on one or two cell types at a time, although the tumor microenvironment is composed of numerous players that likely play distinct and/or complementing functions. Comprehensive approaches that analyze a larger number of cellular or molecular actors simultaneously would therefore be useful.

Conclusion

Our ability to dissect the activity of cells in tissues, in real time and at different spatial scales, has started to yield new insight into how tumor immunity happens *in vivo*. It is hoped that further studies, complementing findings from nonimaging approaches, will eventually elucidate the many immunological processes that promote tumorigenesis, and in turn will help improve immunotherapeutic approaches.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest

•• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

1. Brunner K, Mauel J, Cerottini J, Chapuis B. Quantitative assay of the lytic action of immune lymphoid cells on 51-Cr-labelled allogeneic target cells in vitro: inhibition by isoantibody and by drugs. Immunology 1968;14:181–196. [PubMed: 4966657]
2. Altman J, Moss P, Goulder P, et al. Phenotypic analysis of antigen-specific T lymphocytes. Science 1996;274:94–96. [PubMed: 8810254]
3. Boon T, Coulie P, Van Den Eynde BJ, Van Der Bruggen P. Human T cell responses against melanoma. Annu Rev Immunol 2006;24:175–208. [PubMed: 16551247]
4. Dunn G, Bruce A, Ikeda H, et al. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 2002;3:991–998. [PubMed: 12407406]
5. Dudley ME, Wunderlich J, Robbins PF, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science 2002;298:850–854. [PubMed: 12242449]
6. Romero P, Dunbar P, Valmori D, et al. Ex vivo staining of metastatic lymph nodes by class I major histocompatibility complex tetramers reveals high numbers of antigen-experienced tumor-specific cytolytic T lymphocytes. J Exp Med 1998;188:1641–1650. [PubMed: 9802976]
7. Van Der Bruggen P, Traversari C, Chomez P, et al. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science 1991;254:1643–1647. [PubMed: 1840703]
8. Zippelius A, Batard P, Rubio-Godoy V, et al. Effector function of human tumor-specific CD8 T cells in melanoma lesions: a state of local functional tolerance. Cancer Res 2004;64:2865–2873. [PubMed: 15087405]
9. Lee PP, Yee C, Savage P, et al. Characterization of circulating T cells specific for tumor-associated antigens in melanoma patients. Nat Med 1999;5:677–685. [PubMed: 10371507]
10. Curiel T, Coukos G, Zou L, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med 2004;10:942–949. [PubMed: 15322536]
11. Liyanage U, Moore T, Joo H, et al. Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinoma. J Immunol 2002;169:2756–2761. [PubMed: 12193750]
12. Wang H, Lee DA, Peng G, et al. Tumor-specific human CD4+ regulatory T cells and their ligands: implications for immunotherapy. Immunity 2004;20:107–118. [PubMed: 14738769]
13. Pollard J. Tumour educated macrophages promote tumour progression and metastasis. Nat Rev Cancer 2004;4:71–78. [PubMed: 14708027]
14. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature 2008;454:436–444. [PubMed: 18650914]
15. Murdoch C, Muthana M, Coffelt S, Lewis C. The role of myeloid cells in the promotion of tumour angiogenesis. Nat Rev Cancer 2008;8:618–631. [PubMed: 18633355]
16. Chen M, Pittet M, Gorelik L, et al. Regulatory T cells suppress tumor-specific CD8 T cell cytotoxicity through TGF-beta signals in vivo. Proc Natl Acad Sci U S A 2005;102:419–424. [PubMed: 15623559]
17. Dittmer U, He H, Messer RJ, et al. Functional impairment of CD8(+) T cells by regulatory T cells during persistent retroviral infection. Immunity 2004;20:293–303. [PubMed: 15030773]
18. Lin C, Graca L, Cobbold S, Waldmann H. Dominant transplantation tolerance impairs CD8+ T cell function but not expansion. Nat Immunol 2002;3:1208–1213. [PubMed: 12415266]
19. Soucek L, Lawlor E, Soto D, et al. Mast cells are required for angiogenesis and macroscopic expansion of Myc-induced pancreatic islet tumors. Nat Med 2007;13:1211–1218. [PubMed: 17906636]
20. Shojaei F, Wu X, Zhong C, et al. Bv8 regulates myeloid-cell-dependent tumour angiogenesis. Nature 2007;450:825–831. [PubMed: 18064003]
21. Karnoub A, Dash A, Vo A, et al. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature 2007;449:557–563. [PubMed: 17914389]
22. Weissleder R, Pittet M. Imaging in the era of molecular oncology. Nature 2008;452:580–589. [PubMed: 18385732]
23. Gambhir SS. Molecular imaging of cancer with positron emission tomography. Nat Rev Cancer 2002;2:683–693. [PubMed: 12209157]

24. Phelps M. Inaugural article: positron emission tomography provides molecular imaging of biological processes. Proc Natl Acad Sci U S A 2000;97:9226–9233. [PubMed: 10922074]
25. Bulte JW, Kraitchman D. Iron oxide MR contrast agents for molecular and cellular imaging. NMR Biomed 2004;17:484–499. [PubMed: 15526347]
26. Condeelis J, Segall J. Intravital imaging of cell movement in tumours. Nat Rev Cancer 2003;3:921–930. [PubMed: 14737122]
27. Pittet M, Mempel T. Regulation of T-cell migration and effector functions: insights from in vivo imaging studies. Immunol Rev 2008;221:107–129. [PubMed: 18275478]
28. Ntziachristos V, Tung CH, Bremer C, Weissleder R. Fluorescence molecular tomography resolves protease activity in vivo. Nat Med 2002;8:757–760. [PubMed: 12091907]
29. Mempel T, Pittet M, Khazaie K, et al. Regulatory T cells reversibly suppress cytotoxic T cell function independent of effector differentiation. Immunity 2006;25:129–141. [PubMed: 16860762]
30. Swirski F, Cr B, Figueiredo J, et al. A near-infrared cell tracker reagent for multiscopic in vivo imaging and quantification of leukocyte immune responses. PLoS ONE 2007;2:1075. This study identifies a novel cell-labeling agent that can be used to detect lymphocytes in the whole body with fluorescence molecular tomography, and within tissues at single cell resolution by multiphoton or confocal microscopy.
31. Giepmans B, Adams S, Ellisman M, Tsien R. The fluorescent toolbox for assessing protein location and function. Science 2006;312:217–224. [PubMed: 16614209]
32. Weissleder R, Kelly K, Sun E, et al. Cell-specific targeting of nanoparticles by multivalent attachment of small molecules. Nat Biotechnol 2005;23:1418–1423. [PubMed: 16244656]
33. Contag C, Bachmann M. Advances in in vivo bioluminescence imaging of gene expression. Annu Rev Biomed Eng 2002;4:235–260. [PubMed: 12117758]
34. Mrass P, Takano H, Ng L, et al. Random migration precedes stable target cell interactions of tumor-infiltrating T cells. J Exp Med 2006;203:2749–2761. [PubMed: 17116735]
35. Breart B, Lemaitre F, Celli S, Bousso P. Two-photon imaging of intratumoral CD8+ T cell cytotoxic activity during adoptive T cell therapy in mice. J Clin Invest 2008;118:1390–1397. [PubMed: 18357341] This elegant intravital imaging study characterizes the kinetics of tumor cell killing by cytotoxic T lymphocytes and the contribution of these cells versus myeloid cells in the elimination of tumors.
36. Stinchcombe J, Bossi G, Booth S, Griffiths G. The immunological synapse of CTL contains a secretory domain and membrane bridges. Immunity 2001;15:751–761. [PubMed: 11728337]
37. Boissonnas A, Fetler L, Zeelenberg I, et al. In vivo imaging of cytotoxic T cell infiltration and elimination of a solid tumor. J Exp Med 2007;204:345–356. [PubMed: 17261634] This intravital imaging study investigates the role of cognate antigen for T cell motility and infiltration within tumors.
38. Romero P, Valmori D, Pittet M, et al. Antigenicity and immunogenicity of Melan-A/MART-1 derived peptides as targets for tumor reactive CTL in human melanoma. Immunol Rev 2002;188:81–96. [PubMed: 12445283]
39. Pittet M, Grimm J, Berger C, et al. In vivo imaging of T cell delivery to tumors after adoptive transfer therapy. Proc Natl Acad Sci U S A 2007;104:12457–12461. [PubMed: 17640914] This study shows that nuclear SPECT/CT imaging can be used to follow 111In-oxine-labeled T cells in the whole body and noninvasively.
40. Mantovani A, Sozzani S, Locati M, et al. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol 2002;23:549–555. [PubMed: 12401408]
41. Tiemessen M, Jagger A, Evans H, et al. CD4+CD25+Foxp3+ regulatory T cells induce alternative activation of human monocytes/macrophages. Proc Natl Acad Sci U S A 2007;104:19446–19451. [PubMed: 18042719]
42. Condeelis J, Pollard J. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell 2006;124:263–266. [PubMed: 16439202]
43. De Visser KE, Eichten A, Coussens L. Paradoxical roles of the immune system during cancer development. Nat Rev Cancer 2006;6:24–37. [PubMed: 16397525]

44. Bingle L, Brown N, Lewis C. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. J Pathol 2002;196:254–265. [PubMed: 11857487]
45. Lin EY, Nguyen A, Russell R, Pollard J. Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. J Exp Med 2001;193:727–740. [PubMed: 11257139]
46. Zhang B, Zhang Y, Bowerman N, et al. Equilibrium between host and cancer caused by effector T cells killing tumor stroma. Cancer Res 2008;68:1563–1571. [PubMed: 18316622]
47. Nagaraj S, Gupta K, Pisarev V, et al. Altered recognition of antigen is a mechanism of CD8+ T cell tolerance in cancer. Nat Med 2007;13:828–835. [PubMed: 17603493] This study identifies how tumor-associated macrophages can impair the function of cytotoxic T lymphocytes.
48. Wyckoff J, Wang Y, Lin E, et al. Direct visualization of macrophage-assisted tumor cell intravasation in mammary tumors. Cancer Res 2007;67:2649–2656. [PubMed: 17363585] This intravital imaging identifies that tumor-associated macrophages can directly promote tumor cell intravasation.
49. Orimo A, Gupta P, Sgroi D, et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell 2005;121:335–348. [PubMed: 15882617]
50. Granot D, Addadi Y, Kalchenko V, et al. In vivo imaging of the systemic recruitment of fibroblasts to the angiogenic rim of ovarian carcinoma tumors. Cancer Res 2007;67:9180–9189. [PubMed: 17909023]
51. Fukumura D, Xavier R, Sugiura T, et al. Tumor induction of VEGF promoter activity in stromal cells. Cell 1998;94:715–725. [PubMed: 9753319]
52. Rabinovich G, Gabrilovich D, Sotomayor E. Immunosuppressive strategies that are mediated by tumor cells. Annu Rev Immunol 2007;25:267–296. [PubMed: 17134371]
53. Alvarez D, Vollmann E, Von Andrian UH. Mechanisms and consequences of dendritic cell migration. Immunity 2008;29:325–342. [PubMed: 18799141]
54. Gilboa E. DC-based cancer vaccines. J Clin Invest 2007;117:1195–1203. [PubMed: 17476349]
55. Steinman R. Dendritic cells in vivo: a key target for a new vaccine science. Immunity 2008;29:319–324. [PubMed: 18799140]
56. De Vries IJ, Lesterhuis W, Barentsz J, et al. Magnetic resonance tracking of dendritic cells in melanoma patients for monitoring of cellular therapy. Nat Biotechnol 2005;23:1407–1413. [PubMed: 16258544]
57. Stroh M, Zimmer J, Duda D, et al. Quantum dots spectrally distinguish multiple species within the tumor milieu in vivo. Nat Med 2005;11:678–682. [PubMed: 15880117]
58. Montet X, Figueiredo J, Alencar H, et al. Tomographic fluorescence imaging of tumor vascular volume in mice. Radiology 2007;242:751–758. [PubMed: 17325064]
59. Johnson L, Mercer K, Greenbaum D, et al. Somatic activation of the K-ras oncogene causes early onset lung cancer in mice. Nature 2001;410:1111–1116. [PubMed: 11323676]
60. Kirsch D, Dinulescu D, Miller J, et al. A spatially and temporally restricted mouse model of soft tissue sarcoma. Nat Med 2007;13:992–997. [PubMed: 17676052] This study describes a novel mouse model of soft-tissue sarcoma that mimics human disease and that can be used for molecular imaging.
61. Overwijk W, Tsung A, Irvine K, et al. gp100/pmel 17 is a murine tumor rejection antigen: induction of ``self''-reactive, tumoricidal T cells using high-affinity, altered peptide ligand. J Exp Med 1998;188:277–286. [PubMed: 9670040]
62. Shaner NC, Steinbach P, Tsien R. A guide to choosing fluorescent proteins. Nat Methods 2005;2:905–909. [PubMed: 16299475]
63. Bonasio R, Carman C, Kim E, et al. Specific and covalent labeling of a membrane protein with organic fluorochromes and quantum dots. Proc Natl Acad Sci U S A 2007;104:14753–14758. [PubMed: 17785425]
64. Weissleder R, Ntziachristos V. Shedding light onto live molecular targets. Nat Med 2003;9:123–128. [PubMed: 12514725]

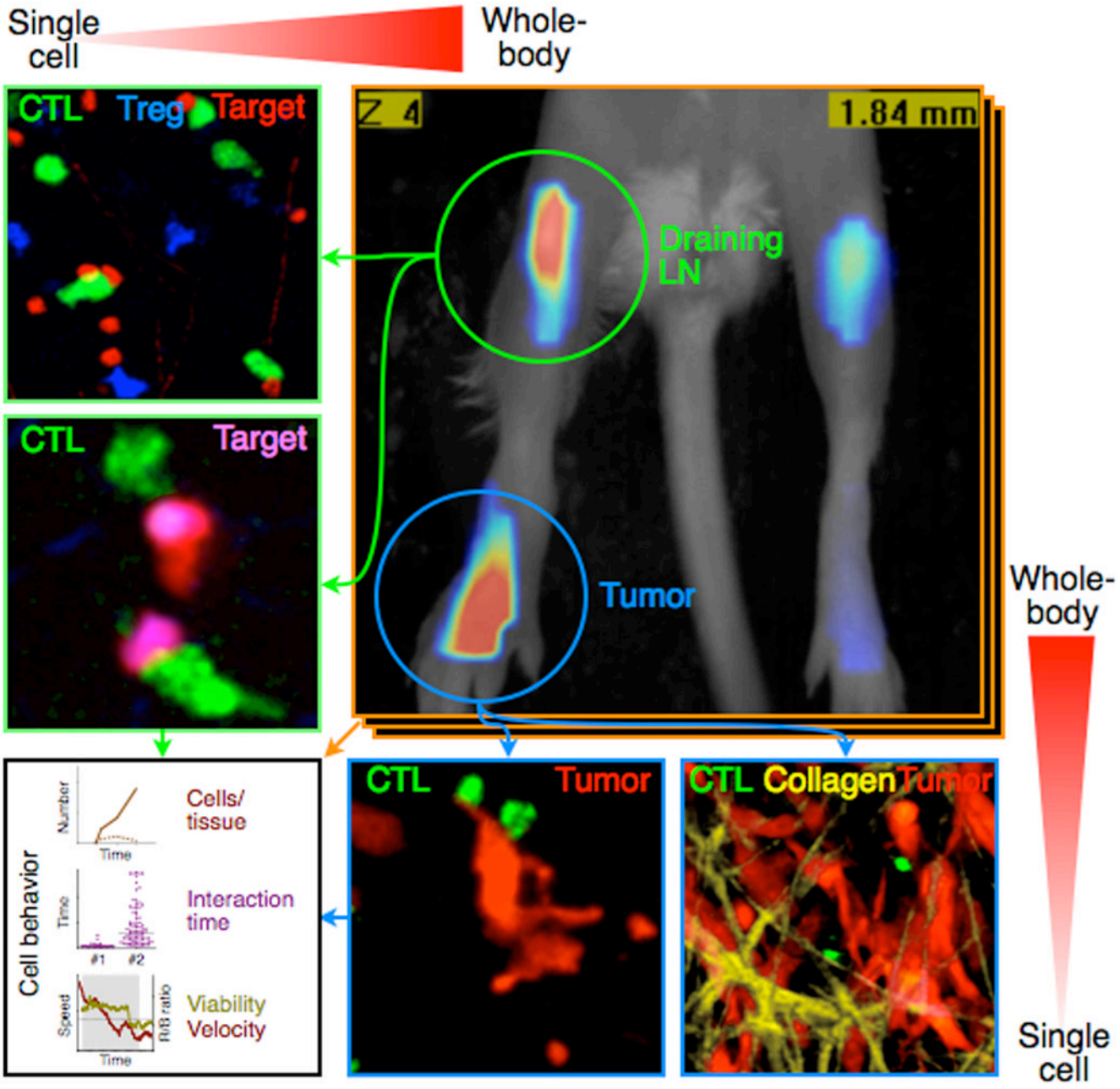


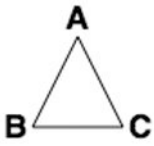
Figure 1. In-vivo imaging of T cells gr1

Tracking of T cells in three dimensions and at different scales can be achieved by using complementary optical imaging approaches. Whole-body imaging uses fluorescence molecular tomography (FMT) that reconstructs quantitative maps of fluorescently labeled cells in three dimensions on the basis of advanced algorithms. Single cell imaging uses intravital multiphoton or confocal microscopy that yields three-dimensional information from light emitted by differentially labeled objects. Here the FMT image in the upper right panel shows tumor-specific cytotoxic T lymphocytes (CTLs) labeled with the far-red dye VT680. The CTLs can be quantified in the tumor-draining lymph node and at the tumor site. The intravital microscopy images (left and bottom panels) show CTLs and other cells types, for example,

tumor-specific T regulatory cells (Treg), antigen-pulsed target B cells (Target), tumor cells (Tumor), as well as collagen fibers (Collagen). The analysis was performed within tissues, either in tumor-draining lymph nodes, or at the tumor site. Analysis in draining lymph nodes identifies CTL,, Treg cells, and target cells within the same microenvironment, and allows one to visualize target cell lysis by CTLs. Analysis in tumors shows physical engagements between CTLs and tumor cells. Time-lapse, multicolor imaging permits to assess the behavior of the different cell types simultaneously, as illustrated in the lower left panel. The images are reproduced with permission from Refs. [29] and [30•].

Study of cellular players in situ with appropriate bioimaging technologies

Type	Microscopic	Meso & Macroscopic
Optical:	Multiphoton intravital microscopy (MP-IVM)	Fluorescence mediated tomography (FMT)
	Confocal intravital microscopy	Fluorescence reflectance imaging (FRI)
	Fiber-optic microscopy	Bioluminescence imaging (BLI)
Nuclear:	–	Positron emission tomograpgy (PET)
		Single-photon emission computed tomography (SPECT)
Magnetic:	Magnetic resonance imaging (MRI)	Magnetic resonance imaging (MRI)



Quantification and modeling of information obtained by bioimaging

Microscopic	Macroscopic
Interaction time	Biodistribution
Motility	Quantification of cell populations
Chemotaxis	
cell integrity (dead/alive)	

Comprehensive investigation of components of the tumor microenvironment

Immune cells	Non-immune cells	Others
All T cell types	Tumor cells	Extracellular matrix
NK cells	Fibroblasts	
All myeloid cell types	Endothelial cells	
	Stem cells	

Figure 2. Three objectives when performing in-vivo molecular imaging gr2

(a) Choice of the appropriate technology to track cells of interest at either microscopic or mesoscopic/macroscopic resolution; (b) quantification of information obtained by bioimaging; and (c) development of more comprehensive approaches for the simultaneous investigation of various cell types in defined microenvironments.

Table 1

Technological improvement for in-vivo imaging of immune cells in cancer

1	Use of genetically inducible mouse models that recapitulate closely human disease
2	Visualization of endogenous cells by means of new mouse models or injectable targeting agents
3	Simultaneous analysis of multiple cell types
4	Ability to track molecules within cells