Published in final edited form as:

Trends Cancer. 2015 September 1; 1(1): 66–75. doi:10.1016/j.trecan.2015.07.008.

Immune response to cancer therapy: mounting an effective antitumor response and mechanisms of resistance

Terry R. Medler¹, Tiziana Cotechini¹, and Lisa M. Coussens^{1,2,*}

¹Department of Cell, Developmental & Cancer Biology, Oregon Health & Science University, Portland, Oregon, USA

²Knight Cancer Institute, Oregon Health & Science University, Portland, Oregon, USA

Abstract

Chemotherapy and radiotherapy have been extensively used to eradicate cancer based on their direct cytotoxic effects on rapidly proliferating tumor cells. Accumulating evidence indicates that these therapies also dramatically affect resident and recruited immune cells that actively support tumor growth. We now appreciate that mobilization of effector CD8⁺ T cells enhances efficacy of chemotherapy and radiotherapy; remarkable clinical advances have been achieved by blocking regulatory programs limiting cytotoxic CD8⁺ T cell activity. This review discusses immune-mediated mechanisms underlying efficacy of chemotherapy and radiotherapy, and provides a perspective on how understanding tissue-based immune mechanisms can be used to guide therapeutic approaches combining immune and cytotoxic therapies to improve outcomes for a larger subset of patients than currently achievable.

Keywords

Checkpoints; Chemotherapy; Radiotherapy; Macrophage; Immunosuppression

Antitumor Immunity: CD8⁺ T cells and their regulation

Surrounding a nest of neoplastic tumor cells is a microenvironment consisting of diverse mesenchymal support cells (fibroblasts, adipocytes, etc.), cells forming hematogenous and lymphatic vasculature, immune cells, and a dynamically-regulated extracellular matrix (ECM), all of which influence neoplastic progression to the malignant and/or metastatic state [1, 2]. Resident or recruited immune cells present within the tumor microenvironment (TME) represent a diverse assemblage of both lymphoid and myeloid cells, and depending on their activation state and phenotype, can either promote or inhibit various aspects of tumor development [3] as well as regulate response to anti-cancer therapy [4, 5].

*Corresponding Author: Lisa M. Coussens, Ph.D., Professor and Chair, Cell, Developmental & Cancer Biology, Mail Code L215, 3181 SW Sam Jackson Park Road, Portland, OR 97239, Tel: 503-494-7811, coussensl@ohsu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Anti-tumor immunity is largely imposed by antigen-specific CD8⁺ T cells [6], although tumoricidal macrophages do play a role [7]. Antigens (Ag), typically foreign substances of environmental, viral, or bacterial origin, products of somatically altered proteins, or debris from dying (apoptotic) cells are processed and presented by major histocompatibility complex (MHC) on antigen presenting cells, including (but not limited to) dendritic cells, macrophages, and B cells. CD8⁺ T cells utilize T cell receptors (TCRs) to recognize MHC-presented peptides and subsequently mount an antigen-specific cytolytic attack [8, 9]. In particular, Ag-TCR engagement ultimately leads to activation and proliferation of CD8⁺ T cells that play a crucial role in autoimmunity, response to pathogens, and tumor suppression [10–13]. Genetic rearrangement of TCRs during T cell development enables the recognition of a broad spectrum of processed Ag in the adult. Individual T cells vary with respect to precise structure of TCRs, and this variation endows each T cell with a different specificity for a distinct foreign entity [8]. There is growing evidence that solid tumors contain T cells specific to tumor Ags, but have either been rendered tolerant or inhibited from killing tumor cells by expression of suppressive cytokines such as interleukin (IL)-10 [14] and arginase [15], expression of ligands for immune checkpoints molecules [16], downregulation of MHC class I on tumor cells [17], or other immunosuppressive factors expressed within the TME [18]. Therefore, a major goal of immune-based therapies is to quell regulatory programs limiting CD8⁺ T cell responses to tumor Ags. For example, selective checkpoint inhibition can unleash endogenous anti-tumor programs; this approach has resulted in dramatic and durable clinical responses for several tumor types. In particular, antibodies blocking cytotoxic T-lymphocyte-associated protein 4 (CTLA- 4) [19–21] or the programmed death (PD)-1 pathway have resulted in durable responses for various malignancies [22–26]. Despite these clinical successes, many patients' endogenous immune responses are insufficient to mediate anti-tumor programs when checkpoints are blocked, possibly due to high level expression of suppressive molecules expressed by regulatory T or B cells, or myeloid cells present within the TME.

Myeloid cells, including various subsets of monocytes, neutrophils, and macrophages, are implicated in T cell suppression [27]. Macrophages exist along a continuum of subtypes in tumors with classically-activated M1-like macrophages on one end, and alternatively-activated M2-like macrophages on the other [28]. In breast cancers, tumors vary with respect to relative proportions of macrophages (in general) and CD8⁺ T cells. High macrophage ratios correlate with decreased overall survival (OS), progression-free survival (PFS), and pathologic complete response to therapy (pCR), with the most significant stratification of these parameters in women with HER2+ and basal/triple negative breast cancer [29, 30]. Several have reported that macrophages present in several types of solid tumors are predominantly immunosuppressive (M2-like) and play a major role in suppressing the actions of CD8⁺ T cells, as well as fostering malignancy by providing pro-growth, survival, and angiogenic molecules critical for rapid tumor development [2, 31–36]. A more extensive summary of the protumorigenic functions of macrophages has been recently reviewed elsewhere [4, 37].

The functional significance of macrophage:T cell ratios has been examined in pre-clinical mouse models of cancer development. The data indicate that either reprogramming immunosuppressive macrophages towards an pro-inflammatory (T_H1) state or eliminating

macrophages altogether, depending on the tumor context, limits tumor growth by fostering the infiltration of interferon (IFN)- γ or granzyme B-producing CD8⁺ T cells [30, 34]. These approaches yield significantly improved outcomes for tumor-bearing mice when delivered in combination with cytotoxic chemotherapy (CTX) or radiotherapy (RT), some targeted therapies, or other immunotherapeutic approaches [29, 38–47]. Discussed below are implications of these findings with regards to how immunomodulatory agents interact with cytotoxic therapies and may drive potent and durable anti-tumor immune responses for improved clinical outcomes.

Immune Response to Chemotherapy and Radiation Therapy

Tumor-intrinsic effects

CTX and RT have tumoricidal properties that are directly linked to their ability to arrest cell cycle progression leading to cell death. Several classes of chemotherapy (e.g., alkylating agents, nitrosureas, platinum agents, antimetabolites, antitumor antibiotics, anthracyclines, epipodophyllotoxins, vinca alkaloids, taxanes, and camptothecin analogs) have been developed, all of which kill proliferating cells by either cell cycle-specific or cell cycle-nonspecific mechanisms [48]. On the other hand, RT, (e.g., ionizing radiation) leads to cell death by inducing single and double strand breaks in DNA, and can be synergistic when used in combination with CTX. While initially thought to be immunologically silent, it is now clear that the massive cell death resulting from these modalities also leads to release of stress molecules and antigens into the TME, and these, in part, sculpt local (and likely systemic) immune responses to “damage” and subsequently impact therapeutic response [49–51].

Tumor-extrinsic immune effects

The fact that CTX and RT exert significant effects on tumor-infiltrating leukocytes was unappreciated in the 1950s and 60s when these modalities were being developed as mainstays for cancer therapy. Although both treatments can lead to transient depletion of resident leukocytes and/or myelosuppression via direct cytotoxic activity, rebound effects following therapy do occur [52] and are known to impact response to therapy.

Immune Effects of RT—While early experimental studies revealed that RT induces tumor regression by CD8⁺ T cell-dependent mechanisms [53, 54], later work specifically demonstrated that release of IFN- γ by CD8⁺ T cells mediates these effects [55, 56]. Following RT, pre-apoptotic exposure of calreticulin on tumor cell surfaces presages release of high-mobility group box 1 (HMGB1) during late apoptosis. HMGB1 subsequently binds toll-like receptor (TLR)4 on dendritic cells (DCs), leading to antigen processing and presentation, as well as cytotoxic T cell activation [57–59]. We now appreciate that dying tumor cells release numerous stress factors in addition to HMGB1, including ATP, heat shock proteins (HSPs), and other danger-associated molecular patterns (DAMPs) that signal through TLRs on DCs, leading to DC maturation and cross-presentation of tumor antigens [60, 61].

In addition to fostering DC maturation and cross-presentation to CD8⁺ T cells, RT also promotes CD8⁺ T cell recruitment into tumors via two mechanisms: 1) CXCR6-expressing CD8⁺ T cells are recruited to tissue in response to CXCL16 production induced by RT [62]; and 2) RT upregulates expression of adhesion molecules E-selectin and ICAM-1 on surrounding vasculature, thereby enhancing leukocyte endothelial transmigration and entry into tissue [63]. The consequences of DC maturation and CD8⁺ T cell mobilization are most clearly appreciated in the abscopal effect following RT where efficacy is observed in distant metastatic sites after irradiation of primary tumors [64]. Both cytotoxic CD8⁺ T cells and Ag-presenting DCs mediate the abscopal effects, at least in animal models. In a syngeneic mammary carcinoma model, tumor growth outside the irradiation field was stunted following RT, but only in combination with Flt3 ligand, a growth factor responsible for recruitment and maturation of DCs from bone marrow [65]. This effect was impaired in mice lacking functional T cells, indicating that cross presentation of tumor antigens to CD8⁺ T cells mediates the phenomenon [65]. However, it should be noted that these results are rarely observed in the clinic with RT monotherapy, likely due to an immunosuppressive TME.

Supporting this rationale are studies demonstrating that tumor-promoting monocytes and macrophages are recruited to TMEs following RT, likely mediated by upregulation of growth factors [30] or chemokines such as CXCL12 in RT-induced hypoxic regions [66, 67]. Recruited macrophages in this scenario are generally immunosuppressive, support angiogenesis, and generate a protumor microenvironment. Efforts to prevent macrophage recruitment or deplete them once they have arrived following RT delays tumor regrowth when combined with RT in squamous [68], mammary [38], and prostate [39] cancers, as well as glioblastoma [66]. Together, these studies indicate that while the capability to produce a potent and durable anti-tumor CD8⁺ T cell response is induced by RT, there is often a macrophage-regulated immunosuppressive TME thwarting those efforts. Thus, combining immunotherapies designed to dampen macrophage-mediated immunosuppression and enhance CD8⁺ T cell anti-tumor responses may prove to be more efficacious than the use of either approach alone.

Immune Effects of CTX—While the effects of the immune system on efficacy of CTX have been intensely examined and recently reviewed [4, 5], the converse effects of CTX on the immune system are less studied. In many instances, treatment with CTX results in an enhanced cytotoxic CD8⁺ T cell response. Dose-dense treatment of ovarian cancer with either cisplatin or paclitaxel yields enhanced IFN- γ production by CD8⁺ T cells in both murine models and in patients [69]. Similarly, treatment of murine mammary carcinoma and fibrosarcoma tumors with doxorubicin results in IFN- γ -producing CD8⁺ T cell proliferation and their recruitment to tumors [70]. As is similarly observed with RT, some types of CTX (e.g., anthracyclines, cyclophosphamide, and oxaliplatin) also activate immunogenic cell death pathways whereby cell surface expression of calreticulin is followed by release of ATP, HMGB1, and HSPs, thereby leading to DC-mediated cross-presentation of tumor antigens to CD8⁺ T cells [71, 72]. Corroborating in vitro evidence indicates that exposure of cancer cells to 5-fluoruracil or doxorubicin stimulates HSP release and promotes engulfment of cell debris by DCs, thereby promoting cross-presentation to CD8⁺ T cells [73, 74].

Similarly, when doxorubicin-treated cancer cells are injected into syngeneic mice, DCs phagocytose cell debris and generate a tumor-specific CD8⁺ T cell antitumor immune response [75].

While CD8⁺ T cells certainly elicit tumor cell death, natural killer (NK) cells also mediate tumor cell death and are affected by CTX, albeit with varying outcomes. NK cell effector functions have been found to be impaired in breast cancer patients treated with high-dose cyclophosphamide [76, 77]. However, dosing may influence this effect, since low-dose cyclophosphamide treatment was instead found to stimulate NK activity in late-stage cancer patients [78]. Moreover, CTX enhances NK recruitment and bioeffector functions in several cancer models [79–81], indicating that CTX can regulate NK cell recruitment and activity, but may be dependent on cancer type or dosing strategy.

In addition to the effects on DCs and NK cells, macrophage phenotype can also be influenced by CTX. Generally speaking, the majority of macrophages infiltrating treatment-naïve tumors exhibit gene expression patterns corresponding to M2-like macrophages [28, 82, 83] that are immunosuppressive in nature towards NK and T cells [84]. Thus, targeting macrophages to relieve immunosuppression by reprogramming their activities towards M1-like states is an attractive therapeutic strategy that is being pursued clinically [4]. Context and tumor-specificity will likely underlie the therapeutic responses of these treatments. Preclinical mouse models have shown that whereas low-dose cyclophosphamide reprograms M2-like macrophages towards an M1-like state in vivo [85], others tumor types may require additional immunomodulation for effective reprogramming [29, 30, 34, 86, 87] and elaboration of functional anti-tumor CD8⁺ T cell responses.

Mechanisms of Immune Resistance: Checkpoints

While the aforementioned mechanisms indicate that RT and CTX can produce robust tumor-specific immune responses through mobilization of CD8⁺ T cells, there are often counter regulatory mechanisms in place that restrict these responses. Most notably, immune checkpoint molecules function to limit activation of CD8⁺ T cells following Ag stimulation by APCs [16]. While these checkpoints are necessary to dampen an active CD8⁺ T cell immune response and to avoid autoimmune pathologies, these molecules are attractive targets for enhancing T cell-mediated anti-tumor programs in the context of cancer. As such, therapies targeting CTLA-4 and PD-1 have been developed and evaluated clinically, and are showing tremendous efficacy in several tumor types [19–23, 88–91].

While the CTLA-4 and the PD-1 pathways both have the capacity to dampen antitumor responses, their mechanisms of action are distinct. T cell expression of CTLA-4 functions to dampen T cell function by outcompeting for binding and costimulation of the TCR by CD28 [92]. Instead of functioning within the TME as is the case with PD-1, CTLA-4 functions to dampen T cell function in secondary lymphoid organs where cross-presentation occurs. It should be noted that CTLA-4 is primarily expressed on CD4⁺ T cells and that efficacy of αCTLA-4 therapy likely relies on T helper function, though direct action of CTLA-4 on CD8⁺ T cells as has been reported [93]. On the other hand, PD-1 is primarily expressed on CD8⁺ T cells and has two ligands that are differentially expressed, PD-L1 and PD-L2. While

PD-L2 is primarily expressed on APCs, PD-L1 is more broadly expressed and is found on neoplastic cells, immune cells, and endothelial cells [16, 94, 95]. Engagement of PD-1 with PD-L1 or PD-L2 within the TME dampens the effector functions after TCR stimulation [96]. Moreover, PD-1 engagement can lead to increased migration of CD8⁺ T cells, thereby reducing the time available for TCR stimulation and effectively rendering tumor cells invisible [97]. For a more thorough and recent review on immune checkpoints, see [98].

Several studies have evaluated patients treated with αCTLA-4 and found that T cell responses to therapy can be monitored in peripheral blood [99]. These data reveal the possibility of identifying circulating biomarkers that may be indicative of tumor response to therapy. By sequencing TCRβ chains to define diversity and frequency of T cell clones in patients with prostate cancer treated with αCTLA-4 therapy, CTLA-4-blockade was found to induce global remodeling of the T cell repertoire, a response that could be monitored in blood [99]. Though CTLA-4-blockade induced both gains and losses in the frequency of specific TCR clones, a gain in clonotype frequency predominated in patients on therapy, thereby revealing increased T cell diversity [99]. Similarly, TCR pattern changes were observed in melanoma patients treated with various immune checkpoint inhibitors within 4-weeks of treatment [100]. Of particular interest, patients with the most favorable outcomes were those whose most frequent TCRs were able to maintain an undiminished frequency during therapy, indicating that some patients have in place a set of T cells primed and ready to attack, needing only immunotherapy to unleash them, and that TCR measurements conducted at two time points can identify patients likely to benefit.

The success of CTLA-4 blockade led to rapid clinical evaluation of monoclonal antibodies targeting the PD-1 pathway. αPD-1 antibodies, like those targeting CTLA-4, have demonstrated significant clinical efficacy in non-small-cell lung carcinoma, melanoma, renal cell carcinoma [22], and Hodgkin's lymphoma [101]. Significantly, αPD-1 antibodies have been found to be efficacious in CTLA4-refractory disease [102]. Similarly, αPD-L1 antibodies have demonstrated efficacy in non-small-cell lung carcinoma, melanoma, renal cell carcinoma [23], and bladder cancer [103]. Since the mechanisms regulating CTLA-4 and PD-1 function are distinct with regards to regulating T cell function [104, 105], combination approaches have also been investigated and two recent clinical trials in advanced melanoma demonstrated greater efficacy using αCTLA-4 combined with αPD-1 [25, 106].

Overcoming resistance

Despite the clinical success of checkpoint inhibition, the majority of patients still fail to respond to therapy, likely due either to a lack of ligand expression or due to compensatory mechanisms limiting productive T cell infiltration [94]. Given that CTX and RT both elicit immunogenic cell death, resulting in cross-presentation of tumor antigens to CD8⁺ T cells, several studies have combined CTX or RT with checkpoint inhibitors to investigate synergy. Indeed, RT enhances TCR diversity and when combined with αPD-1 or αCTLA-4 antibodies, reverses T cell exhaustion and promotes T cell expansion, respectively [107]. Moreover, in mouse models of pancreatic cancer, where macrophage antagonists combined with CTX foster CD8⁺ T cell infiltration of tumors, tumor regression is restricted by

simultaneous upregulation of PD-L1 and CTLA4 [47]. Importantly, PD-1 and/or CTLA4-blockade in this context potently elicited tumor regression, even in larger established tumors [47]. Collectively, these data indicate that myeloid cell reprogramming should also be considered going forward as combinatorial therapy for improving long-term durable anti-tumor response to checkpoint inhibition.

Concluding Remarks

As mainstays of tumor therapy, CTX and RT have profound effects not only on rapidly dividing tumor cells that are the intended targets, but also on cellular components of the TME that in turn regulate overall response to therapy. Since both CTX and RT elicit immunogenic cell death in tumor cells, these can also serve as an endogenous vaccine providing tumor antigens against which CD8⁺ T cells can be primed [108]. Unfortunately, the protumorigenic TME limits productive antitumor immune response and thereby restricts efficacy. Consequently, combating immunosuppression and T cell exhaustion are primary targets for immunotherapy (Figure 1, Key Figure). While many are hailing the emergence of checkpoint inhibitors as a panacea for anticancer therapy since CD8⁺ T cells have the capacity to recognize a virtually unlimited number of tumor Ags, there are still many issues to overcome. While checkpoint blockade is undoubtedly promising, in many cases less than 20% of patients have durable responses to therapy. This raises several questions outlined in the outstanding questions box. Regarding whether checkpoint expression changes in response to therapy, recent studies indicate that one mechanism of resistance to αCTLA-4 treatment in melanoma is mediated by upregulation of PD-L1 by tumor cells [107]. While this is one mechanism by which resistance can occur, there are likely other mechanisms of resistance or immune escape in response to monotherapy, and future directions in both clinical and basic research should aim to understand these pathways. An additional and significant question that remains to be addressed is whether by overcoming counter-regulatory mechanisms by which CD8⁺ T cells exert antitumor responses, therapies will lead to subsequent autoimmune pathology? A meta-analysis of clinical trial data from stage III-IV melanoma using various immunotherapies revealed that 3.4% of all patients subsequently develop autoimmune vitiligo, while patients treated with checkpoint inhibitors fared better with a 2.0% incidence of vitiligo [109]. Importantly, appearance of vitiligo after immunotherapy was associated with response to therapy, where an increase progression-free survival and overall survival was observed [109]. With accumulating clinical data emerging, additional adverse events in several cancer types associated with checkpoint blockade are being identified [110]. For the most part, adverse events can likely be managed with corticosteroids, and interestingly, immunosuppression with corticosteroids does not appear to alter clinical benefits of checkpoint blockade [111]. Efforts are currently underway both in preclinical models and in the clinic to identify efficacious combinations of immunotherapies for a majority of patients. Whether these combined therapies will have broad applicability or will require personalization based on predictive biomarkers remains to be determined. Regardless of the outcome, there is optimism for these rational combinatorial immunotherapeutic strategies and the hope they will provide potent and durable antitumor immune responses a greater number of patients than currently achievable.

Outstanding questions

- Is there significant patient variability for expression of checkpoint inhibitors or is expression cancer-type specific?
- Is there similar intratumoral heterogeneity for checkpoint inhibitors as what has been observed for somatic mutations and gene expression?
- Does checkpoint inhibitor expression vary as a function of neoplastic progression?
- Does checkpoint inhibitor expression change in response to therapy?
- Will checkpoint therapies lead to subsequent autoimmune pathology?

Acknowledgments

The authors thank the members of the Coussens Laboratory for critical discussion during the preparation of this review. TRM acknowledges support from the American Cancer Society – Friends of Rob Kinas, the Medical Research Foundation, and the Cathy and Jim Rudd Career Development Award for Cancer Research. LMC acknowledges support from the NIH/NCI, DOD BCRP Era of Hope Scholar Expansion Award, Susan B. Komen Foundation, *Stand Up To Cancer – Lustgarten Foundation Pancreatic Cancer Convergence Dream Team Translational Research Grant*, and Brenden-Colson Center for Pancreatic Health. We also gratefully acknowledge support from the OHSU Knight Cancer Institute.

References

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011; 144(5):646–74. [PubMed: 21376230]
2. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell*. 2012; 21(3):309–22. [PubMed: 22439926]
3. Coussens LM, Zitvogel L, Palucka AK. Neutralizing tumor-promoting chronic inflammation: a magic bullet? *Science*. 2013; 339(6117):286–91. [PubMed: 23329041]
4. Ruffell B, Coussens LM. Macrophages and Therapeutic Resistance in Cancer. *Cancer Cell*. 2015; 27(4):462–472. [PubMed: 25858805]
5. Klemm F, Joyce JA. Microenvironmental regulation of therapeutic response in cancer. *Trends Cell Biol*. 2015; 25(4):198–213. [PubMed: 25540894]
6. Davis MM, Bjorkman PJ. T-cell antigen receptor genes and T-cell recognition. *Nature*. 1988; 334(6181):395–402. [PubMed: 3043226]
7. Beatty GL, et al. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science*. 2011; 331(6024):1612–6. [PubMed: 21436454]
8. Zoete V, et al. Structure-Based, Rational Design of T Cell Receptors. *Front Immunol*. 2013; 4:268. [PubMed: 24062738]
9. Linnemann C, Mezzadra R, Schumacher TN. TCR repertoires of intratumoral T-cell subsets. *Immunol Rev*. 2014; 257(1):72–82. [PubMed: 24329790]
10. Gros A, et al. PD-1 identifies the patient-specific CD8(+) tumor-reactive repertoire infiltrating human tumors. *J Clin Invest*. 2014; 124(5):2246–59. [PubMed: 24667641]
11. Gubin MM, et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature*. 2014; 515(7528):577–81. [PubMed: 25428507]
12. Tran E, et al. Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer. *Science*. 2014; 344(6184):641–5. [PubMed: 24812403]
13. Linnemann C, et al. High-throughput epitope discovery reveals frequent recognition of neo-antigens by CD4+ T cells in human melanoma. *Nat Med*. 2015; 21(1):81–5. [PubMed: 25531942]

14. Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. *Nat Rev Immunol*. 2010; 10(3):170–81. [PubMed: 20154735]
15. Crittenden MR, et al. Expression of arginase I in myeloid cells limits control of residual disease after radiation therapy of tumors in mice. *Radiat Res*. 2014; 182(2):182–90. [PubMed: 24992164]
16. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012; 12(4):252–64. [PubMed: 22437870]
17. Leone P, et al. MHC class I antigen processing and presenting machinery: organization, function, and defects in tumor cells. *J Natl Cancer Inst*. 2013; 105(16):1172–87. [PubMed: 23852952]
18. Zou W. Immunosuppressive networks in the tumour environment and their therapeutic relevance. *Nat Rev Cancer*. 2005; 5(4):263–74. [PubMed: 15776005]
19. Hodi FS, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010; 363(8):711–23. [PubMed: 20525992]
20. Graff JN, et al. Sustained complete response to CTLA-4 blockade in a patient with metastatic, castration-resistant prostate cancer. *Cancer Immunol Res*. 2014; 2(5):399–403. [PubMed: 24795352]
21. Robert C, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med*. 2011; 364(26):2517–26. [PubMed: 21639810]
22. Topalian SL, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 2012; 366(26):2443–54. [PubMed: 22658127]
23. Brahmer JR, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med*. 2012; 366(26):2455–65. [PubMed: 22658128]
24. Garon EB, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med*. 2015; 372(21):2018–28. [PubMed: 25891174]
25. Postow MA, et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. *N Engl J Med*. 2015; 372(21):2006–17. [PubMed: 25891304]
26. Robert C, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N Engl J Med*. 2015; 372(26):2521–32. [PubMed: 25891173]
27. Gabrilovich D, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nature reviews Immunology*. 2009; 9(3):162–174.
28. Mills CD, et al. M-1/M-2 macrophages and the Th1/Th2 paradigm. *J Immunol*. 2000; 164(12):6166–6173. [PubMed: 10843666]
29. Ruffell B, et al. Macrophage IL-10 Blocks CD8(+) T Cell-Dependent Responses to Chemotherapy by Suppressing IL-12 Expression in Intratumoral Dendritic Cells. *Cancer Cell*. 2014; 26(5):623–37. [PubMed: 25446896]
30. DeNardo DG, et al. Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. *Cancer Discov*. 2011; 1(1):54–67. [PubMed: 22039576]
31. Lin EY, Pollard JW. Tumor-associated macrophages press the angiogenic switch in breast cancer. *Cancer Res*. 2007; 67(11):5064–6. [PubMed: 17545580]
32. Lin EY, et al. Macrophages regulate the angiogenic switch in a mouse model of breast cancer. *Cancer Res*. 2006; 66(23):11238–46. [PubMed: 17114237]
33. Obeid M, et al. Calreticulin exposure dictates the immunogenicity of cancer cell death. *Nat Med*. 2007; 13(1):54–61. [PubMed: 17187072]
34. Pyonteck SM, et al. CSF-1R inhibition alters macrophage polarization and blocks glioma progression. *Nat Med*. 2013; 19(10):1264–72. [PubMed: 24056773]
35. Shree T, et al. Macrophages and cathepsin proteases blunt chemotherapeutic response in breast cancer. *Genes Dev*. 2011; 25(23):2465–79. [PubMed: 22156207]
36. Gocheva V, et al. IL-4 induces cathepsin protease activity in tumor-associated macrophages to promote cancer growth and invasion. *Genes Dev*. 2010; 24(3):241–55. [PubMed: 20080943]
37. Mantovani A, Allavena P. The interaction of anticancer therapies with tumor-associated macrophages. *J Exp Med*. 2015; 212(4):435–445. [PubMed: 25753580]
38. Shiao SL, et al. TH2-Polarized CD4+ T Cells and Macrophages Limit Efficacy of Radiotherapy. *Cancer Immunol Res*. 2015; 3(5):518–25. [PubMed: 25716473]

39. Xu J, et al. CSF1R signaling blockade stanches tumor-infiltrating myeloid cells and improves the efficacy of radiotherapy in prostate cancer. *Cancer Res.* 2013; 73(9):2782–94. [PubMed: 23418320]
40. Strachan DC, et al. CSF1R inhibition delays cervical and mammary tumor growth in murine models by attenuating the turnover of tumor-associated macrophages and enhancing infiltration by CD8 T cells. *Oncoimmunology.* 2013; 2(12):e26968. [PubMed: 24498562]
41. Mitchem JB, et al. Targeting tumor-infiltrating macrophages decreases tumor-initiating cells, relieves immunosuppression, and improves chemotherapeutic responses. *Cancer Res.* 2013; 73(3): 1128–41. [PubMed: 23221383]
42. Escamilla J, et al. CSF1 Receptor Targeting in Prostate Cancer Reverses Macrophage-Mediated Resistance to Androgen Blockade Therapy. *Cancer Res.* 2015; 75(6):950–62. [PubMed: 25736687]
43. Fridlender ZG, et al. Monocyte chemoattractant protein-1 blockade inhibits lung cancer tumor growth by altering macrophage phenotype and activating CD8+ cells. *Am J Respir Cell Mol Biol.* 2011; 44(2):230–7. [PubMed: 20395632]
44. Priceman SJ, et al. Targeting distinct tumor-infiltrating myeloid cells by inhibiting CSF-1 receptor: combating tumor evasion of antiangiogenic therapy. *Blood.* 2010; 115(7):1461–71. [PubMed: 20008303]
45. Chen EP, et al. Myeloid Cell COX-2 deletion reduces mammary tumor growth through enhanced cytotoxic T-lymphocyte function. *Carcinogenesis.* 2014; 35(8):1788–97. [PubMed: 24590894]
46. Mok S, et al. Inhibition of CSF-1 receptor improves the antitumor efficacy of adoptive cell transfer immunotherapy. *Cancer Res.* 2014; 74(1):153–61. [PubMed: 24247719]
47. Zhu Y, et al. CSF1/CSF1R blockade reprograms tumor-infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models. *Cancer Res.* 2014; 74(18):5057–69. [PubMed: 25082815]
48. Malhotra V, Perry MC. Classical chemotherapy: mechanisms, toxicities and the therapeutic window. *Cancer Biol Ther.* 2003; 2(4 Suppl 1):S2–4. [PubMed: 14508075]
49. Kroemer G, et al. Immunogenic cell death in cancer therapy. *Annu Rev Immunol.* 2013; 31:51–72. [PubMed: 23157435]
50. Zitvogel L, Kepp O, Kroemer G. Immune parameters affecting the efficacy of chemotherapeutic regimens. *Nat Rev Clin Oncol.* 2011; 8(3):151–60. [PubMed: 21364688]
51. Zitvogel L, et al. Immunological aspects of cancer chemotherapy. *Nat Rev Immunol.* 2008; 8(1): 59–73. [PubMed: 18097448]
52. Proietti E, et al. Importance of cyclophosphamide-induced bystander effect on T cells for a successful tumor eradication in response to adoptive immunotherapy in mice. *J Clin Invest.* 1998; 101(2):429–41. [PubMed: 9435316]
53. Awwad M, North RJ. Immunologically mediated regression of a murine lymphoma after treatment with anti-L3T4 antibody. A consequence of removing L3T4+ suppressor T cells from a host generating predominantly Lyt-2+ T cell-mediated immunity. *J Exp Med.* 1988; 168(6):2193–206. [PubMed: 2974065]
54. Lee Y, et al. Therapeutic effects of ablative radiation on local tumor require CD8+ T cells: changing strategies for cancer treatment. *Blood.* 2009; 114(3):589–95. [PubMed: 19349616]
55. Lugade AA, et al. Radiation-induced IFN-gamma production within the tumor microenvironment influences antitumor immunity. *J Immunol.* 2008; 180(5):3132–9. [PubMed: 18292536]
56. Burnette BC, et al. The efficacy of radiotherapy relies upon induction of type I interferon-dependent innate and adaptive immunity. *Cancer Res.* 2011; 71(7):2488–96. [PubMed: 21300764]
57. Kepp O, et al. Immunogenic cell death modalities and their impact on cancer treatment. *Apoptosis.* 2009; 14(4):364–75. [PubMed: 19145485]
58. Newcomb EW, et al. The combination of ionizing radiation and peripheral vaccination produces long-term survival of mice bearing established invasive GL261 gliomas. *Clin Cancer Res.* 2006; 12(15):4730–7. [PubMed: 16899624]
59. Reits EA, et al. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. *J Exp Med.* 2006; 203(5):1259–71. [PubMed: 16636135]

60. Zitvogel L, Kepp O, Kroemer G. Decoding cell death signals in inflammation and immunity. *Cell*. 2010; 140(6):798–804. [PubMed: 20303871]
61. Golden EB, et al. Radiation fosters dose-dependent and chemotherapy-induced immunogenic cell death. *Oncoimmunology*. 2014; 3:e28518. [PubMed: 25071979]
62. Matsumura S, et al. Radiation-induced CXCL16 release by breast cancer cells attracts effector T cells. *J Immunol*. 2008; 181(5):3099–107. [PubMed: 18713980]
63. Hallahan D, Kuchibhotla J, Wyble C. Cell adhesion molecules mediate radiation-induced leukocyte adhesion to the vascular endothelium. *Cancer Res*. 1996; 56(22):5150–5. [PubMed: 8912850]
64. Vatner RE, et al. Combinations of immunotherapy and radiation in cancer therapy. *Front Oncol*. 2014; 4:325. [PubMed: 25506582]
65. Demaria S, et al. Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. *Int J Radiat Oncol Biol Phys*. 2004; 58(3):862–70. [PubMed: 14967443]
66. Kioi M, et al. Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice. *J Clin Invest*. 2010; 120(3):694–705. [PubMed: 20179352]
67. Kozin SV, et al. Recruitment of myeloid but not endothelial precursor cells facilitates tumor regrowth after local irradiation. *Cancer Res*. 2010; 70(14):5679–85. [PubMed: 20631066]
68. Ahn GO, et al. Inhibition of Mac-1 (CD11b/CD18) enhances tumor response to radiation by reducing myeloid cell recruitment. *Proc Natl Acad Sci U S A*. 2010; 107(18):8363–8. [PubMed: 20404138]
69. Chang CL, et al. Dose-dense chemotherapy improves mechanisms of antitumor immune response. *Cancer Res*. 2013; 73(1):119–27. [PubMed: 23108141]
70. Mattarollo SR, et al. Pivotal role of innate and adaptive immunity in anthracycline chemotherapy of established tumors. *Cancer Res*. 2011; 71(14):4809–20. [PubMed: 21646474]
71. Apetoh L, et al. The interaction between HMGB1 and TLR4 dictates the outcome of anticancer chemotherapy and radiotherapy. *Immunol Rev*. 2007; 220:47–59. [PubMed: 17979839]
72. Tesniere A, et al. Immunogenic death of colon cancer cells treated with oxaliplatin. *Oncogene*. 2010; 29(4):482–91. [PubMed: 19881547]
73. Buttiglieri S, et al. Influence of drug-induced apoptotic death on processing and presentation of tumor antigens by dendritic cells. *Int J Cancer*. 2003; 106(4):516–20. [PubMed: 12845646]
74. Galetto A, et al. Drug- and cell-mediated antitumor cytotoxicities modulate cross-presentation of tumor antigens by myeloid dendritic cells. *Anticancer Drugs*. 2003; 14(10):833–43. [PubMed: 14597879]
75. Casares N, et al. Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. *J Exp Med*. 2005; 202(12):1691–701. [PubMed: 16365148]
76. Brenner BG, Margolese RG. The relationship of chemotherapeutic and endocrine intervention on natural killer cell activity in human breast cancer. *Cancer*. 1991; 68(3):482–8. [PubMed: 2065267]
77. Sewell HF, et al. Chemotherapy-induced differential changes in lymphocyte subsets and natural-killer-cell function in patients with advanced breast cancer. *Int J Cancer*. 1993; 55(5):735–8. [PubMed: 7902339]
78. Ghiringhelli F, et al. Metronomic cyclophosphamide regimen selectively depletes CD4+CD25+ regulatory T cells and restores T and NK effector functions in end stage cancer patients. *Cancer Immunol Immunother*. 2007; 56(5):641–8. [PubMed: 16960692]
79. Liu P, et al. Administration of cyclophosphamide changes the immune profile of tumor-bearing mice. *J Immunother*. 2010; 33(1):53–9. [PubMed: 19952956]
80. Doloff JC, Waxman DJ. VEGF receptor inhibitors block the ability of metronomically dosed cyclophosphamide to activate innate immunity-induced tumor regression. *Cancer Res*. 2012; 72(5):1103–15. [PubMed: 22237627]
81. Khallouf H, et al. 5-Fluorouracil and interferon-alpha immunochemotherapy enhances immunogenicity of murine pancreatic cancer through upregulation of NKG2D ligands and MHC class I. *J Immunother*. 2012; 35(3):245–53. [PubMed: 22421942]
82. Chittezhath M, et al. Molecular profiling reveals a tumor-promoting phenotype of monocytes and macrophages in human cancer progression. *Immunity*. 2014; 41(5):815–29. [PubMed: 25453823]

83. Sica A, et al. Tumour-associated macrophages are a distinct M2 polarised population promoting tumour progression: potential targets of anti-cancer therapy. *Eur J Cancer*. 2006; 42(6):717–27. [PubMed: 16520032]
84. Pollard JW. Trophic macrophages in development and disease. *Nat Rev Immunol*. 2009; 9(4):259–70. [PubMed: 19282852]
85. Bryniarski K, et al. Influence of cyclophosphamide and its metabolic products on the activity of peritoneal macrophages in mice. *Pharmacol Rep*. 2009; 61(3):550–7. [PubMed: 19605955]
86. Ryder M, et al. Genetic and pharmacological targeting of CSF-1/CSF-1R inhibits tumor-associated macrophages and impairs BRAF-induced thyroid cancer progression. *PLoS One*. 2013; 8(1):e54302. [PubMed: 23372702]
87. Affara NI, et al. B cells regulate macrophage phenotype and response to chemotherapy in squamous carcinomas. *Cancer Cell*. 2014; 25(6):809–21. [PubMed: 24909985]
88. van den Eertwegh AJ, et al. Combined immunotherapy with granulocyte-macrophage colony-stimulating factor-transduced allogeneic prostate cancer cells and ipilimumab in patients with metastatic castration-resistant prostate cancer: a phase 1 dose-escalation trial. *Lancet Oncol*. 2012; 13(5):509–17. [PubMed: 22326922]
89. Hodi FS, et al. CTLA-4 blockade with ipilimumab induces significant clinical benefit in a female with melanoma metastases to the CNS. *Nat Clin Pract Oncol*. 2008; 5(9):557–61. [PubMed: 18665147]
90. Yang JC, et al. Ipilimumab (anti-CTLA4 antibody) causes regression of metastatic renal cell cancer associated with enteritis and hypophysitis. *J Immunother*. 2007; 30(8):825–30. [PubMed: 18049334]
91. Carthon BC, et al. Preoperative CTLA-4 blockade: tolerability and immune monitoring in the setting of a presurgical clinical trial. *Clin Cancer Res*. 2010; 16(10):2861–71. [PubMed: 20460488]
92. Qureshi OS, et al. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. *Science*. 2011; 332(6029):600–3. [PubMed: 21474713]
93. Chambers CA, et al. Secondary but not primary T cell responses are enhanced in CTLA-4-deficient CD8+ T cells. *Eur J Immunol*. 1998; 28(10):3137–43. [PubMed: 9808182]
94. Herbst RS, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature*. 2014; 515(7528):563–7. [PubMed: 25428504]
95. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. *Curr Opin Immunol*. 2012; 24(2):207–12. [PubMed: 22236695]
96. Keir ME, et al. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol*. 2008; 26:677–704. [PubMed: 18173375]
97. Honda T, et al. Tuning of antigen sensitivity by T cell receptor-dependent negative feedback controls T cell effector function in inflamed tissues. *Immunity*. 2014; 40(2):235–47. [PubMed: 24440150]
98. Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer Cell*. 2015; 27(4):450–61. [PubMed: 25858804]
99. Cha E, et al. Improved survival with T cell clonotype stability after anti-CTLA-4 treatment in cancer patients. *Sci Transl Med*. 2014; 6(238):238ra70.
100. Kvistborg P, et al. Anti-CTLA-4 therapy broadens the melanoma-reactive CD8+ T cell response. *Sci Transl Med*. 2014; 6(254):254ra128.
101. Ansell SM, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med*. 2015; 372(4):311–9. [PubMed: 25482239]
102. Hamid O, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *N Engl J Med*. 2013; 369(2):134–44. [PubMed: 23724846]
103. Powles T, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. *Nature*. 2014; 515(7528):558–62. [PubMed: 25428503]
104. Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell*. 2015; 161(2):205–14. [PubMed: 25860605]

105. Topalian SL, Drake CG, Pardoll DM. Immune Checkpoint Blockade: A Common Denominator Approach to Cancer Therapy. *Cancer Cell*. 2015; 27(4):450–461. [PubMed: 25858804]
106. Wolchok JD, et al. Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med*. 2013; 369(2):122–33. [PubMed: 23724867]
107. Twyman-Saint Victor C, et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. *Nature*. 2015; 520(7547):373–7. [PubMed: 25754329]
108. Hannani D, et al. Prerequisites for the antitumor vaccine-like effect of chemotherapy and radiotherapy. *Cancer J*. 2011; 17(5):351–8. [PubMed: 21952286]
109. Teulings HE, et al. Vitiligo-like depigmentation in patients with stage III-IV melanoma receiving immunotherapy and its association with survival: a systematic review and meta-analysis. *J Clin Oncol*. 2015; 33(7):773–81. [PubMed: 25605840]
110. Wolchok JD, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clin Cancer Res*. 2009; 15(23):7412–20. [PubMed: 19934295]
111. Gao J, et al. Review of immune-related adverse events in prostate cancer patients treated with ipilimumab: MD Anderson experience. *Oncogene*. 2015

Trends

- Conventional chemotherapy and radiation therapy alter immune cell function in addition to their direct cytotoxic effects on rapidly proliferating tumor cells.
- CD8⁺ T cells are often found in immunosuppressive tumor microenvironments.
- These immunosuppressive microenvironments are generally tumor subtype-specific and can be mediated by a multitude of immune cells, including macrophages.
- Immune checkpoint inhibitors provide durable responses in a subset of patients for many cancer subtypes.
- Overcoming additional tissue-based mechanisms of immunosuppression embedded within the tumor microenvironment may improve clinical response rates.

Key Figure, Figure 1.

Strategies to bolster antitumor immunity. (A) Apoptotic tumor cells release high-mobility group box 1 (HMGB1), ATP, heat shock proteins (HSPs), and other danger-associated molecular patterns (DAMPs) into the stroma following chemotherapy and/or radiation therapy. DAMPs signal through TLRs on DCs and lead to maturation, trafficking to lymph nodes, and cross-presentation of tumor antigens to cytotoxic T cells. (B) CD8⁺ T cells present within the TME are often rendered tolerant via immunosuppressive factors released by M2 macrophages or a T_H2 microenvironment, as well as expression of immune checkpoint inhibitors by various cells within the microenvironment. By utilizing cancer-type specific immunotherapy, macrophages can be repolarized such that CD8⁺ T cells can be recruited and activated within the TME. (C) Expression of checkpoint inhibitors, such as PD-1, PD-L1, and CTLA-4, limits activity of CD8⁺ T cells. By blocking checkpoint molecules, CD8⁺ T cells are able to mount a cytolytic attack against tumor antigens. By targeting several or all of the pathways outlined above, the immunosuppressive TME can become immune stimulatory and likely offer potent and durable anti-tumor immune responses in the clinic.