Immune Effects of Conventional Cancer Therapeutics

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KEY POINTS

- Conventional chemotherapy, targeted anticancer agents, and radiotherapy have immunological effects that contribute to their activity *in vivo*.
- Immunological effects can be “on-target” (affecting cancer cells directly) or “off-target” (affecting immune cells).
- “On-target” immunostimulatory effects enhance immunogenicity of the cancer cells by improving antigenicity (increased expression of antigens or major histocompatibility complex [MHC] molecules) and/or adjuvanticity (generation of danger signals or damage-associated molecular patterns [DAMPs]).
- “Off-target” immunostimulatory effects are direct when the drug activates antitumor immune effectors or indirect when the drug eliminates or inhibits immunosuppressive cells.
- Cancer therapy can also mediate immunosuppressive effects as it promotes the accumulation or activation of regulatory and suppressor cells or factors.
- Treatments able to induce an immunogenic form of cancer cell death are often synergistic with immune checkpoint inhibitors.
- Rational combinations of conventional chemotherapy and/or radiotherapy with immunotherapy are undergoing clinical testing.

INTRODUCTION

Conventional cancer therapeutics, which include cytotoxic chemotherapy and radiotherapy, were developed with the goal of killing transformed cells while sparing normal cells as much as possible. An improved understanding of the molecular alterations that drive a specific cancer type has fostered the development of targeted therapeutics with increased selectivity and limited toxicity.¹ Technological advances in imaging and radiation dose delivery have also improved tumor targeting by radiotherapy.² At the same time, unequivocal demonstration that the malignant potential of transformed cells is regulated by the immune system fostered the development of anticancer therapeutics that target immune cells.³ The success of the latter approach urges revisiting long-held assumptions about the mechanisms through which conventional cancer therapeutics work and assessing their effects on cells of the immune system and on tumor–host interactions.^{4,5} This information becomes essential in the new era of immuno-oncology, in which several immune checkpoint inhibitors are approved for treatment of a growing list of cancer types and many more immunotherapies are likely to enter routine clinical care in the coming years.⁶ In this new landscape, the immunological effects of conventional and targeted therapeutics used to treat patients before, during, or after immunotherapy need to be clearly understood as they can profoundly influence disease outcome.

Conventional chemotherapy and radiation preferentially affect proliferating cells, a feature not only of cancer but also of cells of the adaptive immune system, and have been considered uniformly immunosuppressive. While this is true for some agents, especially when used

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at high doses, experimental and clinical evidence accumulating over the past 15 years shows that many cytotoxic cancer treatments can stimulate antitumor immune responses and that the latter contribute significantly to their therapeutic activity.^{7–14} Immunostimulatory effects of chemotherapy can be categorized as “on-target” when

they result directly from the interaction of the drug with cancer cells or “off-target” when they result from effects of the drug on immune cells (Figure 29.1). A similar definition applies to targeted therapeutics, at least in part to focal radiotherapy, since radiation affects all compartments of the tumor stroma including immune cells. For

Figure 29.1 Immunostimulatory effects of conventional chemotherapeutics, targeted anticancer agents, and radiation therapy. According to current models, malignant precursors only form clinically manifest tumors as they escape immunosurveillance. Such an escape generally involves the selection of cancer cell variants with reduced immunogenicity (i.e., reduced antigenicity or reduced adjuvanticity) and/or the establishment of robust immunosuppressive mechanisms that operate locally (i.e., within the tumor microenvironment) and systemically (A). Multiple chemotherapeutics, targeted anticancer agents, and radiation therapy support the (re-)activation of tumor-targeting immune responses by (1) inhibiting or depleting immunosuppressive cell populations, (2) boosting the activity of immune effectors cells, and/or (3) restoring the antigenicity or adjuvanticity of neoplastic cells (B).

CTL, CD8⁺ cytotoxic lymphocyte; DAMPs, damage-associated molecular patterns; MDSC, myeloid-derived suppressor cell; NK, natural killer; TAM, tumor-associated macrophage; T_{reg} , CD4⁺CD25⁺Foxp3⁺ regulatory T.

the purpose of discussion in this chapter we will categorize on-target and off-target effects as outlined next and refer the reader to Section I of this book for a more detailed description of the key components and mechanisms of antitumor immune responses.

ON-TARGET IMMUNE EFFECTS

Tumors that become clinically evident have escaped immunological elimination by becoming less immunogenic or by suppressing antitumor immune effectors^{15,16} (Figure 29.1A). Some cytotoxic agents can trigger a number of molecular responses in cancer cells that restore (at least part of) their immunogenicity. The immunogenicity of cancer cells is determined by their antigenicity and adjuvanticity. "Antigenicity" involves the expression of proteins that are not covered by central or peripheral tolerance.¹⁶ Somatic mutations that accumulate in cancer cells are a major (but not the sole) source of the so-called tumor neoantigens.^{17,18} Alternative tumor-specific antigens can also originate from nonmutated proteins that are overexpressed and/or ectopically expressed,¹⁹ splice variants or lipids derived from noncanonical posttranscriptional or posttranslational events,²⁰ re-expression of latent proteins that are normally silenced by DNA methylation during development (e.g., the so-called "oncofetal antigens"),²¹ as well as proteins aberrantly expressed from normally noncoding genomic regions²² or endogenous retroelements.²⁰ Such antigens must be presented to CD8⁺ cytotoxic T lymphocytes (CTLs) in association with major histocompatibility complex class I (MHC-I) molecules. Thus, both the number of antigenic epitopes and MHC-I expression affect cancer cell antigenicity. "Adjuvanticity" refers to the expression or release of danger signals collectively known as "damage-associated molecular patterns" (DAMPs).^{23,24} For the purpose of the present discussion, DAMPs can be broadly subdivided into two (partially overlapping) groups: (a) cell death-associated DAMPs and (b) cell stress-associated DAMPs.²⁴ A stressful cell death, as occurs when cells are infected or exposed to some cytotoxic agents that trigger the premortem activation of the DNA damage response (DDR), endoplasmic reticulum (ER) stress response, and autophagy, results in release of DAMPs that promote the recruitment and activation of dendritic cells (DCs) by binding to pattern recognition receptors (PRRs), and thus initiate the presentation of tumor-derived antigens to T cells.^{24,25} An instance of cell death associated with elevated antigenicity and considerable DAMP emission is therefore defined as immunogenic cell death (ICD).²⁶ Molecules that are expressed by cells coping with (but surviving) therapy include ligands for natural killer (NK) cell-activating receptors like killer cell lectin-like receptor K1 (KLRK1, best known as NKG2D),²⁷ as well as the death receptor FAS/CD95, and adhesion molecules that improve recognition and

killing of the cancer cells by CTLs.²⁸ Although these molecules do not interact with PRRs as bona fide DAMPs do,²⁹ they contribute to the activation of the antitumor immune effectors and are therefore considered here as cell stress-associated DAMPs.

OFF-TARGET IMMUNE EFFECTS

Multiple immunosuppressive mechanisms operate locally (i.e., within the tumor microenvironment) and systemically to hinder tumor rejection by the immune system.¹⁵ Inhibition of DCs, CTLs, and NK cells is mediated by suppressive cytokines, such as transforming growth factor beta 1 (TGF- β 1), and co-inhibitory receptors, like cytotoxic T lymphocyte associated protein 4 (CTLA-4), programmed cell death 1 (PD-1),³ and hepatitis A virus cellular receptor 2 (HAVCR2, best known as TIM-3),³⁰ within a network of interactions with immune cells mediating tolerogenic and suppressive function such as regulatory T (T_{reg}) cells, myeloid-derived suppressor cells (MDSCs), or M2-polarized macrophages.^{31,32} Conventional and targeted anticancer treatments have been shown to mediate immunostimulatory effects by *direct* interaction with immune effector cells or *indirectly* by eliminating or reprogramming immunosuppressive cells (Figure 29.1B).

Conventional Chemotherapy

Increased Antigenicity

Multiple chemotherapeutic agents currently employed in clinical practice have been found to boost the antigenicity of cancer cells by upregulating the levels of surface-expressed MHC-I molecules, an "on-target" immunostimulatory activity that likely contributes to the efficacy of these drugs.⁹ In combination with type I interferon (IFN-I), 5-fluorouracil (an irreversible inhibitor of thymidylate synthase used for the treatment of several solid tumors) as well as doxorubicin (an anthracycline frequently employed in women with breast carcinoma) restored MHC-I expression on murine Panc02 pancreatic cancer cells, and this regimen had prominent therapeutic activity against Panc02 cells growing orthotopically in immunocompetent syngeneic mice.^{33,34} Gemcitabine (a nucleoside analogue mainly used for therapy of pancreatic carcinoma) had similar effects on human non-small cell lung carcinoma (NSCLC) A549 cells, human colorectal carcinoma HCT-116 cells, and human breast carcinoma MCF-7 cells.³⁵ The upregulation of MHC-I molecules induced by gemcitabine was associated with (a) increased tumor-specific T cell cytotoxicity *in vitro*, and this could be inhibited by an MHC-I-targeting monoclonal antibody³⁵; and (b) with increased beta 2 microglobulin expression (which is required for MHC-I exposure), upregulation of immune-proteasomal subunits, and altered antigenic epitopes exposed by tumor cells.³⁶ Along similar lines,

cisplatin (a DNA-damaging agent platinum derivative approved for use in patients with a wide variety of solid tumors) employed at low doses efficiently upregulated surface-exposed MHC-I molecules in cultured mouse lung adenocarcinoma TC-1 cells and in human head and neck squamous cell carcinoma (HNSCC) UMSCC-46 and UMSCC-74A cell lines,^{37,38} as did oxaliplatin (another platinum-based DNA-damaging agent commonly used in combinatorial regimens for the treatment of colorectal cancer).³⁸ Moreover, MHC-I expression was upregulated by two topoisomerase inhibitors employed against different solid cancers, topotecan and irinotecan, in human breast carcinoma ZR-75-1 cells and murine mammary FM3A carcinoma cells, respectively, most likely as a consequence of autocrine/paracrine IFN-I signaling.^{39,40} At least theoretically, a wide panel of DNA-damaging agents, including radiation therapy (RT) employed at suboptimal doses (i.e., doses that are unable to promote cell death),⁴¹ could increase the antigenicity of cancer cells by favoring the generation of mutational neoantigens.¹⁷ These agents include cisplatin and other platinum derivatives (like carboplatin, which is frequently employed against ovarian carcinoma, and oxaliplatin, which is approved for the treatment of colorectal carcinoma),⁴² doxorubicin and other anthracyclines (such as idarubicin, which is also part of the chemotherapeutic options for acute myeloid leukemia [AML]),⁴³ topotecan and other topoisomerase inhibitors (like etoposide, which is employed against multiple hematological and solid neoplasms),⁴⁴ and alkylating agents (such as temozolomide, which is approved for use in glioblastoma and astrocytoma patients).⁴⁵ However, a recent study of gliomas recurring after temozolomide-based chemotherapy demonstrates that hypermutated tumor cells do not necessarily become more sensitive to immunotherapy,⁴⁶ lending further support to the emerging notion that neo-antigen quality may be critical for immunogenicity.^{47–49}

Increased Adjuvanticity

Multiple conventional chemotherapeutic agents mediate “on-target” immunostimulatory effects by increasing the capacity of malignant cells to emit danger signals as they respond to stress.^{9,50} Cell stress-associated DAMPs are emitted by malignant cells that successfully cope with the potentially lethal effects of chemotherapy, and hence survive treatment.⁵¹ Prototypic DAMPs of this class include various ligands for the activating NK-cell receptor NKG2D, including MHC-I polypeptide-related sequence A (MICA), MHC-I polypeptide-related sequence B (MICB), and retinoic acid early transcript 1E (RAET1E), which are upregulated on the membrane of cancer cells experiencing DNA damage.²⁷ On the other hand, cell death-associated DAMPs are emitted by neoplastic cells that are unable to cope with the

damaging effect of chemotherapy and eventually die as they attempt to do so (i.e., they activate adaptive stress responses that are insufficient for the recovery of homeostasis).²³ DAMPs liberated following chemotherapy-induced cancer cell death trigger signaling cascades that ultimately result in the induction of adaptive immune responses.^{24,26,52} The optimal perception of ICD has been reported to involve the following cascade of events: (a) the exposure of calreticulin (CALR) and other ER chaperones on the cell surface, where they facilitate the phagocytosis of dead cells and corpses by antigen-presenting cells (APCs);^{53,54} (b) the secretion of ATP, which functions both as a chemotactic cue for myeloid cells and as a pro-inflammatory signal;^{55,56} (c) the release of annexin A1 (ANXA1), which guides the final approach of myeloid cells toward dying cells;⁵⁷ (d) the liberation of high-mobility group box 1 (HMGB1), a nuclear protein with multipronged immunostimulatory activity;⁵⁸ (e) the activation of an autocrine/paracrine type I IFN signaling pathway, via cyclic GMP-AMP synthase (CGAS), culminating with the release of chemotactic cytokines, including C-X-C motif chemokine ligand 10 (CXCL10, a chemoattractant for T cells);^{59,60} and (f) passive release of nucleic acids which can engage Toll-like receptor 3 (TLR3), TLR7/8, and/or TLR9.⁶⁰

Several chemotherapeutics have been shown to upregulate NKG2D ligands on the surface of cancer cells.^{9,24} 5-Fluorouracil as well as doxorubicin (both in combination with type I IFN) efficiently stimulated the exposure of RAET1E and UL16 binding protein 1 (ULBP1, which also activates NKG2D) on the surface of murine Panc02 cells maintained in vitro.^{33,34} Treatment with doxorubicin plus interleukin-12 (IL-12) also restored expression of the NKG2D ligands MICA, MICB, RAET1E, and ULBP2 in vivo, in multiple murine tumors encompassing 4T1 breast carcinomas, CT26 colorectal carcinomas, Lewis lung carcinomas (LLCs), and K7M3 osteosarcomas, as well as in a genetically engineered model of mammary carcinoma, which was coupled to CD8⁺ CTL-dependent tumor regression.⁶¹ Gemcitabine at doses not affecting cell proliferation upregulated surface-exposed MICA and MICB in two out of six human pancreatic cancer cell lines tested in vitro (i.e., PANC-1 and HPAF-II cells), but this failed to correlate with increased T cell cytotoxicity, possibly as a result of the concomitant release of soluble MICA and/or MICB.⁶² Gemcitabine also upregulated the expression of ULBP2, ULBP5, and ULBP6, as well as other NKG2D ligands, and of death receptor FAS/CD95 in human HCT-116 colorectal carcinoma cells and A549 NSCLC cells, and weakly increased the expression of FAS/CD95 on the surface of MCF7 breast carcinoma cells.⁶³ Docetaxel (a taxane employed for the treatment of various carcinomas) promoted the exposure of MICA, MICB, ULBP1, and/or ULBP2 on cultured human breast carcinoma BT474 and MDAMB361 cells,

as well as in MDAMB361 cells grown in SCID mice, which was accompanied by a 15% to 40% increase in NK cell-dependent antibody-mediated cellular cytotoxicity.⁶⁴ Along similar lines, dacarbazine (a mainstay in the treatment of melanoma) robustly upregulated multiple NKG2D ligands including MICA, MICB, RAET1E, and/or ULBP1 in cultured mouse melanoma B16F10 cells and human melanoma Mel-C cells (at both the mRNA and protein level), correlating with the ability of dacarbazine to improve the efficacy of checkpoint blockers in immunocompetent mice bearing B16F10 melanomas.⁶⁵

In addition, multiple chemotherapeutics commonly employed in clinical practice have been shown to promote bona fide ICD, as monitored in gold-standard vaccination experiments involving immunocompetent mice and syngeneic tumors.⁶⁶ These agents include (but may not be limited to) doxorubicin, idarubicin, and epirubicin (yet another anthracycline used for the treatment of breast carcinoma, most often in the context of combinatorial chemotherapeutic regimens)^{67–69}; oxaliplatin^{70–74}; cyclophosphamide (an alkylating agent approved for the therapy of hematological and solid neoplasms)^{75–77}; bortezomib (a proteasome inhibitor used for the treatment of multiple myeloma)⁷⁸; and pemetrexed (an antimetabolite approved for use against pleural mesothelioma and NSCLC).⁷⁹ Recently, other chemotherapeutic agents that are under clinical development for cancer therapy, such as lurbinectedin (a synthetic alkaloid analogue) and PT-112 (a platinum-pyrophosphate conjugate), have been shown to induce several markers of ICD in different tumor models.^{80,81} Of note, ICD is accompanied by such an adjuvanticity that chemotherapeutic ICD inducers appear to synergize with checkpoint blockers to induce potent therapeutic responses even in tumors that are normally insensitive to checkpoint blockade.^{73,79–85} A considerable number of clinical trials is currently focusing on the safety and efficacy of ICD-inducing chemotherapeutics plus immune checkpoint blockers (ICBs) in patients affected by a variety of solid and hematological tumors.^{86,87}

Direct Immunostimulation

Although it may seem counterintuitive, some conventional chemotherapeutics mediate “off-target” immunostimulatory effects by delivering activating signals to the myeloid cell populations that initiate anticancer immune responses and/or to lymphoid immune effectors.⁹ Thus, while dose-intensive cyclophosphamide has been employed for decades as part of myelo- and lymphoablative preconditioning (to prepare patients for hematopoietic stem cell transplantation),⁸⁸ metronomic cyclophosphamide has multipronged immunostimulatory effects. Low-dose cyclophosphamide has been shown to support therapeutically relevant Th1 and Th17 responses in immunocompetent mice

developing KRAS^{G12D}-driven lung cancer in the absence of functional p53 through a gut microbiota-dependent mechanism.⁸⁹ This regimen promoted CTL activity and the expansion of circulating NK cells in C57BL/6 mice implanted with syngeneic GL261 gliomas, an effect that contributed to the efficacy of treatment.⁷⁶ Along similar lines, metronomic cyclophosphamide synergized with a tumor lysate-based vaccine in the eradication of aggressive mouse AgN2a neuroblastomas growing in syngeneic hosts, an activity that also depended on CD8⁺ CTL expansion.⁹⁰ In combination with radiotherapy, 5-fluorouracil promoted tumor infiltration by CD8⁺ CTLs in a cohort of 52 locally advanced rectal cancer patients,⁹¹ and an increased number of CD8⁺ tumor-infiltrating lymphocytes (TILs) expression have also been found in a cohort of 69 patients with esophageal squamous cell carcinoma treated with 5-fluorouracil and cisplatin. This effect has been linked to compensatory upregulation of the immunosuppressive ligand CD274 (best known as PD-L1).⁹² Of note, other chemotherapeutic agents can upregulate the expression of PD-L1 on cancer cells or tumor-infiltrating myeloid cells in preclinical⁸⁵ and clinical settings,^{93–96} most likely reflecting increased tumor infiltration by CD8⁺ CTLs. Moreover, low-dose 5-fluorouracil enabled a peptide-based anticancer vaccine to mediate therapeutic effects in immunocompetent mice bearing syngeneic E.G7 lymphoma cells, an effect that was linked to enhanced CD8⁺ CTL activity.⁹⁷

In a cohort of 28 pancreatic cancer patients, gemcitabine administration was associated with considerably increased numbers of circulating CD11c⁺ DCs and CD14⁺ monocytes (both of which are known to contribute to natural and therapy-elicited anticancer immunosurveillance).^{98,99} Similarly, gemcitabine restored defective cross-presentation by tumor-infiltrating DCs in a model of mouse AB1 mesothelioma engineered to express an exogenous immunoreactive antigen,^{100,101} as did paclitaxel (a taxane used for the treatment of multiple solid tumors) in mice bearing transgenic breast carcinomas.¹⁰² Oxaliplatin potentially boosted the anticancer activity of neutrophils and macrophages in immunocompetent mice bearing syngeneic EL4 lymphomas via a microbiota-dependent circuitry, thus resembling metronomic cyclophosphamide.¹⁰³ Moreover, oxaliplatin also favored the activation of DCs by increasing IL-12 production and concomitantly decreasing PD-L1 expression by DCs themselves.¹⁰⁴ Neoadjuvant paclitaxel favored the accumulation of TILs in a cohort of 25 breast carcinoma patients, correlating with clinical responses to treatment.¹⁰⁵ Finally, pemetrexed stimulated IFN- γ production by circulating NK cells from pancreatic cancer patients, an immunostimulatory effect that was abrogated by concurrent gemcitabine treatment and associated with the depletion of CD45RO⁺ memory T cells.¹⁰⁶ Pemetrexed also increased T cell activation *in vitro* and in preclinical

models of colorectal carcinoma by simultaneously inducing ICD and mediating direct “on-target” effects on T cells.⁷⁹ Taken together, these observations suggest that the direct immunostimulatory effects of specific chemotherapeutics may be sensitive to contextual variables including the presence of additional drugs.

Indirect Immunostimulation

Arguably the most common mechanism through which conventional chemotherapeutics employed at metronomic doses promote therapeutically relevant immunostimulation is by inhibiting or depleting immunosuppressive cell populations.⁹ Thus, it appears possible to find doses and/or schedules of chemotherapeutic agents that preferentially deplete immunosuppressive cells over immune effector cells *in vivo*. Molecular mechanisms are understood in only a subset of cases. Human CD4⁺CD25⁺Foxp3⁺ T_{reg} cells have been shown to be particularly sensitive to cyclophosphamide-driven cell death as they do not express ATP-binding cassette, subfamily B (MDR/TAP), member 1 (ABCB1), which normally extrudes the active metabolite of the drug.¹⁰⁷ On the contrary, why other immunosuppressive cells succumb so easily in response to a wide panel of chemotherapeutic agents employed at low doses remains unknown.⁹

Metronomic cyclophosphamide has been reported to preferentially deplete CD4⁺CD25⁺Foxp3⁺ T_{reg} cells in nine end-stage cancer patients, correlating with the restoration of NK-cell and CD8⁺ CTL activity.¹⁰⁸ A similar effect has been seen in C57BL/6 mice bearing syngeneic GL261 gliomas⁷⁶ and in rats bearing syngeneic colorectal carcinomas.¹⁰⁹ Conversely, in mice bearing syngeneic 4T1 breast carcinoma cells and A20 B-cell lymphoma cells, low-dose metronomic cyclophosphamide slightly increased circulating T_{reg} cells as it decreased circulating B and effector T cells.¹¹⁰ Despite these latter observations, cyclophosphamide and an anti-PD-L1 antibody synergized in the control of 4T1 and A20 tumors.¹¹⁰ A T_{reg} cell-depleting activity has also been ascribed to docetaxel, in a cohort of 40 NSCLC patients;¹¹¹ gemcitabine, in 53 subjects with pancreatic cancer¹¹² and in 40 NSCLC patients (receiving gemcitabine plus cisplatin);¹¹³ and vinorelbine (a vinca alkaloid employed for the treatment of solid tumors), in 14 NSCLC patients treated with this agent in combination with cisplatin.¹¹⁴ 5-Fluorouracil was reported to deplete circulating MDSCs from immunocompetent mice bearing carcinogen-driven colorectal cancer¹¹⁵ or syngeneic EL4 lymphomas,¹¹⁶ as well as from 23 patients with stage IV colorectal carcinoma,¹¹⁶ an indirect immunostimulatory effect that was abolished by the concomitant administration of irinotecan but not oxaliplatin.¹¹⁵ In mice bearing FM3A breast carcinomas, irinotecan depleted T_{reg} cells when used as monotherapy, but an increase in CD8⁺ T cells in tumors or lymph nodes was

only observed when used in combination with an anti-PD-L1 antibody.⁴⁰ Finally, doxorubicin could increase the percentage of CTLs secreting IFN- γ through reducing the accumulation of T_{reg} cells in LLC and 4T1 breast carcinomas only when coadministered with IL-12, while the administration of doxorubicin alone resulted in T_{reg} cell accumulation in the same models.¹¹⁷

Gemcitabine caused a decrease in the percentage of circulating MDSCs in a variety of syngeneic tumor models, including CT26 colorectal carcinomas,¹¹⁸ A20 lymphomas,¹¹⁹ WEHI-164 fibrosarcomas,¹²⁰ AB12 mesotheliomas,¹²¹ L1C2 bronchoalveolar carcinomas,¹²¹ and K7M2 osteosarcomas¹²⁰ established in BALB/c mice, as well as LLC or TC-1 lung adenocarcinomas,^{121,122} L1C2 bronchoalveolar carcinomas,¹²¹ AE-17 mesothelioma,¹²¹ and EL4 lymphoma¹¹⁶ established in C57BL/6 mice. Along similar lines, paclitaxel was capable of depleting circulating MDSCs from mice bearing transgene-driven melanoma, accounting for most of the therapeutic efficacy of the drug in this setting.¹²³ Moreover, low levels of MDSCs have been found in the peripheral blood of patients with gastric cancer treated with paclitaxel.¹²⁴

Finally, the experimental platinum-pyrophosphate conjugate PT-112 has been linked to a reduction in immunosuppressive cells (i.e., MDSCs, tumor-associated macrophages, and T_{reg} cells) and a concomitant increase in CD8⁺ CTLs in the tumor microenvironment of CT26 colon carcinomas.⁸¹ A similar indirect immunostimulatory effect has been ascribed to oxaliplatin, which reduced peritoneal metastasis by decreasing the number of tumor-associated macrophages and MDSCs in the spleen of BALB/c mice bearing syngeneic CT26 colon carcinomas,¹²⁵ and to a nanoparticle formulation of doxorubicin that causes the repolarization of murine M2-polarized immunosuppressive tumor-associated macrophages into their M1-polarized tumoricidal counterparts in 4T1 mammary carcinoma established in BALB/c mice.¹²⁶ Interestingly, gemcitabine and paclitaxel have also been shown to cause the M2-to-M1 repolarization of human and murine macrophages *in vitro* and in tumor samples from ovarian cancer patients.^{127,128} However, it remains unclear to which extent such a conversion occurs in pancreatic cancer patients treated with gemcitabine-based chemotherapy *in vivo*, and whether such indirect immunostimulatory effects are therapeutically relevant in the clinic. Irrespectively, these observations corroborate the notion that a wide panel of chemotherapeutics currently employed in clinical practice can inhibit immunosuppressive cell populations, especially when employed according to metronomic regimens.

Overall, these data highlight the multiple effects of conventional chemotherapeutics on the antitumor host response (Table 29.1). While it remains to be established to which degree the therapeutic response to chemotherapy in patients is the result of direct tumor cytotoxicity

Table 29.1 Immunological Effects of Conventional Chemotherapy (Examples)

EFFECT	AGENT	SETTING	NOTES	REF.
<i>"On-target" effects</i>				
Increased antigenicity	5-Fluorouracil (plus type I IFN)	Orthotopic Panc02 mouse pancreatic carcinoma	Restored MHC class I expression	[33]
	Cisplatin	Mouse lung adenocarcinoma TC-1 cells	Restored MHC class I expression	[37]
		Human HNSCC UMSSC-46 and UMSSC-74A cells	Restored MHC class I expression	[38]
	Doxorubicin (plus type I IFN)	Mouse pancreatic carcinoma Panc02 cells	Restored MHC class I expression	[34]
	Gemcitabine	Human breast carcinoma MCF-7 cells	Restored MHC class I expression	[35, 36]
		Human NSCLC A549 cells	Restored MHC class I expression	[35, 36]
		Human colorectal cancer HCT-116 cells	Restored MHC class I expression	[35, 36]
	Irinotecan	Transplantable FM3A mouse breast carcinoma	Restored MHC class I expression	[40]
	Oxaliplatin	Human HNSCC UMSSC-46 and UMSSC-74A cells	Restored MHC class I expression	[38]
	Topotecan	Human breast carcinoma ZR-75-1 cells	Restored MHC class I expression	[39]
Increased adjuvanticity	5-Fluorouracil (plus type I IFN)	Mouse pancreatic carcinoma Panc02 cells	Restored RAET1E and ULBP1 expression	[33]
	Bortezomib	Human multiple myeloma U266 cells	ICD induction	[78]
	Cyclophosphamide	Transplantable EL4 mouse lymphoma and GL261 mouse glioma	ICD induction	[75, 76]
		Mouse glioma GL261 and CT-2A cells	ICD induction	[77]
	Dacarbazine	Human melanoma Mel-C cells and mouse melanoma B16F10 cells	Restored MICA, MICB, RAET1E, and/or ULBP1 expression	[65]
	Docetaxel	Human breast carcinoma BT474 and MDAMB361 cells	Restored MICA, MICB, ULBP1, and/or ULBP2 expression	[64]
	Doxorubicin	Various human and mouse cancer cells	ICD induction	[67, 68]
	Doxorubicin (liposome-microbubble)	Mouse lung carcinoma LL/2 cells	ICD induction	[69]
		Mouse colon carcinoma CT26 cells	ICD induction	[69]
	Doxorubicin (plus type I IFN)	Mouse pancreatic carcinoma Panc02 cells	Restored RAET1E and ULBP1 expression	[34]
	Doxorubicin (plus IL-12)	Various transgenic and transplantable mouse tumors	Restored RAET1E, MICA/B, and ULBP2 expression	[61]
	Gemcitabine	Human pancreatic carcinoma PANC-1 and HPAF-II cells	Restored MICA and MICB expression	[62]

(continued)

Table 29.1 Immunological Effects of Conventional Chemotherapy (Examples) (*continued*)

EFFECT	AGENT	SETTING	NOTES	REF.
	Lurbinectedin	Various human and mouse cancer cells	ICD induction	[80]
	Gemcitabine	Human breast carcinoma MCF-7 cells	Restored CD95 expression	[63]
		Human NSCLC A549 cells	Restored ULBP2/5/6 and CD95 expression	[63]
		Human colorectal cancer HCT-116 cells	Restored ULBP2/5/6 and CD95 expression	[63]
	Oxaliplatin	Various human and mouse cancer cells	ICD induction	[70–73]
	Pemetrexed	Mouse colorectal carcinoma Colon26 and MC38 cells	ICD induction	[79]
	PT-112	Mouse breast carcinoma TSA cells	ICD induction	[81]
<i>“Off-target” effects</i>				
Direct immunostimulation	5-Fluorouracil	Rectal cancer patients	Tumor infiltration by CD8 ⁺ CTLs	[91]
		Transplantable E.G7 mouse lymphoma	Enhanced CD8 ⁺ CTL activity	[97]
	5-Fluorouracil plus cisplatin	Esophageal squamous cell cancer patients	Tumor infiltration by CD8 ⁺ CTLs	[92]
	5-Fluorouracil plus oxaliplatin	Transplantable Colon26 and MC38 mouse colorectal carcinomas	Enhanced CD8 ⁺ CTL activity	[85]
	5-Fluorouracil plus docetaxel plus a platinum-based agent	HNSCC patients	Tumor infiltration by CD8 ⁺ CTLs	[93]
	Cyclophosphamide	Endogenous KRAS ^{G12D} -driven lung cancer	Improved T _H 1 and T _H 17 responses, dependent on the gut microbiota	[89]
		Transplantable AgN2a mouse neuroblastomas	CD8 ⁺ CTL expansion	[90]
		Transplantable GL261 mouse gliomas	Enhanced CD8 ⁺ CTL activity and peripheral NK cell expansion	[76]
	Gemcitabine	Pancreatic cancer patients	Increased circulating CD11C ⁺ DCs and CD14 ⁺ monocytes	[98]
		Transplantable AB1 mouse mesothelioma	Restored cross-presentation by tumor-infiltrating DCs	[100, 101]
	Oxaliplatin	Transplantable MC38 mouse colon carcinoma	Induced DCs phenotypic maturation	[104]

(continued)

Table 29.1 Immunological Effects of Conventional Chemotherapy (Examples) (*continued*)

EFFECT	AGENT	SETTING	NOTES	REF.
	Paclitaxel	Breast carcinoma patients	Increased levels of TILs, correlating with clinical responses	[105]
		Transgenic mouse breast carcinoma	Restored cross-presentation by tumor-infiltrating DCs	[102]
	Pemetrexed	Pancreatic cancer patients	Enhanced IFN- γ -secretory activity in circulating NK cells	[106]
		Primary T cells and transplantable Colon26 and MC38 mouse colon carcinomas	Activated T cells and enhanced CD8 ⁺ CTL activity	[79]
Indirect immunostimulation	5-Fluorouracil	Colorectal carcinoma patients	Depletion of circulating MDSCs	[115]
		Mouse carcinogen-driven colorectal cancer	Depletion of MDSCs, abolished by irinotecan coadministration	[115]
		Transplantable EL4 mouse lymphoma	Depletion of circulating MDSCs	[116]
	Cyclophosphamide	Advanced cancer patients	Depletion of T _{reg} cells, linked to NK cell and CD8 ⁺ CTL activity	[108]
		Transgenic rat colorectal carcinoma	Depletion of T _{reg} cells	[109]
		Transplantable GL261 mouse glioma	Depletion of T _{reg} cells	[76]
	Docetaxel	NSCLC patients	Depletion of T _{reg} cells	[111]
	Doxorubicin (plus IL-12)	Transplantable LLC lung and 4T1 breast carcinomas	Depletion of T _{reg} cells linked to CD8 ⁺ CTL activity	[117]
	Doxorubicin (nanoparticles)	Transplantable 4T1 breast carcinoma	M2-to-M1 repolarization linked to CD8 ⁺ CTL activity	[126]
	Gemcitabine	Pancreatic cancer patients	Depletion of T _{reg} cells	[112]
		Human M2-polarized macrophages	M2-to-M1 repolarization	[127]
		Transplantable K7M2 mouse osteosarcomas and WEHI-164 mouse fibrosarcomas	Depletion of T _{reg} cells	[120]
		Various transplantable mouse tumors	Depletion of circulating MDSCs	[116, 118–122]
	Gemcitabine (plus cisplatin)	NSCLC patients	Depletion of T _{reg} cells	[113]
	Irinotecan	Transplantable FM3A mouse breast carcinoma	Depletion of T _{reg} cells	[40]
	Oxaliplatin	Transplantable CT26 mouse colon carcinoma	Depletion of TAMs and of MDCs in the spleen, linked to CD8 ⁺ CTL activity	[125]

(continued)

Table 29.1 Immunological Effects of Conventional Chemotherapy (Examples) (*continued*)

EFFECT	AGENT	SETTING	NOTES	REF.
	Paclitaxel	Transgenic mouse melanoma	Depletion of circulating MDSCs	[123]
		Gastric cancer patients	Depletion of circulating MDSCs	[124]
		Ovarian cancer patients	M2-to-M1 repolarization	[128]
		Murine M2-polarized macrophages	M2-to-M1 repolarization	[128]
	PT-112	Transplantable CT26 and MC38 mouse colon carcinomas	Depletion of MDSCs, TAMs, and T _{reg} cells, linked to CD8 ⁺ CTL activity	[81]
	Vinorelbine (plus cisplatin)	NSCLC patients	Depletion of T _{reg} cells	[114]

CTL, cytotoxic T lymphocyte; DC, dendritic cell; HNSCC, head and neck squamous cell carcinoma; ICD, immunogenic cell death; IFN, interferon; LCC, Lewis lung carcinoma; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex; MICA, MHC class I polypeptide-related sequence A; MICB, MHC class I polypeptide-related sequence B; NK, natural killer; NSCLC, non-small-cell lung carcinoma; RAET1E, retinoic acid early transcript 1E; TAM, tumor-associated macrophages; T_H, helper T; TIL, tumor-infiltrating lymphocyte; T_{reg}, CD4⁺CD25⁺Foxp3⁺ regulatory T; ULBP1, UL16 binding protein 1; ULBP2, UL16 binding protein 2.

Source: Modified with permission from Galluzzi L, Buqué A, Kepp O, et al. Immunological effects of conventional chemotherapy and targeted anticancer agents. *Cancer Cell*. 2015;28(6):690–714. doi:10.1016/j.ccell.2015.10.012

versus the induction of antitumor immunity, it is clear that the immunomodulatory effects of a given chemotherapy can influence its interaction with immunotherapeutic agents, either positively or negatively.

Targeted Therapy

Similar to conventional chemotherapy, targeted anticancer agents can have unintended immunomodulatory effects that are the result of their on-target action (inhibition of a mutated or overexpressed oncogene product) or may result from the interaction of the drug with other targets expressed by immune cells. These effects can have positive or negative influences on antitumor immune responses and are important yet incompletely understood determinants of clinical efficacy. In support of this notion, in a mouse model of oncogene addiction, durable responses to specific targeting of an oncogene were shown to require the contribution of T cells.¹²⁹

The tyrosine kinase inhibitor (TKI) imatinib mesylate developed against the oncogenic fusion protein BCR-ABL is the prototype-targeted anticancer therapeutic and one of the most successful clinically. Initially developed for BCR-ABL⁺ chronic myelogenous leukemia (CML),¹³⁰ imatinib was later shown to also have activity against the oncogenic kinase KIT, which is expressed by gastrointestinal stromal tumors (GISTs),¹³¹ and platelet-growth factor receptor beta (PDGFRB), which is expressed by several solid tumors.¹³² Induction of antileukemia T cell responses was reported in nine of 14 CML patients in remission after imatinib treatment.¹³³ In another study, BCR-ABL-specific T cells were detected in 10 of 10 CML

patients in maintenance with imatinib, more frequently in the bone marrow than in the peripheral blood. There was a correlation between BCR-ABL-specific T cells and lower minimal residual disease, and absence at leukemia relapse.¹³⁴ Moreover, ibrutinib, which targets Bruton tyrosine kinase, has been reported to increase diversification of the T cell compartment by increasing the TCR repertoire diversity in patients with chronic lymphocytic leukemia.¹³⁵

An impact of kinase targeting on host immune responses has also been observed in the context of GIST. In a mouse model of GIST driven by an activated *KIT* mutation, the therapeutic activity of imatinib was shown to be partially dependent on antitumor CD8⁺ T cells. The activation of antitumor immunity was due to downregulation of the immunosuppressive enzyme indoleamine 2,3-dioxygenase 1 (IDO1) in cancer cells, resulting in reduced T_{reg} cells and increased effector CTL infiltration into the tumor, a change that correlated with imatinib sensitivity in human GISTs.¹³⁶ Thus, activation of antitumor immunity is an *on-target* effect of imatinib in GISTs, which can be categorized as enhancing their *adjuvanticity*. In contrast, an *off-target* inhibitory activity of this drug against colony-stimulating factor 1 receptor (CSF1R) was likely responsible for decreased tumor-associated macrophage (TAM) recruitment to imatinib-treated GISTs. Despite such reduction, TAM interaction with apoptotic cancer cells in treated tumors was shown to promote M2 polarization, resulting in increased immunosuppression.¹³⁷ Consistent with this data, coadministration of imatinib and an anti-CD40 antibody activated TAMs and resulted in superior antitumor activity.¹³⁸ In other

studies, the ability of imatinib to inhibit KIT on DCs was shown to promote DC-mediated NK-cell activation, culminating in antitumor effects against imatinib-resistant GISTs.^{139,140} Imatinib also induced the M2-to-M1 repolarization of macrophages by TLR engagement and the M1-mediated NK-cell activation in neuroblastoma and CML samples, as did the imatinib-like drug nilotinib.¹⁴¹ Recent findings also demonstrate that imatinib can augment antitumor immunity by inducing selective depletion of T_{reg} cells and increasing the number of effector $CD8^+$ T cells in patients with CML or melanoma. Mechanistically, imatinib appears to inhibit the lymphocyte-specific protein tyrosine kinase LCK proto-oncogene, Src family tyrosine kinase (LCK), which is highly expressed by T cells other than T_{reg} cells, rendering the latter more sensitive to apoptosis upon TCR inhibition in the presence of imatinib.¹⁴² The analysis of samples from dermatofibrosarcoma patients after treatment with imatinib also demonstrated that PDGFRB inhibition favors antigen presentation, linked to enhanced infiltration of the tumor by T cells and strong PD-L1 expression on the malignant cells themselves.¹⁴³ Consistent with this, coadministration of PD-1/PD-L1 blockers correlated with superior therapeutic activity in a model of GISTs.¹⁴⁴ ICBs also synergized with crizotinib, a TKI used to treat NSCLC, which acted as a potent ICD stimulator when combined with non-ICD-inducing chemotherapeutics like cisplatin, and induced *off-target* effects by promoting immune infiltration in NSCLC tumors established in immunocompetent mice.¹⁴⁵ These data highlight the complex and unsuspected effects of a molecularly targeted drug on the intended and unintended targets, which contribute to, and in some circumstances could hinder, the therapeutic response via immunological mechanisms.¹³²

Many other TKIs used in the clinical practice for the treatment of different malignancies have been found to mediate direct and indirect effects on the immune system.⁹ For example, dasatinib, a second-generation TKI active against BCR-ABL, SRC, KIT, PDGFR, and ephrin tyrosine kinases, reduced the levels of MDSCs and T_{reg} cells in a mouse melanoma model, leading to improved T cell responses to a tumor vaccine, an *off-target indirect immunostimulatory* effect.¹⁴⁶ A similar reduction of MDSCs and T_{reg} cells was reported for sorafenib, another broad-spectrum TKI, in mice and patients with renal cell carcinoma,^{147,148} and sunitinib, which also upregulated CXCL10 and CXCL11 on the tumor endothelium, improving the recruitment of T cells in mouse melanoma and hepatocellular models, effects that are at least in part the consequence of the antiangiogenic activity of these drugs.^{149,150} Erlotinib and gefitinib, which target epidermal growth factor receptor (EGFR), were shown to increase the expression of NKG2D ligands on lung cancer cells, increasing their susceptibility to NK

cell-mediated lysis, an example of an *on-target* increase in *adjuvant activity*.¹⁵¹ *Off-target immunostimulatory* effects have also been reported for lenvatinib, which showed potent antitumor activity in the tumor microenvironment of murine colorectal and hepatocellular tumors, characterized by reduced TAMs and increased percentage of CTLs secreting IFN- γ and granzyme B. Moreover, combination treatment of lenvatinib plus anti-PD-1 further increased these effects resulting in greater antitumor activity.^{152,153}

Activating mutations in B-Raf proto-oncogene (*BRAF*), encoding a serine/threonine kinase of the mitogen-activated protein kinase (MAPK) pathway, are frequent in melanoma and can be successfully targeted by specific *BRAF* inhibitors vemurafenib, encorafenib, and dabrafenib, alone or in combination with inhibitors of downstream mitogen-activated protein kinase kinase 7 (MAP2K7, best known as MEK, such as trametinib, cobimetinib, and binimetinib), but responses are generally short-lived.^{154,155} Treatment of melanoma cells with *BRAF* and MEK inhibitors was shown to increase expression of melanocyte differentiation antigens, resulting in improved recognition by T cells, an *on-target* increase in cancer cell *immunogenicity*. In a recent study, combined inhibition of *BRAF* and MEK also led to DC activation following the release of HMGB1 by melanoma cells.¹⁵⁶ Although a previous study demonstrated that MEK inhibitor can impair T cell function,¹⁵⁷ MEK inhibition has recently been linked to a blockage of naïve $CD8^+$ T cell expansion and priming but an increased number of antigen-specific effector $CD8^+$ T cells in the tumor microenvironment (TME), and limited T cell exhaustion in vitro upon TCR stimulation.¹⁵⁸ These findings have been confirmed in mouse models of AML as well as in primary samples of AML patients sensitive to trametinib.¹⁵⁹ Moreover, the MEK inhibitor selumetinib combined with the PD-L1 blocker atezolizumab augmented antitumor immunity by upregulating MHC-I expression, boosting the production of several cytokines (i.e., IFN- γ , IL-6, IL-1 β , and TNF), and reducing PD-L1 expression in a panel of NSCLC cell lines.¹⁶⁰ Other studies also suggest that T cell impairment may not be significant in vivo since patients co-treated with *BRAF* and MEK inhibitors showed increased infiltration of the tumor by T cells, which was associated with increased expression of PD-L1 (which is reflective of local IFN- γ secretion).^{161,162} Importantly, responses to *BRAF* inhibitors correlated with the presence of an oligoclonal $CD8^+$ T cell infiltrate in pretreatment biopsies, suggesting a contribution of the immune system to clinical responses, while reduced levels of intratumoral $CD8^+$ T cells were seen at progression.^{163,164} Interestingly, *BRAF* inhibition has also been shown to have *off-target direct immunostimulatory* effects on mouse and human NK cells, leading to increased phosphorylation of ERK1/2, CD69 upregulation, and improved effector functions. In a mouse model of *BRAF*^{V600E}-driven melanoma, NK cells and perforin

1 (PRF1) were required for therapeutic effects of BRAF inhibitors.¹⁶⁵ Moreover, BRAF and MEK inhibitors combined with a PD-1 blocker decreased TAM and T_{reg} cell accumulation, improved IFN- γ release, and enhanced antigen presentation in a preclinical melanoma model, and increased the frequency of long-lasting antitumor responses in patients with BRAF-mutated melanoma.¹⁶⁶

Improved antitumor immunity and synergy with ICBs has been described for MEK inhibitors in different preclinical models of tumors driven by mutant KRAS proto-oncogene, GTPase (KRAS).^{167,168} Activating mutations of KRAS, which impact several signaling pathways including BRAF-MEK signaling and sustain carcinogenesis as well as treatment resistance in multiple oncological settings,¹⁶⁹ have recently been considered as novel targets to impair tumor growth and enhance antitumor immunity. Interestingly, pharmacological inhibition of KRAS^{G12C} with AMG510 resulted in tumor regression linked to the activation of ICD in several KRAS^{G12C}-driven tumor models. In addition, AMG510 improved the therapeutic efficacy of MEK inhibitors and a PD-1 blocker in KRAS^{G12C}-expressing mouse colorectal tumors growing in immunocompetent syngeneic mice.^{170,171}

Some of the therapeutics targeting growth factor receptors that are overexpressed by cancer cells, including HER2/ERBB2 and EGFR, are monoclonal antibodies (e.g., trastuzumab, cetuximab). Not surprisingly, these agents not only mediate direct cytostatic or cytotoxic effects on cancer cells, but also engage immunological mechanisms such as antibody-dependent cellular cytotoxicity (ADCC), which likely contributes to their efficacy.¹⁷² Interestingly, EGFR-targeting antibodies were shown to induce ICD and facilitate the elicitation of antitumor T cells in mouse models,^{173,174} and the clinically relevant antibodies cetuximab and panitumumab induced ICD via a mechanism dependent on the mutational status of the EGFR signaling pathway and involving inhibition of the unfolded protein response, leading to ER stress, CALR translocation to the cell surface, and phagocytosis of the cancer cells by DCs.¹⁷³ Trastuzumab in combination with chemotherapy induced humoral responses to the intracellular domain of HER2 and other tumor-associated antigens, including carcinoembryonic antigen (CEA) and p53, in breast cancer patients, an effect that was associated with improved survival.¹⁷⁵ Trastuzumab-mediated ADCC was enhanced when conjugated to an anthracycline derivative, an effect that was linked to *off-target immunostimulatory* effects and synergy with an anti-PD-1 antibody in a murine orthotopic breast cancer model resistant to other HER2-targeted therapies.¹⁷⁶ Cetuximab has also been attributed *off-target immunostimulatory* effects by activating human CD8⁺ T and NK cells in patients with colorectal cancer, correlating with T_{reg} cell inhibition,¹⁷⁷ as well as systemic

effects linked to improved infiltration of liver metastases in colorectal cancer patients.¹⁷⁸

Epigenetic modifiers are another group of therapeutics with potentially important effects on antitumor immunity. For instance, decitabine, an inhibitor of DNA methyltransferases, has been reported to upregulate interferon signaling, antigen processing and presentation, and cytokine/chemokine-dependent signal transduction, as well as cancer-testis antigen expression in breast, colorectal, and ovarian cancer cells.¹¹¹ These data were confirmed in human samples from patients with colorectal and ovarian cancer responding to low-dose decitabine.¹⁷⁹ Such a response was attributed to the upregulation of hypermethylated endogenous retroviruses coupled to cytosolic sensing of double-stranded (ds) RNA by TLR3 and mitochondrial antiviral signaling protein.¹⁸⁰ The antitumor activity of histone deacetylase (HDAC) inhibitor trichostatin A, which is similar to the U.S. Food and Drug Administration (FDA)-approved HDAC inhibitor vorinostat, was shown to depend (at least in part) on the inhibition of intratumoral CD4⁺ T cell apoptosis via downregulation of FAS ligand.¹⁸¹

DDR targeting can also mediate immunostimulatory effects and several reports are evaluating the therapeutic efficacy of combining these agents with immunotherapy.⁸⁷ For example, the FDA-approved poly(ADP-ribose) polymerase 1 inhibitors olaparib and rucaparib showed *on-target immunostimulatory* effects by inducing the accumulation of single-stranded (ss) DNA and micronuclei into the cytosol of NSCLC and triple-negative breast cancer cells.^{182,183} In turn, this activated CGAS and consequent type I IFN secretion (linked to increased PD-L1 expression) and recruitment of CD8⁺ T cells into the tumor.^{182,183} Moreover, coadministration of ICBs led to superior disease control in tumors with DDR deficiency (including tumors with *BRCA1* or *BRCA2* mutations).¹⁸⁴

Cyclin-dependent kinase 4 (CDK4) and CDK6 are frequently upregulated in human malignant cells.¹⁸⁵ These proteins are involved in cell-cycle regulation and can be specifically targeted with CDK4/6 inhibitors, including palbociclib, ribociclib, and abemaciclib, all of which have recently been approved for the treatment of patients with hormone receptor (HR)-positive breast cancer.¹⁸⁶ Their therapeutic activity has been largely attributed to their ability to induce cell-cycle arrest in malignant cells, but accumulating reports also highlighted a role for cell-cycle inhibitors in promoting *on-target* and *off-target immunostimulatory* effects.¹⁸⁷ In particular, CDK4/6 inhibition can induce *on-target* effects by enhancing antigen presentation via MHC-I on breast and colorectal cells.^{188,189} Malignant cells treated with CDK4/6 inhibitors can also secrete type III IFN plus multiple inflammatory cytokines as a part of the senescence-associated secretory phenotype (SASP),^{188,189} resulting in the modulation of

anticancer immune responses.¹⁹⁰ Moreover, CDK4/6 inhibitors can induce *off-target immunostimulatory* effects by promoting effector T cell proliferation upon nuclear factor of activated T cell 1 activation and downregulating T_{reg} cell proliferation.^{188,189,191} Recent findings also demonstrated that the combination of palbociclib with a MEK inhibitor (trametinib) favors a SASP that recruits NK cells with anticancer activity or favors a vascular remodeling that enables CD8⁺ CTL infiltration in preclinical models of KRAS-driven lung or pancreatic adenocarcinomas, respectively.^{192,193} Enhanced T cell activation downstream of genome destabilization has been recently documented for pharmacological CDK7 inhibitors.¹⁹⁴ Moreover, targeting CDK8 resulted in NK cell-dependent antitumor responses in mice bearing B16F10 melanoma.¹⁹⁵ Finally, superior tumor eradication has been observed in different mouse tumor models following coadministration of cell-cycle inhibitors with ICBs.^{188,189,191,193,194,196,197}

Radiation Therapy

Radiation has been known for a long time to have dose-dependent pro-inflammatory effects.¹⁹⁸ In fact, the multifraction radiation regimens traditionally used in clinical practice, which consist of small daily doses given for several weeks (1.5–2.2 Gy/day to a total dose of 50–80 Gy depending on the tumor type), were developed to minimize inflammatory reactions in normal tissue incorporated in the radiation field to account for differences in the daily reproducibility of the patient position. Technological advances have dramatically expanded the range of doses that can be safely delivered to the tumor with each fraction by improving the precision of tumor targeting with stereotactic body radiotherapy (SBRT).^{2,199} One or a few high radiation doses (above 8 Gy and up to 30 Gy) are used to “ablate” the tumor by increasing the amount of cancer cell death. However, experimental evidence shows that the ability of radiation to activate antitumor immunity is an important determinant of radiation response,^{200,201} and hence that the quality of cancer cell death, namely the induction of ICD, may be more important than its quantity.^{10,202} In some preclinical studies, the local response to radiation given at doses in the SBRT range (single dose of 20–30 Gy) was largely dependent on T cells and a single large dose was more effective at inducing antitumor T cells than several small doses,^{11,203} but other studies reported activation of antitumor T cells with a few comparatively lower dose fractions (e.g., 2 Gy X 5, 3 Gy X 5, or 8 Gy X 3),^{204–206} suggesting some degree of model-dependency in this regard.²⁰⁷

Overall, the influence of the dose and fractionation regimen on the immunogenic effects of radiation therapy remains incompletely understood. The direct comparison of different radiation doses and fractionation schedules in a few mouse tumor models showed that hypofractionated

regimens of 8 Gy X 3 or 6 Gy X 5 (but not single-dose radiation of 20 or 30 Gy) were able to induce a potent immune response leading to systemic tumor regression when combined with ICBs.^{206,208} However, when the immunogenicity of radiation is assessed by the immunological control of the tumor in experimental models, several factors (many of which are model-specific) may contribute to outcome, including intrinsic radiosensitivity, immune contexture, and the type of immunotherapy combined with radiation.²⁰⁹

Besides contributing to the control of the irradiated lesion, radiation-induced antitumor T cells can reject metastases outside of the radiation field, a phenomenon known as abscopal effect.^{207,210–212} Tumor responses in non-irradiated metastases have been reported in patients receiving radiation alone but are very rare.^{213,214} However, when radiation was used in patients treated with immunotherapy, either to palliate progressing tumors or in the setting of clinical trials testing combinations of radiation and immune modulators, abscopal responses were detected at increased frequency.^{215–222} The ability of focal tumor radiotherapy in combination with ICBs to induce abscopal responses in patients with NSCLC has been tested prospectively in various studies. In patients with chemorefractory metastatic NSCLC, which is poorly sensitive to ICBs targeting CTLA-4 used alone or combined with chemotherapy,²²³ fractionated radiation (6 Gy X 5 and 9.5 Gy X 5) combined with the anti-CTLA-4 agent ipilimumab induced objective abscopal responses in 18% of individuals, accompanied by evidence of tumor-specific CD8⁺ T cell responses.²¹⁹ In another study, metastatic NSCLC patients were randomized to receive the anti-PD1 agent pembrolizumab alone or combined with focal radiation (8 Gy X 3), and the latter treatment was associated with superior overall response rate and median overall survival,²²⁴ supporting the hypothesis that local radiotherapy can play a role in metastatic disease by sensitizing tumors to immunotherapy.⁸ However, results have not been conclusive across different clinical studies,^{225–228} highlighting the need to improve the current understanding of the immunological effects of radiotherapy.

Overall, radiation seems to mediate both on-target and off-target immunomodulatory effects that influence its therapeutic activity and identify novel targets for the development of combinatorial treatment regimens with superior clinical efficacy (Figure 29.2).

Immunostimulatory Effects of Radiation

Increased Antigenicity

Multiple studies have shown that radiation enhances the expression of MHC-I molecules as well as some tumor antigens in mouse and human cancer cells maintained in vitro.^{229,230} In one study analyzing the

Figure 29.2 Immunostimulation and immunosuppression by radiation therapy. Malignant cells can die in response to radiation therapy as a consequence of irreparable damage to macromolecules and organelles. Importantly, such a cytotoxic response only affects a fraction of the tumor, implying that it is not sufficient to eradicate established neoplasms. Alongside, radiation therapy also mediates immunostimulatory (A) and immunosuppressive (B) effects, originating not only within cancer cells, but also within the stromal, endothelial, and immunological tumor compartments. The balance between such immunostimulatory and immunosuppressive effects determines whether radiation therapy can ultimately stimulate a therapeutically relevant anticancer immune response resulting in tumor eradication.

CD73 (official name: NT5E), 5'-nucleotidase ecto; CTL, CD8⁺ cytotoxic lymphocyte; DAMP, damage-associated molecular pattern; DC, dendritic cell; DR, death receptor; ICAM1, intercellular adhesion molecule 1; ICD, immunogenic cell death; IFN, interferon; MDSC, myeloid-derived suppressor cell; NK, natural killer; NKG2D (official name KLRK1), killer cell lectin-like receptor K1; PD-L1 (official name, CD274); TAA, tumor-associated antigen; TAM, tumor-associated macrophage; TGF β 1, transforming growth factor β 1; T_{reg}, CD4⁺CD25⁺Foxp3⁺ regulatory T; TREX1, three prime repair exonuclease 1; VCAM1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor.

effects of radiation on expression of MHC-I and two tumor antigens, that is, CEA and mucin-1 (MUC-1), exposure to a single radiation dose of 10 or 20 Gy induced the upregulation of MHC-I and MUC-1 in eight of 23 human colon, lung, and prostate carcinoma cell lines tested, and CEA in 16 of them.²⁸ In mouse colorectal carcinoma MC38 cells growing in syngeneic immunocompetent mice, MHC-I upregulation was

shown to occur *in vivo* after a single 10 Gy radiation dose, improving tumor rejection by adoptively transferred tumor-specific CTLs.²³¹ Similar findings have been obtained in human NSCLC A549 and H1975 cell lines maintained *in vitro*, with a plateau effect at 20 Gy single-dose radiation.²³²

In another study, the mechanisms of radiation-induced MHC-I upregulation were investigated using

human melanoma cells.²³¹ Upregulation was detected at doses of 4 Gy or above and plateaued between 10 and 25 Gy. In the first few hours postradiation (clean-up phase), increased expression of surface MHC-I was supported by degradation of cellular proteins damaged by reactive oxygen species (ROS) generated by radiation, resulting in a larger pool of peptides available for loading onto MHC-I molecules. In a second phase (repair phase), activation of mechanistic target of rapamycin was required to sustain the increased availability of antigenic peptides, which largely originated from overall enhanced protein synthesis resulting in enhanced generation of defective ribosomal products. Importantly, some peptides displayed on surface MHC-I molecules by irradiated cells were not detected in the immunopeptidome of non-irradiated cells, and originated from proteins synthesized in response to DNA damage.²³¹ These data suggest that radiation modulates cancer cell antigenicity by altering the repertoire of epitopes displayed by MHC-I. This may result in increased expression of genes that encode immunogenic mutations, thus exposing neoantigens to the immune system.²³⁰ Evidence in support of this hypothesis comes from the identification of CD8⁺ T cell clones specific for an immunogenic mutation encoded in karyopherin subunit alpha 2 (*KPNA2*), a gene upregulated by radiation, in an NSCLC patient with complete response to radiation and ipilimumab.²¹⁹

The upregulation of MHC-I *in vivo* is not always the result of an on-target effect of radiation. For instance, in the B16 mouse melanoma model, host-produced IFN- γ was required for MHC-I upregulation in tumors treated with a single dose of 15 Gy.²³³ This effect has also been observed in an orthotopic HNSCC murine model in which a single radiation dose of 10 Gy promoted IFN- γ secretion by CD4⁺ and CD8⁺ T cells, ultimately increasing tumor immunogenicity.²³⁴

Regardless of the mechanisms involved, which include a stable increase in expression of MHC-I heavy chain mRNA in mouse melanoma cells after prolonged exposure to 2 Gy daily radiation doses,²³⁵ the increased antigenicity of irradiated cancer cells can contribute to their ability to serve as vaccines, especially when doses that are not ablative but may sensitize tumor cells to CTLs are used. In fact, in a mouse intracranial GL261 glioma model, peripheral vaccination with irradiated GM-CSF-transduced GL261 tumor cells had little effect, and whole brain radiotherapy with two doses of 4 Gy only minimally extended survival. However, when combined, the two treatments cured 40% to 80% of the mice.²³⁶ Interestingly, restored MHC-I expression was seen in invading glioma cells at the edge of the tumor growing in the irradiated brain, suggesting that it may have contributed to complete tumor elimination by vaccine-elicited T cells.

Increased Adjuvanticity

Similar to chemotherapy, radiation has been shown to increase the capacity of malignant cells to emit danger signals. Cell stress-associated danger signals expressed on cells that survive irradiation include several NKG2D ligands, death receptors, adhesion molecules, and co-stimulatory molecules.²³⁷ Ionizing radiation induced the expression of NKG2D ligands ULBP1 and RAET1E in mouse ovarian cancer cells,²³⁸ and MICB, ULBP1, and ULBP2 in several human cancer cells, increasing their sensitivity to NK cell-mediated lysis.²³⁹ Radiation also promoted the immunogenicity of different glioma cell lines, including glioma stem-like cells, *in vitro* and *in vivo*, by upregulating the expression of several NKG2D ligands at the mRNA and protein levels and enhancing DDR.²⁴⁰ Consistently, decreased NKG2D ligand expression coupled to increased PD-L1 expression has been found in radioresistant NSCLC cell lines, protecting these cells from the cytotoxic action of NK cells.²⁴¹ Sensitivity of cancer cells to NK cell-mediated lysis was also increased by radiation-induced release of diablo IAP-binding mitochondrial protein (DIABLO) from mitochondria, which increases granzyme-induced cytotoxicity by blocking X-linked inhibitor of apoptosis (XIAP).²⁴² Moreover, inhibition of XIAP has been related to enhanced radiation-induced antigen presentation, induction of ICD, and recruitment of T cells in mouse models of HNSCC.²⁴³ In the 4T1 mouse breast cancer model, *in vivo* irradiation increased the expression of intercellular adhesion molecule 1 (ICAM1) and induced the exposure of RAET1E on the surface of cancer cells. The interaction of RAET1E with NKG2D expressed on CD8⁺ T cells was critical for formation of stable immune synapses between these immune effectors and cancer cells in mice treated with an anti-CTLA-4 antibody.²⁴⁴ Expression of death receptor FAS/CD95 was induced in MC38 colorectal mouse tumor cells after *in vivo* irradiation with a single 8 Gy radiation dose and increased tumor rejection by CD8⁺ T cells.²⁴⁵ Similar findings have been obtained in models of pancreatic adenocarcinoma treated with CAR T cells.²⁴⁶ In this setting, low-dose (1–2 Gy) irradiation caused the upregulation of TNF receptor superfamily member 10b (TNFRSF10B, best known as DR5) on the surface of malignant cells, ultimately resulting in some degree of antigen-independent cytotoxicity by CAR T cells.²⁴⁶ Finally, irradiation of human colorectal cancer cells *in vitro* resulted in the upregulation of OX40L and 41BBL through epigenetic modulation, resulting in improved CTL survival, activation, and effector functions.²⁴⁷

Radiation has also been shown to induce the expression and release of many pro-inflammatory cytokines and chemokines by cancer cells. *In vitro*, a single radiation dose of 20 Gy induced IL-1 β release by leukemia cells.²⁴⁸ Human sarcoma cells produce TNF in response

to a single radiation dose of 5 Gy.²⁴⁹ CXCL16 was induced in vitro in human breast cancer cells and murine mammary, prostate, and colon carcinoma cells by a single dose of 12 Gy.^{250,251} Moreover, radiation-induced CXCL16 was required for optimal infiltration of 4T1 mouse breast tumors by activated CD8⁺ T cells in mice treated with anti-CTLA-4 antibody.²⁵⁰ Other than CXCL16, CXCL10, CCL2, and CCL5 also increased in B16 melanoma tumors after 30 Gy of radiation given in two fractions, resulting in increased CD8⁺ T cells and decreased CD11b⁺Gr1⁺ MDSCs tumor infiltration.²⁵²

Importantly, radiation-induced cancer cell death is associated with the generation of at least some of the DAMPs that characterize ICD induced by chemotherapy: CALR translocation to the cell surface, ATP secretion, and HMGB1 release, all of which have been shown to manifest in a radiation dose-dependent manner.^{53,58,218,253,254} In addition, DNA released by cancer cells succumbing to radiation in vivo can be taken up by tumor-infiltrating DCs and provide a critical signal by activating IFN-I production via CGAS and stimulator of interferon response cGAMP interactor 1 (STING1),²⁵⁵ an effect that may depend, at least in part, on tumor-derived exosomes.²⁵⁶ At least in some settings, STING1 activation and consequent IFN-I production also occurs in irradiated cancer cells downstream of the accumulation of double-stranded (ds) DNA in the cytosol.²⁵⁷ The ability of cancer cells to produce IFN-I is dependent on multiple factors, including expression levels of CGAS and STING1, the status of the cofactors and posttranslational modifications that modulate their functions, and the radiation dose and fractionation schedule.²⁵⁸ One of the regulatory mechanisms limiting the secretion of IFN-I by irradiated cells is mediated by three prime repair exonuclease 1 (TREX1), which degrades cytosolic dsDNA and is upregulated at least in some cancer cells by radiation doses above 12 Gy. Conversely, production of IFN-I following CGAS-STING1 activation appears to be optimal when radiation is given in hypofractionated doses (8 Gy X 3). Upregulation of IFN-inducible chemokines (i.e., CXCL9, CXCL10, CXCL11, and CXCL16) and consequent recruitment of conventional type 1 DCs (cDC1s) and effector CD8⁺ T cells to the irradiated tumor were also seen in mouse tumors following fractionated radiation doses that optimally induce IFN-I.²⁰⁶ Thus, in vivo radiation amplifies a pathway responsible for priming of spontaneous antitumor immunity to immunogenic tumors.^{10,259}

Off-Target Immunostimulatory Effects

Some of the stromal cells present within the irradiated tumor have been shown to respond to radiation with phenotypic changes that improve tumor infiltration of effector T cells. Most of the studies have analyzed changes in tumor endothelium, showing upregulation of vascular cell adhesion molecule (VCAM1), which was

associated with increased tumor-specific CD8⁺ T cell infiltration into irradiated mouse B16 melanomas.²⁰⁴ Ionizing radiation also induced ICAM1 and VCAM1 expression on lymphatic endothelial cells in MC38 and B16 tumors grown in mice, an effect that was partially mediated by TGF- β 1.²⁶⁰ In another study based on a mouse model of spontaneous pancreatic islet carcinogenesis, radiation used at a low dose of 0.5 to 2 Gy normalized the aberrant vasculature by re-programming macrophages into a M1 phenotype, resulting in endothelial activation and recruitment of adoptively transferred T cells.²⁶¹ Interestingly, a recent study using longitudinal in vivo imaging of mouse tumors demonstrated that a significant fraction of tumor-infiltrating T cells survives therapeutic doses of radiation and exhibits increased motility and cytokine secretion as compared to T cells from unirradiated tumors.¹³ These data indicate that, unlike circulating naïve T cells, tumor-resident T cells respond to radiation with increased effector functions rather than apoptosis.

Overall, the previously described multiple immune activating signals elicited by radiation explain the ability of radiation to convert the tumor into an “*in situ* vaccine” and induce antitumor immune responses that have been shown to contribute to control of the irradiated tumor in experimental studies.^{200,262} However, the immune activation is usually insufficient to overcome negative regulatory networks that can also be upregulated by radiation, precluding the development of systemic responses capable of mediating abscopal effects. This could also be because radiation dose and fractionation schedules for inducing the optimal antitumor immune response and for reducing toxicity are not well defined and may vary between tumors with different genomic alterations and immune contextures.¹⁹⁹ Clearly, evidence for development of systemically detectable antitumor T cells in radiotherapy-treated patients is tenuous,²⁶³ and abscopal responses are rarely seen in patients, as well as in tumor-bearing mice treated with radiation alone,²⁰⁹ suggesting that the immunostimulatory effects of radiation are often insufficient to generate robust antitumor immune responses that overcome existing and radiation-driven immunosuppressive barriers.

Immunosuppressive Effects of Radiation

Radiation causes DNA damage that activates several stress responses including a cytoprotective DDR.²⁶⁴ As previously described, the removal of cytosolic DNA fragments by TREX1 can limit the secretion of type I IFN by cancer cells.²⁰⁶ In this context, ATR serine/threonine kinase (ATR), a regulator of the DDR, has been shown to decrease IFN-I secretion by cancer cells, PD-L1 upregulation on tumor cells, and CD8⁺ T cell exhaustion. Enhancing DNA damage and accumulation of micronuclei by ATR pharmacological inhibition in combination

with radiation significantly increased radiation-mediated IFN-I responses and MHC-I antigen presentation in lung cancer cells, and potentiated CD8⁺ T cell activity in mouse models of KRAS-mutant cancer.^{265–267} Another DNA damage regulator, ATM serine/threonine kinase (ATM), has been shown to activate NF-κB signaling pathway in radioresistant cancer cells,²⁶⁸ resulting in the secretion of pro-inflammatory cytokines but limited STING-mediated type I IFN production.^{269,270} As a consequence of DDR or ROS generation by damaged mitochondria, cancer cells can also activate autophagy as a mechanism of defense in response to radiation,^{202,264} and radiation-induced autophagy can act as an immunosuppressive mechanism by removing the cytosolic sources of dsDNA including micronuclei^{271,272} and limiting CGAS-STING pathway activation.²⁷³ Moreover, CGAS-STING-dependent type I IFN production can be prevented by caspase 9 (CASP9) and CASP3, both of which are activated in cells succumbing to radiation-driven ICD.^{5,264,274,275} In particular, both *Casp9*^{−/−} and *Casp3*^{−/−} cells growing in immunocompetent mice are more sensitive to radiation and generated a superior abscopal response in combination with ICBs.^{212,275,276} In addition, CASP3 mediated prostaglandin E2 release by irradiated cancer cells, which is known to favor the establishment of an immunosuppressive tumor microenvironment.²⁷⁷

Radiation has been shown to upregulate expression of a number of growth factors and chemokines that promote the influx of myeloid cells into the tumor and foster their differentiation into immunosuppressive and tumor-promoting MDSCs and/or M2-polarized TAMs.³² In the MycCaP mouse model of prostate carcinoma, radiation increased CSF1 release by cancer cells, leading to a systemic increase in circulating MDSCs, which were recruited to the tumor. Response of the irradiated tumor was improved by administration of an inhibitor of CSF1R. Increased levels of CSF1 were also found in the serum of prostate cancer patients after radiotherapy.²⁷⁸ In a model of pancreatic ductal adenocarcinoma, radiation upregulated the chemokine CCL2, which increased the recruitment of Ly6C⁺CCR2⁺ monocytes and their differentiation into pro-tumorigenic and pro-angiogenic TAMs.²⁷⁹ Induction of TAMs in irradiated pancreatic tumors in mice also correlated to fewer CD8⁺ T cells and increased numbers of effector CD4⁺ T cells and T_{reg} cells.²⁸⁰ Genetic or antibody-mediated CCL2 blockade, neutralization of macrophage colony-stimulating factor, and treatment with anti-CCR2 antibody alleviated immunosuppression, improving tumor response to radiation.^{279–281} Interestingly, CCL2 was not induced by radiation in MycCaP prostate carcinomas, indicating that different tumors rely on different pathways to recruit myeloid cells in response to radiation.²⁷⁸ Another radiation-induced pathway that promotes immunosuppression involves the upregulation of hypoxia-inducible factor 1 and consequently vascular endothelial growth factor A (VEGFA),²⁸² which

promotes tumor infiltration by MDSCs and T_{reg} cells.²⁸³ In vivo treatment with an aptamer platform that targets radiation-induced VEGFA and 4-1BB (an immune-stimulatory receptor expressed on activated CD8⁺ T cells) potentiated both local tumor control and abscopal responses and reduced tumor-infiltrating T_{reg} cells.²⁸⁴ T_{reg} cells have also been shown to be more radioresistant than conventional T cells, resulting in a relative increase postradiation,^{285,286} although tumor-infiltrating effector T cells also appear to be more radioresistant than their circulating counterparts.¹³ Moreover, radiation promotes the expansion and enhances the suppressive function of suppressive tumor-infiltrating T_{reg} cells, characterized by higher expression of CTLA-4, CD137, and Helios, in several murine tumor models (B16/F10, RENCA, and MC38).²⁸⁷ T_{reg} cell depletion improved tumor control achieved by radiation in different cancer models.^{288,289} Finally, STAT3 inhibition decreased T_{reg} cells, MDSCs, and M2 macrophages in response to radiotherapy, but enhanced effector T cells and M1 macrophages, improving tumor growth delay in preclinical models of HNSCC.²⁸⁹

Many tumors are rich in TGF-β1 but the vast majority is bound to latency-associated peptide (LAP) and inactive. Radiation-induced ROS causes TGF-β1 dissociation from LAP and its activation,²⁹⁰ an event that plays a major role in inhibiting DC activation and priming of tumor-specific T cells. When TGF-β1 was neutralized by an antibody, radiation elicited T cell responses to multiple tumor antigens and abscopal effects in mouse models of metastatic breast cancer.²⁹¹ Abscopal responses were not documented in a prospective randomized clinical study testing systemic TGF-β blockade (fresolimumab) in two doses in the context of local hypofractionated radiation in a cohort of 23 patients with metastatic breast cancer. However, patients receiving the higher dose of fresolimumab showed changes consistent with decreased immunosuppression and improved CD8⁺ T cell memory and experienced longer median overall survival compared to the patients treated with the lower fresolimumab dose.²⁹²

In vivo upregulation of PD-L1 by radiation has been reported in cancer cells and infiltrating myeloid cells in murine tumors.^{205,234,241,291,293} In most cases, PD-L1 upregulation was driven by IFN-γ produced by radiation-induced antitumor T cells and acted as a barrier to tumor rejection.^{205,234} However, CASP9 activation in irradiated cells has also been mechanistically linked to PD-L1 upregulation.²⁷⁵ Other than PD-L1, the adenosine-generating enzyme CD73 and adenosine are upregulated in irradiated mouse and human breast cancer cells.²⁹⁴ The CD73-adenosine axis on tumor cells is well known to impair antitumor T cell responses²⁹⁵ and CD73 blockade with radiotherapy was able to restore DC infiltration in settings in which induction of IFN-I by radiotherapy alone was suboptimal.²⁹⁴

Other factors that may exert an immunosuppressive effect in the context of some tumors include the complement system, which was activated in a mouse model

of lymphoma and mediated fast clearance of apoptotic cells reducing the generation of inflammatory signals,²⁹⁶ as well as galectin-1 (as demonstrated in a mouse lung carcinoma model).²⁹⁷ In summary, radiation can elicit a variety of immunosuppressive mechanisms that, at least potentially, limit its therapeutic efficacy and thus offer valuable targets for the development of combinatorial therapeutic regimens (Figure 29.2).

POTENTIAL ROLE OF THE GENETIC BACKGROUND OF THE HOST

Accumulating clinical data indicate that the genetic background of cancer patients can influence disease outcome as it influences the ability of malignant cells to elicit an immune response following chemotherapy, targeted therapy, or radiation therapy.²⁹⁸ Loss-of-function single nucleotide polymorphisms (SNPs) affecting *TLR3* (encoding the receptor for cancer cell-derived ssRNA), *TLR4* (encoding the receptor for extracellular HMGB1), *P2RX7* (encoding the immunostimulatory receptor for extracellular ATP), and *FRP1* (encoding the receptor for extracellular ANXA1) have all been associated with reduced disease-free or overall survival in cohorts of breast carcinoma patients treated with neoadjuvant anthracycline-based chemotherapy.^{55,57,58,299} Along similar lines, SNPs in *FPR1* and *TLR4* have been linked to poor disease outcome in two independent cohorts of colorectal carcinoma patients.^{299,300} Moreover, a genetic variant of advanced glycosylation end-product specific receptor (*AGER*; another HMGB1 receptor best known as *RAGE*) has been associated with poor response to chemotherapy among NSCLC patients.³⁰¹ Finally, SNPs in *IDO1* and *CD24* (encoding an adhesion molecule involved in the interaction between DCs and T cells) have been linked to decreased 5-year survival rate in patients with resected colorectal liver metastases.³⁰² Altogether, these observations exemplify the potential effect of the genetic background of the host on the elicitation of anticancer immune responses by different treatment modalities.

CONCLUSION

Growing evidence indicates that the therapeutic effect of cytotoxic treatments is mediated by the interaction of stressed and dying cancer cells with the host immune system. Success likely depends on the balance between activating and suppressive signals generated by treatment, which is determined by the interaction of at least three factors: (1) the effects of therapy on cancer cells and immune cells; (2) the intrinsic immunogenicity of the cancer cells; and (3) germline polymorphisms in key immune players. Moreover, accumulating evidence supports a key role for the gut microbiota and a number of environmental and metabolic factors in the immunological fitness of the host and hence its capacity to mount

robust anticancer responses to treatment.^{303–306} Finally, some neoplasms (the so-called “cold tumors”) are able to prevent infiltration by immune cells compromising the immunostimulatory effects of chemotherapy, radiotherapy, and targeted therapy, and the efficacy of immunotherapy.^{5,307} To which degree cancer cell death and the disruption of the tumor microenvironment imposed by chemotherapy and/or radiotherapy can turn cold tumors into hot ones remains to be determined.

As previously mentioned, the immunogenicity of a tumor is determined by antigenicity and adjuvanticity. Some tumors are more antigenic due to the presence of large numbers of mutations, some of which will encode neoantigens.^{16,17} Others have mutations in MHC-I genes resulting in irreversible loss of expression.^{308–312} Oncogenic pathways activated in different tumors are emerging as important regulators of tumor adjuvanticity,^{313,314} and polymorphisms in genes encoding DAMP receptors such as *TLR4* and *P2RX7* affect immune cell activation.^{7,55,58} Each of these factors provides opportunities to improve treatment outcome by devising personalized combinations of agents and ultimately induces a robust and persistent antitumor T cell memory response that not only contributes to cancer control but provides life-long protection from recurrence in the patient.³¹⁵ Multiple clinical trials testing combinations of immune checkpoint inhibitors and other immunotherapies with chemotherapy,⁸⁶ targeted anticancer agents,^{316–319} or radiotherapy^{320,321} are ongoing. Research to improve our understanding of the immune effects of currently used drugs, agents that are still under development, and the various radiation doses and fractionation regimens employed is urgently needed to accelerate progress and achieve therapeutic success in ever more, and eventually all, cancer patients.

KEY REFERENCES

Only key references appear in the print edition. The full reference list appears in the digital product on Springer Publishing Connect: connect.springerpub.com/content/book/978-0-8261-3743-2/part/part02/chapter/ch29

5. Galluzzi L, Chan TA, Kroemer G, et al. The hallmarks of successful anticancer immunotherapy. *Sci Transl Med*. 2018;10(459):eaat7807. doi:10.1126/scitranslmed.aat7807
14. Rodriguez-Ruiz ME, Vanpouille-Box C, Melero I, et al. Immunological mechanisms responsible for radiation-induced abscopal effect. *Trends Immunol*. 2018;39(8):644–655. doi:10.1016/j.it.2018.06.001
26. Galluzzi L, Vitale I, Warren S, et al. Consensus guidelines for the definition, detection and interpretation of immunogenic cell death. *J Immunother Cancer*. 2020;8(1):e000337. doi:10.1136/jitc-2019-000337
219. Formenti SC, Rudqvist NP, Golden E, et al. Radiotherapy induces responses of lung cancer to CTLA-4 blockade. *Nat Med*. 2018;24(12):1845–1851. doi:10.1038/s41591-018-0232-2
258. McLaughlin M, Patin EC, Pedersen M, et al. Inflammatory microenvironment remodelling by tumour cells after radiotherapy. *Nat Rev Cancer*. 2020;20(4):203–217. doi:10.1038/s41568-020-0246-1