

Mechanism of Action of Conventional and Targeted Anticancer Therapies: Reinstating Immunosurveillance

Laurence Zitvogel,^{1,2,4,11} Lorenzo Galluzzi,^{5,6,11} Mark J. Smyth,^{7,8} and Guido Kroemer^{3,5,6,9,10,*}

¹INSERM, U1015, 94805 Villejuif, France

²Center of Clinical Investigations CBT507

³Metabolomics and Cell Biology Platforms

Institut Gustave Roussy, 94805 Villejuif, France

⁴Université Paris Sud/Paris XI, 94270 Le Kremlin Bicêtre, France

⁵Université Paris Descartes/Paris V, Sorbonne Paris Cité, 75006 Paris, France

⁶Equipe 11 labellisée par la Ligue Nationale contre le Cancer, Centre de Recherche des Cordeliers, 75006 Paris, France

⁷Immunology in Cancer and Infection Laboratory, Queensland Institute of Medical Research, Herston 4006, Queensland, Australia

⁸School of Medicine, University of Queensland, Herston 4006, Queensland, Australia

⁹INSERM, U848, 94805 Villejuif, France

¹⁰Pôle de Biologie, Hôpital Européen Georges Pompidou, AP-HP, 75015 Paris, France

¹¹These authors contributed equally to this work

*Correspondence: kroemer@orange.fr

<http://dx.doi.org/10.1016/j.immuni.2013.06.014>

Conventional chemotherapeutics and targeted antineoplastic agents have been developed based on the simplistic notion that cancer constitutes a cell-autonomous genetic or epigenetic disease. However, it is becoming clear that many of the available anticancer drugs that have collectively saved millions of life-years mediate therapeutic effects by eliciting *de novo* or reactivating pre-existing tumor-specific immune responses. Here, we discuss the capacity of both conventional and targeted anticancer therapies to enhance the immunogenic properties of malignant cells and to stimulate immune effector cells, either directly or by subverting the immunosuppressive circuitries that preclude antitumor immune responses in cancer patients. Accumulating evidence indicates that the therapeutic efficacy of several antineoplastic agents relies on their capacity to influence the tumor-host interaction, tipping the balance toward the activation of an immune response specific for malignant cells. We surmise that the development of successful anticancer therapies will be improved and accelerated by the immunological characterization of candidate agents.

Introduction

Over decades, the development of antineoplastic drugs has been based on the conviction that cancer would constitute a cell-autonomous genetic and epigenetic disease (Hanahan and Weinberg, 2011). Thus, chemotherapeutics have long been viewed as a sort of antibiotics for malignant cells, i.e., chemicals designed to preferentially target cancer cells and either limit their proliferation or—preferably—cause their death. Based on this consideration, academic and industrial drug developers generated agents that mediate cytostatic and/or cytotoxic effects *in vitro*, on cultured human tumor cells, tested them on human cancer xenografts growing in immunodeficient mice (a step rendered obligatory by the US National Cancer Institute in 1979) (Zitvogel et al., 2008), assessed their pharmacological and toxicological profiles (in preclinical models and in healthy individuals), and eventually tested their actual therapeutic potential in clinical trials.

This “pipeline” has allowed for the introduction of highly successful anticancer drugs into clinical practice, seemingly justifying its overall design. For instance, specific therapeutic regimens cure a very high fraction of patients affected by some hematological malignancies, such as acute lymphoblastic leukemia (Pui et al., 2009). Along similar lines, the use of anthracyclines and oxaliplatin for the (adjuvant) treatment of breast and

colorectal cancer patients, respectively, has saved millions of life-years and has significantly improved the long-term perspectives of these individuals (André et al., 2004; Poole et al., 2006). Finally, imatinib, which represents the first “targeted” anticancer agent ever developed to specifically inhibit oncogenic signaling cascades (including those driven by constitutively active ABL, KIT, and PDGFR), has dramatically prolonged the life of tens of thousands of patients with chronic myeloid leukemia (CML) or gastrointestinal stromal tumors (GISTs) (Corless et al., 2011; Druker et al., 2006).

Irrespective of these and other undeniable successes, the traditional pipeline for the development of antineoplastic agents is characterized by an enormous rate of attrition, meaning that the vast majority (more than 95%) of agents that have been selected in preclinical assays fails to exert robust therapeutic effects in phase I/II clinical trials (Ocana et al., 2011). Moreover, this traditional approach has been unable to identify drugs that would significantly affect the clinical course of common and quickly lethal malignancies such as lung and pancreatic cancer. It is therefore tempting to ask whether the theoretical basis underlying the current approach to the development of antineoplastic agents, namely, the vision of cancer as a purely cell-autonomous disease, is correct or—at least—practical. Indeed, this vision neglects the increasingly more accepted model

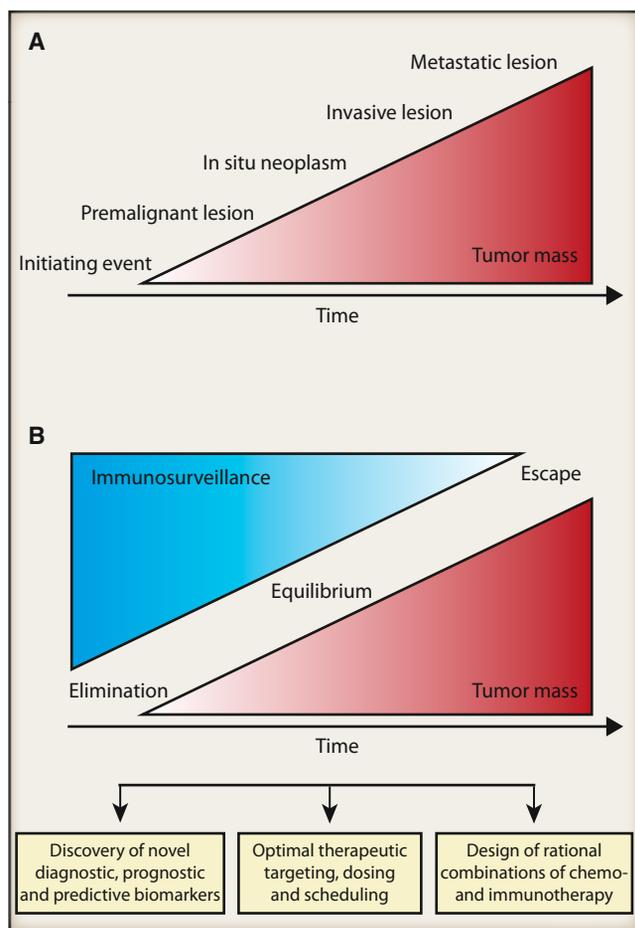


Figure 1. Opposed Views of Oncogenesis and Tumor Progression
 (A) Cancer has been viewed as a merely cell-intrinsic disease that develops and progresses because of the accumulation of genetic and epigenetic alterations.
 (B) According to the immunoeediting model, clinically manifest neoplasms evolve through three distinct steps of interaction with the immune system: (1) initially, malignant cells are recognized and eradicated by immune effector cells (elimination phase), (2) at a later stage, small tumors are still held in check by (increasingly less proficient) immune responses (equilibrium phase), and (3) finally, neoplastic cells lose their antigenic properties or establish potent immunosuppressive networks, thus avoiding any control by the immune system (escape phase). The in-depth knowledge of the mutual interactions between developing tumors and the immunosurveillance system will allow for the identification of novel diagnostic, prognostic, and predictive biomarkers, the discovery of novel targets and the optimization of therapeutic protocols (in terms of doses and schedules), and the design of rational therapeutic regimens combining chemo- and immunotherapeutic agents.

postulating that malignant cells are initially held in check by the immune system (a control mechanism that is generally referred to as immunosurveillance) and can grow into clinically manifest tumors only if they lose the immunogenic determinants that make them recognizable by immune effectors (immunoselection) or if they actively inhibit immune responses (immunosuppression) (Finn, 2008; Schreiber et al., 2011). Furthermore, the therapeutic effects of the most successful anticancer agents originate, at least in part, from elicitation of novel or the reactivation of pre-existing antitumor immune responses (Galluzzi et al., 2012c; Zitvogel et al., 2011).

Here, we evaluate the capacity of cytotoxic chemotherapeutics and targeted anticancer agents that are currently employed in clinical practice to reinstate immunosurveillance. In particular, we differentiate between the direct immunogenic effects that such therapeutic regimens exert on tumor cells and their capacity to interact with the host immune system, resulting in the reactivation of immune effectors or in the relief of immunosuppressive mechanisms. Most knowledge in this domain stems from preclinical studies. Nonetheless, we briefly evaluate clinical evidence supporting the notion that several successful anticancer drugs mediate therapeutic effects by reinstating immunosurveillance.

Clinical Evidence for Therapy-Induced Immunosurveillance

The observation that cancer develops more frequently and more aggressively in immunodeficient, as opposed to immunocompetent, mice (Vesely et al., 2011) is paralleled by epidemiological studies showing that transplant recipients (which are subjected to chronic pharmacological immunosuppression) exhibit an increased incidence of various neoplasms, including tumors that a priori do not have a viral etiology (Bererhi et al., 2012; Tjon et al., 2010; von Boehmer et al., 2012). These epidemiological observations indicate that the immunosurveillance theory, originally referring to mouse models of immunodeficiency (which are usually far more severe than human immunodeficiency syndromes) (Vesely et al., 2011), does apply to human tumors, too. The “three E” theory postulates that premalignant lesions are generally eliminated by immune effectors, that small tumors are in equilibrium with an ongoing (but increasingly less proficient) anticancer immune response, and that neoplastic cells finally escape from the immune control and form sizeable lesions (Figure 1; Schreiber et al., 2011). Hence, tumors are usually diagnosed at the escape stage, which can occur by two distinct, yet not mutually exclusive, mechanisms: (1) the selection of malignant cells that have become unrecognizable by the immune system (Matsushita et al., 2012; Senovilla et al., 2012) and (2) the induction of a plethora of local and systemic immunosuppressive mechanisms, encompassing the secretion of specific cytokines such as interleukin-10 (IL-10) and the accumulation of immunomodulatory cell types including FOXP3⁺ regulatory T (Treg) cells, myeloid-derived suppressor cells (MDSCs), and M2 macrophages (Coussens et al., 2013).

Robust evidence indicates the naturally occurring, pretherapy anticancer immune responses significantly influence disease progression in cancer patients. Thus, several parameters of the immune infiltrate at diagnosis have been shown to correlate with prognosis (and hence with the outcome of conventional anticancer therapies) in patients affected by distinct types of tumors (Fridman et al., 2012). In colorectal cancer (the most extensively characterized tumor in this respect), the density, composition, function, and architecture of immune cells infiltrating primary lesions as well as metastatic sites, the so-called tumor immune contexture, predict patient survival more accurately than any other parameter, including the conventional tumor node metastasis (TNM) classification (Galon et al., 2006; Mlecnik et al., 2011). Along similar lines, an immunological score based on the abundance of CD8⁺ T cells surrounding colorectal cancer hepatic metastases has been shown to reliably predict

chemosensitivity (Halama et al., 2011). Some successful regimens used for the treatment of colorectal cancer (such as the combination of 5-fluorouracil with oxaliplatin or irinotecan) can reduce the frequency of circulating Treg cells (Maeda et al., 2011), thereby facilitating the elicitation of a primary or the boosting of a secondary anticancer immune response. However, this does not formally demonstrate whether local tumor-specific immunity is elicited by chemotherapy. This enigma has been resolved in the setting of breast carcinoma (BC).

The frequency of Treg cells infiltrating BC biopsies predicts the long-term fate of patients receiving postoperative (adjuvant) chemotherapy (Bates et al., 2006). FOXP3⁺ cells surrounding neoplastic lesions appear to be particularly active, and their abundance predicts disease relapse better than that of FOXP3⁺ cells infiltrating the tumor bed (Gobert et al., 2009). Along similar lines, the infiltration of primary BC lesions by cells displaying a CD4^{lo}CD8^{hi}CD68^{lo} profile (CD68 is a marker of macrophages and plasmacytoid dendritic cells) is positively associated with overall and relapse-free survival, irrespective of lymph node involvement (DeNardo et al., 2011). The independent prognostic value of intratumoral CD8⁺ T cell counts in BC patients has been confirmed in additional studies (Mahmoud et al., 2011). Moreover, the amount of hematoxylin/eosin-detectable lymphocytes infiltrating BC lesions at diagnosis constitutes an independent predictive biomarker for the success of induction (neo-adjuvant) chemotherapy, measured in terms of pathological complete responses (pCRs) (Denkert et al., 2010; Loi et al., 2013a; West et al., 2011). Accordingly, the expression of a set of immune function-related genes (including several genes associated with Th1 cells and interferon responses) or that of the constant chain of immunoglobulin κ (IGKC) has been shown to predict pCRs in patients affected by all major BC subtypes, thereby increasing the predictive power of clinicopathological parameters (Ignatidis et al., 2012; Schmidt et al., 2012).

Several longitudinal studies have addressed the impact of chemotherapy on the immune cells infiltrating BC lesions. Thus, paclitaxel-based neoadjuvant chemotherapy has been shown to increase the frequency of HE-detectable BC-infiltrating lymphocytes, more so in patients who responded to chemotherapy (Demaria et al., 2001). Moreover, accumulating evidence indicates that chemotherapy stimulates the infiltration of BCs by myeloid and granzyme B-expressing cells while increasing the intratumoral CD8⁺ to CD4⁺ T cell ratio (Ruffell et al., 2012). Of note, an elevation in the intratumoral CD8⁺ to FOXP3⁺ T cell ratio after one single cycle of anthracycline-based neoadjuvant chemotherapy predicts the pCR to the entire chemotherapeutic regimen (six cycles) (Ladoire et al., 2011; Senovilla et al., 2012). These results may indicate that chemotherapy-elicited immune responses are ultimately responsible for tumor eradication.

Immunological parameters also influence the fate of patients receiving imatinib or similar tyrosine kinase inhibitors. For instance, several biomarkers of natural killer (NK) cell activation associate with the long-term therapeutic effects of imatinib in GIST patients. These biomarkers include the expression of specific isoforms of the NK cell-activating receptor NKp30 (Delahaye et al., 2011), the imatinib-induced production of interferon- γ (IFN- γ) by circulating NK cells (Borg et al., 2004; Ménard et al., 2009), as well as the frequency of tumor-infiltrating NK cells (Rusakiewicz et al., 2013). Moreover, GIST infiltration by T cells

constitutes an independent prognostic marker and the prolonged administration of imatinib is associated with the loss of MHC class I variants by malignant cells, presumably constituting the result of T cell-mediated immunoeediting (Rusakiewicz et al., 2013). In CML patients, imatinib-based therapeutic regimens induce robust T cell responses as well as the secretion of tumor-specific circulating IgMs (Catellani et al., 2011; Chen et al., 2008).

Altogether, these clinical findings suggest that—at least in some settings—tumor-specific immune responses dictate the fate of cancer patients. This notion is supported by ample preclinical evidence indicating that transplantable, chemically induced as well as oncogene-driven mouse cancers respond more efficiently to a vast range of therapeutic modalities in immunocompetent, as opposed to immunodeficient, hosts (Table 1). A comprehensive compendium of chemotherapeutic agents that mediate immunostimulatory effects can be found in Galluzzi et al. (2012c) and Vanneman and Dranoff, (2012). As discussed below, successful anticancer therapies can reinstate immunosurveillance by modifying the propensity of malignant cells to elicit an immune response or by exerting direct immunostimulatory effects (Figure 2).

Immunostimulation via Effects on Cancer Cells

Tumor debulking by surgery, radiotherapy, or chemotherapy obviously reduces the systemic immunosuppressive activity of malignant cells (Zitvogel et al., 2011). Beyond such a general effect, clinically employed antineoplastic agents may stimulate immunosurveillance by acting on cancer cells in several ways, for example (1) by increasing the expression or presentation of tumor-associated antigens on the surface of cancer cells (antigenicity), (2) by causing tumor cells to emit danger signals that stimulate innate or cognate immune responses by operating as adjuvants (immunogenicity), or (3) by augmenting the propensity of tumor cells to be recognized and killed by immune effectors (susceptibility) (Figure 3).

Enhanced Antigenicity

Cisplatin and gemcitabine broaden the range of tumor antigens eliciting cytotoxic T lymphocyte (CTL) responses in vivo. Thus, untreated ovalbumin-expressing mesotheliomas growing in syngeneic C57BL/6 mice initially elicited an immune response against the dominant epitope SIINFEKL that extended to a broad spectrum of antigenic peptides upon chemotherapy, a phenomenon known as “epitope spreading” (Jackaman et al., 2012). Conversely, some antineoplastic agents such as cyclophosphamide, gemcitabine, oxaliplatin, paclitaxel, and γ irradiation are known to exacerbate the antigenicity of cancer cells by increasing the expression of MHC class I molecules (Chen and Emens, 2013; Liu et al., 2010; Reits et al., 2006). Many chemotherapeutics including cisplatin, etoposide, paclitaxel, topotecan, and vinblastine stimulate tumor cells to produce IFN- β , which in turn operates as an autocrine factor to stimulate MHC class I expression (Wan et al., 2012). Moreover, several anticancer agents can specifically upregulate the expression of tumor-associated antigens, including carcinoembryonic antigen (which is responsive to 5-fluorouracil), various cancer testis antigens (which are upregulated by 5-aza-2'-deoxycytidine and γ irradiation), and melanoma-associated antigens (which respond to the BRAF inhibitor vemurafenib) (Chen and Emens, 2013; Frederick et al., 2013; Sharma et al., 2011).

Table 1. Examples of FDA-Approved Anticancer Agents whose Efficacy Is Reduced by Immune Deficiencies

Agent	Tumor	Immune Defects	Reference
5-fluorouracil	EL4 lymphomas	<i>Nu/Nu</i> genotype	Vincent et al., 2010
anthracyclines	CT26 colorectal carcinomas, MCA205 fibrosarcomas, MCA-induced tumors	<i>Nu/Nu</i> genotype, depletion of CD8 ⁺ or γ/δ T cells, blockade of CD11b, neutralization of IL-1, IL-17, or IFN- γ	Apetoh et al., 2007b; Casares et al., 2005; Ghiringhelli et al., 2009; Ma et al., 2011, 2013; Mattarollo et al., 2011; Obeid et al., 2007
ATRA \pm arsenic trioxide	murine APLs	SCID phenotype	Westervelt et al., 2002
arsenic trioxide	CT26 colorectal cancers	<i>Nu/Nu</i> genotype	Thomas-Schoemann et al., 2012
cisplatin + digoxin	MCA205 fibrosarcomas	<i>Nu/Nu</i> genotype	Menger et al., 2012
cyclophosphamide	AB1-HA mesotheliomas	<i>Ifngr2^{-/-}</i> , <i>Tnfsf10^{-/-}</i> , depletion of CD8 ⁺ or NK cells	van der Most et al., 2009
dasatinib	P815 mastocytomas	depletion of CD4 ⁺ or CD8 ⁺ T cells	Yang et al., 2012
gemcitabine	AB12 mesotheliomas, EJ-6-2 fibrosarcomas, EL4 lymphomas, TC1 insulinomas	<i>Nu/Nu</i> genotype	Suzuki et al., 2007; Vincent et al., 2010
imatinib	AK7 mesotheliomas, B16 melanomas, RMA-S lymphomas	depletion of NK cells	Borg et al., 2004
	GISTs developing in <i>Kit^{V558/+}</i> mice	<i>Rag1^{-/-}</i> , depletion of CD8 ⁺ T cells	Balachandran et al., 2011
mitomycin C + digoxin	MCA205 fibrosarcomas	<i>Nu/Nu</i> genotype	Menger et al., 2012
oxaliplatin	CT26 colorectal carcinomas, MCA205 fibrosarcomas	<i>Nu/Nu</i> genotype	Michaud et al., 2011; Tesniere et al., 2010
paclitaxel	<i>Ret</i> -driven melanomas	depletion of CD8 ⁺ T cells	Sevko et al., 2013
PLX4720 (BRAF inhibitor)	SM1WT1 melanomas	<i>Ccr2^{-/-}</i> , <i>Ifng^{-/-}</i> <i>Prf1^{-/-}</i> , depletion of CD8 ⁺ T cells	Knight et al., 2013

Abbreviations are as follows: APL, acute promyelocytic leukemia; ATRA, all-*trans* retinoid acid; IFN, interferon; IL, interleukin; MCA, 3-methylcholanthrene; NK, natural killer; SCID, severe combined immunodeficient.

Irradiation can greatly alter the repertoire of MHC class I-restricted peptides that are expressed on the surface of malignant cells, an effect that may stem—at least in part—from mammalian target of rapamycin (mTOR)-transduced signals (Reits et al., 2006). This is in line with the fact that the inhibition of mTOR induces major alterations in the composition of the MHC class I immunopeptidome (Caron et al., 2011). MHC class I-associated peptides preferentially derive from so-called defective ribosomal products (DRiPs), i.e., short, abortive polypeptides that frequently originate from the translation of microRNA (miRNA)-bound mRNAs (Granados et al., 2012). This suggests that the probability of a given peptide to be presented on the surface of tumor cells depends on (1) the primary sequence of the corresponding gene (which is often mutated in cancer cells), (2) the abundance of the corresponding transcript, and (3) the levels of regulatory miRNAs. Thus, chemotherapy-induced modifications in the expression of mRNAs and miRNAs (Salmena et al., 2011) may profoundly influence the antigenicity of malignant cells. This hypothesis warrants further in-depth evaluation.

Improved Immunogenicity

In response to chemotherapeutics, malignant cells can dispatch a series of signals to alert not only their neighbors but also the entire organism of incipient danger. Thus, cancer cells that succumb to antineoplastic agents emit an entire spectrum of cell death-associated molecules (CDAMs), some of which exert potent adjuvant effects (Galluzzi et al., 2012a; Zitvogel et al., 2010). For a long time, apoptosis has been considered as an

immunologically silent (if not tolerogenic) cell death mode, whereas necrosis was ascribed with proinflammatory and immunogenic properties. In contrast to this dogma, however, some instances of apoptosis—such as those induced by radiotherapy and some chemotherapeutics—appears to be more efficient than necrosis—as induced by freeze-thawing or osmotic lysis—at eliciting tumor-specific adaptive immune responses, reflecting the fact that the clearance of apoptotic and necrotic cells in vivo is rather different (Kroemer et al., 2013; Krysko and Vandenabeele, 2010; Ravichandran, 2011). On the other hand, the robust inflammatory responses associated with necrosis may even exert protumorigenic effects (Coussens et al., 2013). This said, cryoablation-induced necrosis has recently been shown to yield robust antitumor immunity if accompanied by appropriate immunostimulatory measures (such as the neutralization of cytotoxic T lymphocyte antigen 4 [CTLA4]) (Waitz et al., 2012), implying that neither of the main cell death modalities is intrinsically tolerogenic (Galluzzi et al., 2012e). Thus, the capacity of dying and dead cancer cells to elicit a specific immune response appears to be dictated by CDAMs rather than by intrinsic features of apoptosis and necrosis. A number of CDAMs exposed on the surface of dying cells or released into their microenvironment, including calreticulin (CRT), ATP, high mobility group box 1 (HMGB1), and several other factors, exert potent immunostimulatory effects.

CRT is an abundant chaperone of the endoplasmic reticulum (ER) that can be exposed on the cell surface in response to

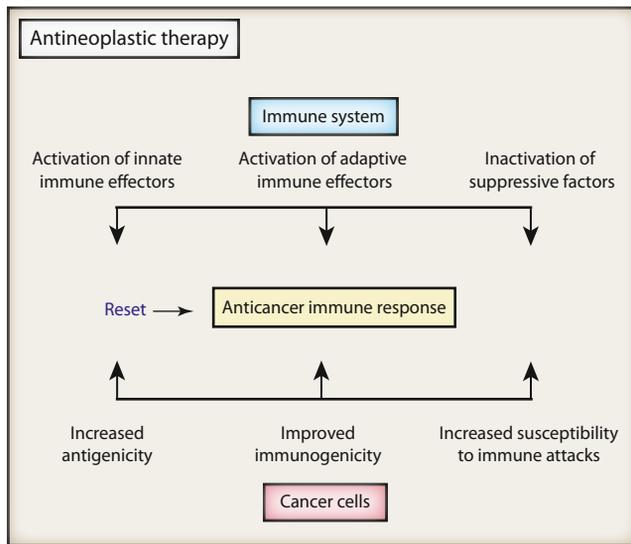


Figure 2. Effects of Conventional and Targeted Antineoplastic Agents on Tumor-Specific Immune Responses

As it depletes effector and suppressor immune cells, chemotherapy “resets” the immune system, thereby establishing a new immune repertoire. In addition, chemotherapy can mediate a multipronged immunostimulatory effect, thereby reinstating anticancer immunosurveillance. On one hand, antineoplastic agents can increase the antigenicity of malignant cells, improve their immunogenicity, and augment their susceptibility to immune attacks. On the other hand, chemotherapy can directly promote the activation of immune effector cells and/or inhibit immunosuppressive networks.

multiple perturbations of reticular homeostasis. The CRT exposure pathway elicited by anthracyclines in tumor cells is complex and relies on paracrine signals (for the most part conveyed by chemokines such as IL-8), as well as on the activation of a multipronged signal transduction cascade. Such signaling pathway involves (1) the phosphorylation of eukaryotic initiation factor 2 α (eIF2 α) by the ER stress-sensing kinase PKR-related ER kinase (PERK), (2) the activation of components of the apoptotic pathway (including caspase-8, BAX, and BAK), (3) the anterograde transport of ER-derived vesicles through the Golgi apparatus, and (4) the SNAP receptor (SNARE)-dependent exocytosis of these vesicles (Panaretakis et al., 2009; Sukkurwala et al., 2013). Other stimuli (e.g., hypericin-based photodynamic therapy) can cause CRT exposure through a distinct, more rapid pathway that relies on PERK but not on caspase-8 (Garg et al., 2012). Once on the cell surface, CRT serves as an “eat-me” signal, stimulating the engulfment of dying tumor cells and their apoptotic debris by macrophages and immature dendritic cells (DCs) (Gardai et al., 2005; Ma et al., 2013; Obeid et al., 2007).

Chemotherapeutic agents that are able to trigger immunogenic cell death (ICD), i.e., a functionally peculiar type of cellular demise that can stimulate protective anticancer immune responses (Kroemer et al., 2013), are also efficient inducers of CRT exposure. This applies to anthracyclines (e.g., doxorubicin, mitoxantrone), oxaliplatin, and mafosfamide, the active metabolite of cyclophosphamide (Obeid et al., 2007; Schiavoni et al., 2011; Tesniere et al., 2010). Conversely, antineoplastic agents that fail to induce CRT exposure (e.g., cisplatin, mitomycin C) are intrinsically incapable of provoking ICD and thus promote suboptimal therapeutic effects. Structurally related

agents such as oxaliplatin and cisplatin differ in their ability to induce CRT exposure and ICD. Whereas oxaliplatin can trigger an ER stress response leading to CRT exposure independently from its effects on nuclear DNA (Panaretakis et al., 2009), cisplatin is a relatively weak inducer of ER stress (Tesniere et al., 2010). Thus, cytoplasmic off-target effects may determine the differential capacity of DNA-damaging agents to induce ICD. Salubrinal (Obeid et al., 2007), thapsigargin (Martins et al., 2011), and cardiac glycosides (Menger et al., 2012) have been successfully employed to restore CRT exposure (they all operate as ER stress inducers), thereby improving the therapeutic profile of cisplatin or mitomycin C. Of note, malignant cells that have been depleted of CRT by RNA interference (RNAi) generate lesions that fail to respond to chemotherapy *in vivo*, unless recombinant CRT is exogenously provided *in vivo* (Panaretakis et al., 2009). Conversely, cancer cells engineered to express a variant of CRT that is constitutively exposed on the cell surface are intrinsically immunogenic and can form tumors *in vivo* only if they lose CRT expression (Senovilla et al., 2012). These results underscore the important contribution of cell surface-exposed CRT to anticancer immune responses.

ATP molecules released by dying cells constitute a potent chemotactic signal for myeloid cells including monocytes/macrophages (Elliott et al., 2009) and DC precursors (Ma et al., 2013). Cancer cells respond to ICD inducers by secreting ATP through a mechanism that involves the caspase-dependent activation of pannexin 1 channels, lysosomal exocytosis, and plasma membrane blebbing (Elliott et al., 2009; Martins et al., 2013). The upregulation of autophagy is required for ATP release by dying cancer cells (Michaud et al., 2011), presumably because autophagy maintains high ATP concentrations within autophagosomes in the course of stress responses (Martins et al., 2013). Thus, autophagy-deficient tumors exposed to chemotherapy are unable to attract tumor-infiltrating leukocytes and therefore fail to induce therapeutic anticancer immune responses (Michaud et al., 2011). Of note, autophagy is frequently disabled during early oncogenesis (Morselli et al., 2009; White, 2012), perhaps helping incipient tumors to evade immunosurveillance.

The transfection-enforced expression of CD39 (an ectonucleotidase that degrades ATP to ADP and AMP) on tumor cells abolishes the therapeutic activity of multiple antineoplastic agents (Michaud et al., 2011, 2012). Another ectonucleotidase, CD73, can convert AMP into adenosine, which exerts prominent immunosuppressive effects (Beavis et al., 2012). The pharmacological inhibition of CD39 improves the response to chemotherapy of autophagy-deficient tumors, presumably by increasing pericellular ATP concentrations and thereby restoring immune infiltration (Michaud et al., 2011). Similarly, CD73-null mice exhibit improved antitumor immune responses and are relatively resistant to the development of metastases (Beavis et al., 2012; Stagg et al., 2011), an effect that appears to involve both hematopoietic and nonhematopoietic cell compartments (Stagg et al., 2011). In line with these notions, high expression levels of CD39 and CD73 correlate with poor disease outcome in patients affected by chronic lymphocytic leukemia (Pulte et al., 2011), colorectal carcinoma (Wu et al., 2012), and estrogen receptor-negative, progesterone receptor-negative, and HER2-negative (triple negative) BC. In this latter context, robust CD73 expression

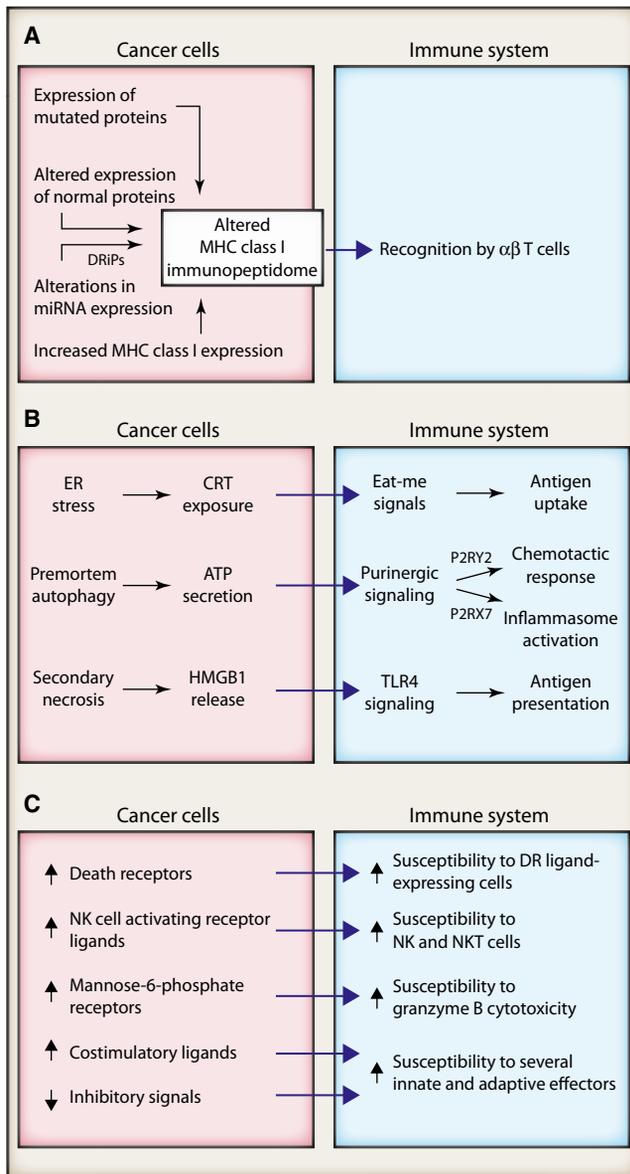


Figure 3. Effects of Anticancer Agents on Tumor Antigenicity, Immunogenicity, and Susceptibility to Immune Attacks

(A) Conventional chemotherapeutics as well as targeted anticancer agents can stimulate the expression of MHC class I molecules and expand the range of antigenic epitopes exposed on the surface of malignant cells (“epitope spreading”). This alters the MHC class I immunopeptidome of cancer cells and thereby increases their propensity to be recognized by $\alpha\beta$ T cells.

(B) Several distinct antineoplastic agents improve the immunogenicity of malignant cells as they stimulate them to emit various immunostimulatory signals, including calreticulin (CRT), ATP, and high mobility group box 1 (HMGB1). Upon binding to specific receptors on the surface of dendritic cells and other cells of the immune system, these signals promote the uptake, processing, and presentation of antigens, favor chemotaxis, and stimulate the secretion of immunostimulatory cytokines.

(C) Chemotherapy can alter the surface proteome of cancer cells so that they become more susceptible to the cytotoxic activity of several innate and adaptive immune effectors. Abbreviations are as follows: DR, death receptor; DRIps, defective ribosomal products; ER, endoplasmic reticulum; NK, natural killer; NKT, natural killer T; TLR4, Toll-like receptor 4.

appear to limit the efficacy of anthracycline-based neoadjuvant therapy (Loi et al., 2013b).

Extracellular ATP acts on purinergic receptors of distinct types, including metabotropic P2RY2 and ionotropic P2RX7 receptors. P2RY2-conveyed signals are required for the recruitment, persistence, and differentiation of DC precursors and neutrophils into the tumor bed (Elliott et al., 2009; Ma et al., 2013). In addition, by binding P2RX7 receptors on the surface of DCs, ATP stimulates the NLRP3 inflammasome-dependent production of IL-1 β , thereby skewing the polarization of subsequent T cell responses toward a cytotoxic T_C1 pattern (Ghiringhelli et al., 2009). Antibodies that neutralize IL-1 β as well as recombinant IL-1 receptor antagonists abolish the efficacy of anticancer chemotherapy and prevent tumor infiltration by IL-17-producing $\gamma\delta$ T cells and IFN- γ -producing CD8⁺ $\alpha\beta$ T cells (Ghiringhelli et al., 2009; Ma et al., 2011; Mattarollo et al., 2011). Moreover, anthracycline-treated BC patients with a loss-of-function P2RX7 allele have been shown to develop metastases more rapidly than individuals bearing two normal copies of the gene (Ghiringhelli et al., 2009). Taken together, these observations suggest that ATP and purinergic receptor signaling play a decisive role in chemotherapy-elicited anticancer immune responses.

HMGB1 is a nonhistone chromatin-binding factor that can be released by dead cells through permeabilized nuclear and plasma membranes. Perhaps depending on posttranslational modifications including redox-dependent changes (Kazama et al., 2008; Venereau et al., 2012), extracellular HMGB1 can interact with several distinct pattern recognition receptors, including advanced glycosylation end product-specific receptor (AGER) and Toll-like receptor 4 (TLR4) (Bianchi, 2009). In the context of anticancer chemotherapy, the binding of extracellular HMGB1 to TLR4 on the surface of DCs stimulates a MYD88 (but not TRIF)-dependent signaling pathway, leading to optimal antigen presentation (Apetoh et al., 2007b). Presumably, this is due to the fact that TLR4 signals prevent the premature lysosomal degradation of engulfed apoptotic debris and tumor-associated antigens (Apetoh et al., 2007b). Tumors that lack HMGB1 and evolve in normal hosts, as well as tumors that do express HMGB1 and grow in *Tlr4*^{-/-} or *Myd88*^{-/-} mice, fail to respond to anthracycline-based chemotherapy (Apetoh et al., 2007b; Yamazaki et al., 2013). In this context, therapeutic responses can be restored by a variety of strategies, including the provision of alternative DC activation signals (e.g., the TLR3 agonist poly(I:C), semisynthetic TLR4 agonists such as dendrophilin A and S) and the short-term inhibition of lysosomal degradation with chloroquine (Apetoh et al., 2007b; Yamazaki et al., 2013).

BC progression is often accompanied by the loss of tumoral HMGB1 expression (Yamazaki et al., 2013), a trait that has been correlated with poor survival in esophageal squamous cell carcinoma patients (Suzuki et al., 2012). Along similar lines, a loss-of-function *TLR4* allele has been shown to negatively affect the prognosis of BC patients receiving adjuvant anthracyclines (Apetoh et al., 2007b) and stage II colorectal cancer patients treated with oxaliplatin-based chemotherapy (Tesniere et al., 2010). Thus, HMGB1 and TLR4 play a major role in the elicitation of anticancer immune responses.

Anticancer agents such as cyclophosphamide, oxaliplatin, and gemcitabine can induce tumor cells to emit signals that stimulate DC maturation (Liu et al., 2010; Schiavoni

et al., 2011). In this setting, the depletion of CRT or HMGB1 usually does not prevent DC maturation (L.Z. and G.K., data not shown), pointing to the existence of alternative players in this process. These factors may include HSP90, which is exposed on the surface of myeloma cells responding to the proteasomal inhibitor bortezomib (Spisek et al., 2007), as well as several mitochondrial products that are released upon necrotic plasma membrane permeabilization (Galluzzi et al., 2012a). Several antineoplastic agents have been shown to induce the differentiation of DC precursors and/or to promote DC maturation, including anthracyclines (van de Ven et al., 2012), vinca alkaloids (Tanaka et al., 2009), and other agents (Kaneno et al., 2009). Whether these effects require a fraction of cells to die (thereby releasing factors that stimulate DC differentiation or maturation) has not been clarified. In response to anthracycline-based chemotherapy, DC precursors have been shown to infiltrate the tumor bed, localize in the close proximity of nests of dying cancer cells, and mature (Ma et al., 2013). This may be critical for the re-establishment of immunosurveillance, as indicated by the fact that tumor-infiltrating immature DCs expressing PD-L1 have been involved in local immunosuppressive networks (Engelhardt et al., 2012; Krempski et al., 2011).

Increased Susceptibility to Immune Attacks

There are multiple mechanisms through which antineoplastic agents can increase the susceptibility of malignant cells to the cytotoxic activity of immune effectors.

A large panel of chemotherapeutics including most DNA-damaging agents has been shown to stimulate the expression of death receptors, including FAS (also known as CD95) and TNF-related apoptosis-inducing ligand (TRAIL) receptors 1 and 2 (TRAIL-R1 and TRAIL-R2), on the surface of tumor cells. In the presence of their ligands, death receptors elicit an intracellular signaling cascade leading to apoptotic or necrotic cell death (Vandenabeele et al., 2010). Hence, chemotherapy may sensitize cancer cells to the induction of cell death by FAS ligand (FASL) or TRAIL, which are produced by a variety of immune effectors (Ashkenazi and Dixit, 1998; Hellwig and Rehm, 2012). TRAIL appears to induce immunogenic cell death (Panaretakis et al., 2009), thereby amplifying ongoing anticancer immune responses. FAS signaling also favors the secretion of multiple cytokines and chemokines, including CXCL1, CCL2, IL-6, and IL-8. IL-8 not only promotes an ATP-independent chemotactic response of phagocytes toward apoptotic cells (Cullen et al., 2013), but also stimulates CRT exposure (Sukkurwala et al., 2013). Therefore, it is possible that death receptor signaling can induce hallmarks of immunogenic cell death indirectly, via the production of IL-8.

A range of different stress conditions can stimulate the exposure of ligands for NK cell-activating receptors (e.g., NKG2D, DNAM-1) on the surface of malignant cells (Chan et al., 2010; Raulet et al., 2013). In doing so, distinct DNA-damaging agents (Soriani et al., 2009), histone deacetylase inhibitors (Berghuis et al., 2012), and lenalidomide (Benson et al., 2011) reportedly increase the susceptibility of cancer cells to lysis by specific lymphocyte populations (mostly NK, NKT, and $\gamma\delta$ T cells) (Chan et al., 2010; Raulet et al., 2013). Whether this mechanism contributes to the eradication of neoplastic cells responding to chemotherapy *in vivo* remains to be elucidated.

Various anticancer agents including paclitaxel, cisplatin, and doxorubicin have been shown to sensitize mouse cancer cell lines to the cytotoxic functions of CTLs by increasing the expression of mannose-6-phosphate receptor (M6PR) on the cell surface. M6PR augments the permeability of the plasma membrane to granzyme B, one of the main CTL effector molecules, and thereby renders tumor cell killing by CTLs independent from perforin (Ramakrishnan et al., 2010). In line with this notion, perforin is not absolutely required for chemotherapy to exert optimal antineoplastic effects (Kroemer et al., 2013), in particular in models in which the therapeutic outcome clearly depends on CD8⁺ T cells (Ghiringhelli et al., 2009). The redistribution of M6PR to the surface of neoplastic cells exposed to chemotherapy appears to be linked to autophagy, as indicated by the fact that the siRNA-mediated knockdown of the essential autophagic mediator Atg5 abolishes this effect (Ramakrishnan et al., 2012). Thus, autophagy may be required for optimal chemotherapeutic responses not only as it promotes the emission of a chemotactic and immunostimulatory signal (ATP, see above), but also as it increases the susceptibility of tumor cells to lysis by CTLs.

Chemotherapy can induce the expression of costimulatory molecules such as CD80 (also known as B7-1) on the surface of malignant cells or downregulate immunosuppressive molecules such as PDL1, PDL2, and VTCN1 (also known as B7-H1, B7-DC, and B7-H4, respectively) (Chen and Emens, 2013). This is the case of lenalidomide, which has been shown to upregulate various costimulatory molecules (i.e., CD80, CD83, CD86) on the surface of tumor cells while downregulating PDL1 (Chanan-Khan et al., 2012), and various platinum derivatives, which reportedly reduce the cell surface expression of PDL2 by activating STAT6 (Lesterhuis et al., 2011).

GISTs are often driven by gain-of-function mutations in the gene coding for the tyrosine kinase KIT and therefore can be treated with imatinib. The administration of imatinib to GIST-bearing mice results in the downregulation of indoleamine 2,3-dioxygenase, an enzyme that catalyzes the synthesis of kynurenine. Because kynurenine constitutes an obligate trophic factor for Treg cells, imatinib limits tumor infiltration by these immunosuppressive cells and hence promotes anticancer immune responses mediated by T and NK lymphocytes (Balachandran et al., 2011; Rusakiewicz et al., 2013).

Taken together, these observations indicate that successful antineoplastic agents can elicit therapeutic immune responses as they increase the antigenicity of malignant cells, their immunogenicity, or their susceptibility to effector mechanisms.

Effects of Anticancer Agents on the Immune System

Many anticancer agents are clinically employed at their maximum tolerated dose, at which they can exert potent myelosuppressive and/or lymphoablative side effects, most often resulting in a transient state of systemic immunosuppression. Such regimens can deplete both effector and suppressor cells and can impose the creation of new immune repertoires (Finn, 2012), thus “resetting” the immune system. Nevertheless, anticancer agents used at clinically efficient doses (which are usually well below the maximum tolerated dose) may mediate rapid immunostimulatory effects (Galluzzi et al., 2012c). For instance, the vaccination of cancer patients receiving standard-of-care chemotherapy can result in vigorous immune responses

(Lesterhuis et al., 2010; Rousseau et al., 2012), contradicting the notion that chemotherapeutics invariably promote severe immunosuppression. Moreover, the results of multiple clinical studies suggest that the CTLA4-targeting antibody ipilimumab can be advantageously combined with various antineoplastic agents such as fotemustine and temozolomide (for the treatment of metastatic melanoma patients) or paclitaxel plus carboplatin (in individuals bearing non-small-cell lung carcinoma) (Lynch et al., 2012; Maio et al., 2013). Obviously such combinatorial treatments would not be effective if chemotherapy resulted in severe immunosuppression.

Paradoxically, the two most commonly used taxanes, paclitaxel and docetaxel, have been shown to mediate immunostimulatory effects in mice, hence increasing immune responses against the model antigen ovalbumin or an influenza H1N1 virus-specific vaccine (Chen et al., 2012; Yuan et al., 2010). Paclitaxel enhances the efficacy of various other immunotherapeutic regimens, including multiple immunocytokines (e.g., IL-2, IL-7, or IL-12 variants targeted to the tumor vasculature by a specific monoclonal antibody) (Moschetta et al., 2012; Pasche et al., 2012) as well as HER2 and vascular endothelial growth factor (VEGF) peptide mimics (Foy et al., 2012). In doing so, paclitaxel allows for the activation of immune responses that—at least in some murine models of cancer—successfully eradicate malignant cells (Moschetta et al., 2012; Pasche et al., 2012). Along similar lines, docetaxel has been shown to improve the therapeutic potential of an immunotherapeutic regimen combining adoptive T cell transfer and DC-based vaccination (Galluzzi et al., 2012b, 2012d; Kodumudi et al., 2012). Backing up these preclinical observations, sporadic evidence from clinical trials suggests that cancer patients who have previously been treated with immunotherapy respond better to salvage chemotherapy than do patient who have not (Vanneman and Dranoff, 2012).

Antineoplastic agents may promote tumor-specific immune responses in several ways. However, in many studies it is difficult to distinguish whether the apparent immunostimulatory effects of chemotherapy, which are paralleled by changes in the immune infiltrate (most frequently an increase in effector cells coupled to a reduction in immunosuppressive cells) are truly therapeutic or simply represent a consequence of tumor debulking. Therefore, we focus on anticancer agents that have been ascribed with immunostimulatory activity in the absence of tumors.

Stimulation of Innate Immune Effectors

Bisphosphonates (e.g., zoledronate, which is mainly used for the treatment of osteolytic metastases) activate caspase-1 in DCs by depriving them of prenylpyrophosphates. In doing so, bisphosphonates stimulate DCs to produce IL-1 β and IL-18, which in turn favor the secretion of IFN- γ by NK cells and $\gamma\delta$ T cells (Nussbaumer et al., 2011). Subcytotoxic doses of doxorubicin, methotrexate, mitomycin C, and paclitaxel directly upregulate the antigen-presenting functions of isolated DCs as well as the expression of CD40, CD80, CD86, and MHC class II molecules on their surface (Shurin et al., 2009).

In vivo, imatinib reportedly reduces the growth of mouse tumor cells that are refractory to its antineoplastic activity in vitro, an effect that is abolished upon NK cell depletion (Borg et al., 2004; Taieb et al., 2006). Accordingly, imatinib potently stimulates NK cells in vivo (but not in vitro) via an indirect mechanism

that involves the inhibition of KIT signaling in DCs (Borg et al., 2004; Delahaye et al., 2011). Dasatinib, another tyrosine kinase inhibitor, has also been shown to mobilize NK cells, thereby improving their cytotoxicity in CML patients (Mustjoki et al., 2013).

Activation of T Cells

The BRAF inhibitors GSK2118436 and vemurafenib increase the infiltration of human melanomas by CD4⁺ and CD8⁺ granzyme-B-expressing $\alpha\beta$ T cells, correlating with a reduction in tumor size (Wilmott et al., 2012). The RNAi-mediated depletion of oncogenic BRAF^{V600E} can reduce the secretion of immunosuppressive soluble factors such as IL-6, IL-10, and VEGF by melanoma cells (Sumimoto et al., 2006). Along similar lines, PLX4720, another BRAF-targeting agent, has been shown to downregulate the production of CCL2 by both transplantable BRAF^{V600E}-driven and de novo melanomas, resulting in robust tumor infiltration by CD8⁺ T cells and prominent antineoplastic effects (Knight et al., 2013). These observations suggest that (1) at least in some settings, the chemotactic functions of CCL2 (which recruits potentially immunosuppressive macrophages) predominate over its immunostimulatory capacity and (2) BRAF inhibitors mediate immunostimulatory effects by targeting tumor cells. In line with this notion, vemurafenib reportedly improves the effector functions of tumor antigen-specific T cells adoptively transferred to melanoma-bearing mice through mechanisms that cannot be recapitulated with isolated T cells (Koya et al., 2012).

Other chemotherapeutic agents may directly stimulate immune effectors. For instance, cyclophosphamide promotes the differentiation of human and murine Th17 cells, in vitro and in vivo, an effect that can be detected among circulating as well as tumor-infiltrating lymphocytes (Viaud et al., 2011). Taxanes stimulate the production of IFN- γ and IL-2 by T cells, presumably favoring T cell polarization toward a Th1 cell profile (Tsavaris et al., 2002). Bisphosphonates increase the proliferation and cytotoxic activity of V γ 9V δ 2 T cells by acting either on DCs or on tumor cells, presumably owing to the accumulation of isopentenyl pyrophosphate (Cabillic et al., 2010). Such V γ 9V δ 2 T cells can directly kill malignant cells (Benzaïd et al., 2011) or elicit CTL responses specific for tumor-associated antigens (Altwater et al., 2012). At present, it is not known whether bisphosphonates preferentially act on neoplastic lesions or whether they predominantly exert systemic effects.

Inhibition of Myeloid-Derived Suppressor Cells and M2 Macrophages

MDSCs and M2 macrophages exert robust tumor-supporting functions as they contribute to the establishment of an immunosuppressive local microenvironment (Coussens et al., 2013). Several chemotherapeutic agents appear to reduce the amount or inhibit the activity of these cells, tipping the balance toward the activation of antitumor immune responses.

Gemcitabine, a nucleoside analog, specifically reduces splenic MDSCs (Gr-1⁺/CD11b⁺ cells) in tumor-bearing mice, while leaving the effector functions of local NK cells unaffected (Suzuki et al., 2005). Similar MDSC-depleting effects in both the spleen and the tumor microenvironment have been ascribed to another nucleoside analog, 5-fluorouracil (Vincent et al., 2010). Moreover, gemcitabine increases the absolute and relative abundance of circulating CD14⁺ monocytes and CD11c⁺ myeloid DCs in pancreatic carcinoma patients (Soeda et al., 2009).

Paclitaxel promotes the differentiation of MDSCs into DCs *in vitro* (Michels et al., 2012). Along similar lines, docetaxel has been reported to decrease splenic MDSCs in mice bearing mammary tumors (Kodumudi et al., 2010) as well as intratumoral MDSCs in mice developing transgene-induced melanomas, an effect that was paralleled by the reduction of local proinflammatory and immunosuppressive factors such as transforming growth factor β (TGF- β) and IL-10 (Sevko et al., 2013). The MDSC-depleting activity of docetaxel is linked to the induction of cell death in MDSCs bearing the M2 marker CD206 (but not in their counterparts expressing the M1 marker CCR7) (Sevko et al., 2013) as well as to the acquisition of macrophage/DC markers such as MHC class II molecules, CD11c, and CD86 by splenocytes (Kodumudi et al., 2012). Docetaxel also inhibits the repopulation of the spleen by MDSCs upon total body irradiation (Kodumudi et al., 2012). By inhibiting ABL, imatinib can reduce the Fc γ R-mediated phagocytosis of murine macrophages, and this may contribute to its broad anti-inflammatory activity (Greuber and Pendergast, 2012). Whether this particular effect contributes to the antineoplastic potential of imatinib remains to be determined.

Suppression of FOXP3⁺ Regulatory T Cells

Cyclophosphamide is now widely acknowledged as an agent that subverts the immunosuppressive functions of Treg cells, especially when used at relatively low, so-called metronomic, doses (Ghiringhelli et al., 2007; Le and Jaffee, 2012). Arsenic trioxide, paclitaxel, and the VEGF receptor (VEGFR) inhibitor sunitinib have also been shown to reduce the numbers of Treg cells, at least in some experimental settings (Le and Jaffee, 2012). Similarly, oxaliplatin (but not irinotecan, 5-fluorouracil, or gemcitabine) combined with IL-12 can increase the CTL to Treg cell ratio within the hepatic metastases and in the spleen of mice bearing transplantable colon carcinomas (Gonzalez-Aparicio et al., 2011). The CTL to Treg cell ratio also increases in melanomas responding to PLX4720 (Knight et al., 2013) as well as in BCs responding to anthracycline-based chemotherapy (Ladoire et al., 2011; Senovilla et al., 2012, 2013). Both cyclophosphamide and gemcitabine (but neither 5-fluorouracil nor oxaliplatin) inhibit the differentiation of Treg cells from human peripheral blood mononuclear cells stimulated with IL-2 and TGF- β *in vitro* (Kan et al., 2012), suggesting that these chemotherapeutics may exert direct Treg cell-inhibitory functions.

Driven by the high production of angiogenic factors, including VEGF, the tumor vasculature develops rapidly and accumulates structural abnormalities such as tortuosity, dilation, and hyperpermeability (Jain, 2005). These local alterations, which result in patchy hypoperfusion, may increase tumor infiltration by Treg cells and M2 macrophages. In addition, high circulating levels of VEGF may (1) promote Treg cell proliferation, (2) stimulate MDSC accumulation in peripheral immune organs, and (3) inhibit the maturation of DC precursors. These effects can be reversed by agents that selectively target the VEGF-VEGFR signaling axis, such as VEGF-blocking antibodies (e.g., bevacizumab), or inhibitors of VEGFR tyrosine kinase activity (e.g., lenvatinib) (Goel et al., 2011). These molecules “normalize” the tumor vasculature and revert tumor-induced alterations of the peripheral immune system, including the elevation of circulating Treg cells (Huang et al., 2013).

Altogether, the findings reviewed here support the notion that at least part of the therapeutic efficacy of successful antineoplastic agents originates from their capacity to functionally interact with one or several components of the immune system.

Concluding Remarks

There is convincing preclinical and accumulating clinical evidence in support of the notion that successful antineoplastic therapies reinstate immunosurveillance. Thus, both conventional chemotherapeutics and targeted anticancer agents appear to stimulate the antigenicity of malignant cells, their immunogenicity, or their susceptibility to immune attack. These alterations are not inherent to the capacity of antineoplastic agents to kill (or permanently block the proliferation of) cancer cells, but rather reside in their potential to induce a cell death-independent stress response that may mimic that elicited by viral infection. As a constant leitmotif, anticancer agents that induce robust ER stress responses and/or autophagy mediate efficient immunostimulation by directly acting on malignant cells (Kroemer et al., 2013). In addition, chemotherapy can have various ancillary effects on the immune system, ranging from a generalized “reset” as a result of severe lympho- and myelodepletion to more subtle alterations in the equilibrium between effector and suppressor cells. Several successful antineoplastic agents seem to reinstate immunosurveillance by influencing the tumor-host equilibrium at multiple levels. For instance, cyclophosphamide not only triggers the immunogenic demise of malignant cells but also stimulates the differentiation of Th17 cells and subverts the immunosuppressive functions of Treg cells. This may reflect the pleiotropic activity of compounds that act on ubiquitous structures (such as microtubules for taxanes or topoisomerase II and mitochondria for anthracyclines) or broadly expressed targets (such as ABL, KIT, and PDGFR for imatinib).

We surmise that chemotherapeutic agents that have been positively selected for their clinical efficacy are actually those that most effectively reinstate cancer immunosurveillance. Dose-intensification approaches, which often result in major immunosuppressive side effects, have been largely unsuccessful, and it is plausible that the doses and administration schedules that are currently being used for chemotherapeutics have been selected because they were compatible with anticancer immune responses. Moreover, the fact that different tumor types are nowadays treated with distinct therapeutic regimens might reflect peculiarities in the immunological features of such neoplasms, extending beyond cancer cell-intrinsic properties and pharmacokinetic issues. Nonetheless, the selection and optimization processes that have yielded current anticancer drugs have been entirely empirical, and hence have systematically neglected possible immune (side) effects. It is therefore tempting to speculate that the introduction of immunological procedures into the development of anticancer agents, including the systematic use of realistic animal models in which cancers evolve in an immunocompetent setting, for instance, as well as the application of suitable immunomonitoring techniques to patients, will speed up the development of novel antineoplastic agents (Figure 4). It is difficult to make firm recommendations on the preferential use of genetically engineered versus engraftment models of cancer, because both approaches have specific advantages and disadvantages. In particular, although engraftment

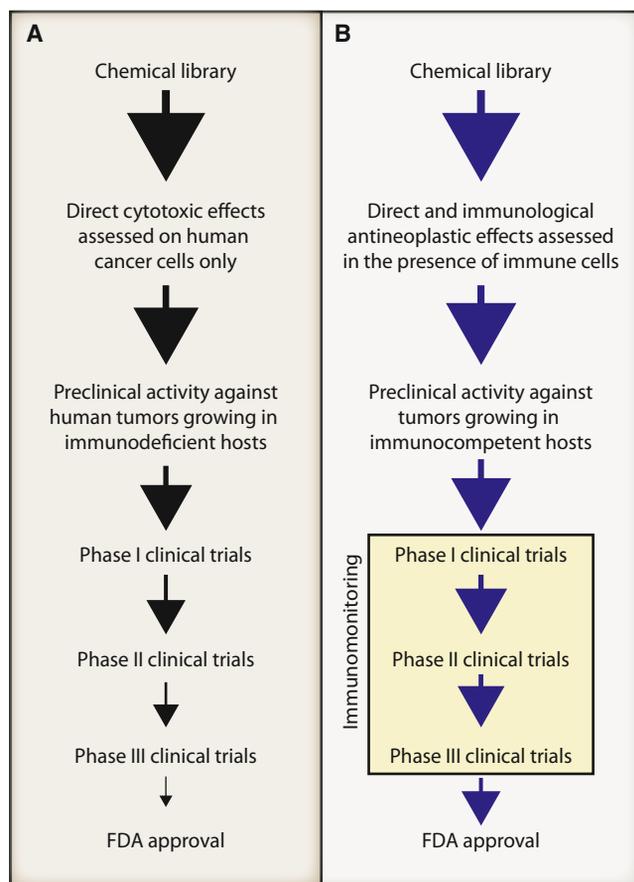


Figure 4. Integrating Immunological Parameters into the Development of Anticancer Agents

(A) The conventional pipeline for the development of novel antineoplastic agents is based on the old vision that cancer would represent a merely cell-intrinsic disease. In this setting, putative therapeutic effects mediated by the immune system are neglected, resulting in high attrition rates, in particular when candidate agents enter clinical trials.

(B) Testing the therapeutic efficacy of candidate antineoplastic drugs in immunocompetent animal models will minimize the clinical development of agents with unwarranted side effects on the immune system while promoting that of compounds that reinstate anticancer immunosurveillance. Along similar lines, carefully monitoring immunological parameters in cancer patients enrolled in clinical trials is expected to guide the discovery of novel biomarkers, the optimization of doses and schedules, and the rational design of combinatorial chemoimmunotherapeutic regimens.

models are simple and highly reproducible, transgene-driven lesions generally offer more “realistic” scenarios than do engrafted tumors. Nonetheless, the tissue-wide overexpression of an oncogene may overwhelm natural immunosurveillance mechanisms. For example, *ErbB2*-driven murine breast cancers respond to chemotherapy independently from the adaptive immune system (Ciampricotti et al., 2012), contrasting with multiple clinical studies that underscore the contribution of tumor-infiltrating lymphocytes to therapeutic outcome (DeNardo et al., 2011; Denkert et al., 2010; Ladoire et al., 2011; Loi et al., 2013a; Senovilla et al., 2012; West et al., 2011).

An in-depth characterization of the immunological effects of currently used anticancer agents may pave the way to the design of rational combination therapies in which maximal immunosti-

mulation is sought by the simultaneous or sequential coadministration of several chemo- and immunotherapeutic agents. In particular, agents acting on distinct facets of malignant cells or the immune system (Figure 2) should stimulate antitumor immune responses in a synergistic fashion. Thus, chemotherapeutic agents that stimulate the antigenicity and immunogenicity of malignant cells or increase their susceptibility to immune attacks may be advantageously combined with immunotherapeutic regimens designed to activate immune effectors or to inhibit immunosuppressive mechanisms. Prominent examples of the therapeutic potential of this approach include (1) the sequential administration of doxorubicin, cyclophosphamide, and an allogeneic granulocyte macrophage colony-stimulating factor (GM-CSF)-secreting anticancer vaccine to breast carcinoma patients (Emens et al., 2009), (2) the combination of paclitaxel with macrophage-depleting agents for the treatment of breast cancer (DeNardo et al., 2011), and (3) the use of paclitaxel, carboplatin, and ipilimumab as a first-line intervention against advanced lung cancer (Lynch et al., 2012).

Similar to all other biological systems, immune responses involve a limited number of molecules and cell types that mediate their effects in a highly context-dependent manner, like letters in words and words in phrases. Thus, it would be a reductionist (and probably futile) exercise to attribute general roles to single entities with respect to inflammation-induced tumor progression and anticancer immune responses (Coussens et al., 2013). Rather, the same molecules and cell types may play ambiguous roles depending on tumor type, immune contexture, and/or precise therapeutic strategy. This is well exemplified by IL-1 β , whose neutralization negatively affects the outcome of anthracycline-based chemotherapy, because IL-1 β produced by DCs is required for anticancer immune responses (Ghiringhelli et al., 2009; Mattarollo et al., 2011), yet can improve the therapeutic potential of 5-fluorouracil and gemcitabine, because IL-1 β produced by MDSCs can participate in tumor-promoting inflammatory reactions (Bruchard et al., 2013). Similarly, extracellular HMGB1 generally boosts chemotherapy-elicited immune responses (Apetoh et al., 2007a), yet can also act as a proinflammatory factor that drives tumor progression (He et al., 2013). Finally, IL-17 may have a dual influence on chemotherapy-elicited immune responses against malignant cells: a positive one if IL-17 is produced by $\gamma\delta$ T cells (Ma et al., 2011) and a negative one when IL-17 is secreted by Th17 cells (Bruchard et al., 2013).

These complexities underscore the need for an ever more profound comprehension of the dynamic changes in the tumor microenvironment and in systemic immune responses as neoplasms evolve, progress, and respond to therapy. An improved knowledge of these aspects will facilitate the rational design of highly efficient, synergistic regimens that combine anticancer agents and immunotherapies.

ACKNOWLEDGMENTS

The authors are supported by the Ligue contre le Cancer (équipe labellisée), Agence National de la Recherche, AXA Chair for Longevity Research, Cancéropôle Ile-de-France, Institut National du Cancer (INCa), Fondation Bettencourt-Schueller, Fondation de France, Fondation pour la Recherche Médicale, the European Commission (ArtForce), the European Research Council, the LabEx Immuno-Oncology, the SIRIC Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (Socrate) and Cancer Research

and Personalized Medicine (Carpem), the Paris Alliance of Cancer Research Institutes, the National Health and Medical Research Council of Australia, the Susan G. Komen Foundation, and the Victorian Cancer Agency.

REFERENCES

- Altwater, B., Pscherer, S., Landmeier, S., Kailayangiri, S., Savoldo, B., Juregens, H., and Rossig, C. (2012). Activated human $\gamma\delta$ T cells induce peptide-specific CD8+ T-cell responses to tumor-associated self-antigens. *Cancer Immunol. Immunother.* *61*, 385–396.
- André, T., Boni, C., Mounedji-Boudiaf, L., Navarro, M., Tabernero, J., Hickish, T., Topham, C., Zaninelli, M., Clingan, P., Bridgewater, J., et al.; Multicenter International Study of Oxaliplatin/5-Fluorouracil/Leucovorin in the Adjuvant Treatment of Colon Cancer (MOSAIC) Investigators. (2004). Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. *N. Engl. J. Med.* *350*, 2343–2351.
- Apetoh, L., Ghiringhelli, F., Tesniere, A., Criollo, A., Ortiz, C., Lidereau, R., Mariette, C., Chaput, N., Mira, J.P., Delaloge, S., et al. (2007a). The interaction between HMGB1 and TLR4 dictates the outcome of anticancer chemotherapy and radiotherapy. *Immunol. Rev.* *220*, 47–59.
- Apetoh, L., Ghiringhelli, F., Tesniere, A., Obeid, M., Ortiz, C., Criollo, A., Mignot, G., Maiuri, M.C., Ullrich, E., Saulnier, P., et al. (2007b). Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat. Med.* *13*, 1050–1059.
- Ashkenazi, A., and Dixit, V.M. (1998). Death receptors: signaling and modulation. *Science* *281*, 1305–1308.
- Balachandran, V.P., Cavnar, M.J., Zeng, S., Bamboat, Z.M., Ocuin, L.M., Obaid, H., Sorenson, E.C., Popow, R., Ariyan, C., Rossi, F., et al. (2011). Imatinib potentiates antitumor T cell responses in gastrointestinal stromal tumor through the inhibition of IdO. *Nat. Med.* *17*, 1094–1100.
- Bates, G.J., Fox, S.B., Han, C., Leek, R.D., Garcia, J.F., Harris, A.L., and Banham, A.H. (2006). Quantification of regulatory T cells enables the identification of high-risk breast cancer patients and those at risk of late relapse. *J. Clin. Oncol.* *24*, 5373–5380.
- Beavis, P.A., Stagg, J., Darcy, P.K., and Smyth, M.J. (2012). CD73: a potent suppressor of antitumor immune responses. *Trends Immunol.* *33*, 231–237.
- Benson, D.M., Jr., Bakan, C.E., Zhang, S., Collins, S.M., Liang, J., Srivastava, S., Hofmeister, C.C., Efebera, Y., Andre, P., Romagne, F., et al. (2011). IPH2101, a novel anti-inhibitory KIR antibody, and lenalidomide combine to enhance the natural killer cell versus multiple myeloma effect. *Blood* *118*, 6387–6391.
- Benzaïd, I., Mönkkönen, H., Stresing, V., Bonnelye, E., Green, J., Mönkkönen, J., Touraine, J.L., and Clézardin, P. (2011). High phosphoantigen levels in bisphosphonate-treated human breast tumors promote Vgamma9Vdelta2 T-cell chemotaxis and cytotoxicity in vivo. *Cancer Res.* *71*, 4562–4572.
- Bererhi, L., Pallet, N., Zuber, J., Anglicheau, D., Kreis, H., Legendre, C., and Candon, S. (2012). Clinical and immunological features of very long-term survivors with a single renal transplant. *Transpl. Int.* *25*, 545–554.
- Berghuis, D., Schilham, M.W., Vos, H.I., Santos, S.J., Kloess, S., Buddingh', E.P., Egeler, R.M., Hogendoorn, P.C., and Lankester, A.C. (2012). Histone deacetylase inhibitors enhance expression of NKG2D ligands in Ewing sarcoma and sensitize for natural killer cell-mediated cytotoxicity. *Clin. Sarcoma Res.* *2*, 8.
- Bianchi, M.E. (2009). HMGB1 loves company. *J. Leukoc. Biol.* *86*, 573–576.
- Borg, C., Terme, M., Taïeb, J., Ménard, C., Flament, C., Robert, C., Maruyama, K., Wakasugi, H., Angevin, E., Thielemans, K., et al. (2004). Novel mode of action of c-kit tyrosine kinase inhibitors leading to NK cell-dependent antitumor effects. *J. Clin. Invest.* *114*, 379–388.
- Bruchard, M., Mignot, G., Derangère, V., Chalmin, F., Chevriaux, A., Végran, F., Boireau, W., Simon, B., Ryffel, B., Connat, J.L., et al. (2013). Chemotherapy-triggered cathepsin B release in myeloid-derived suppressor cells activates the Nlrp3 inflammasome and promotes tumor growth. *Nat. Med.* *19*, 57–64.
- Cablic, F., Toutirais, O., Lavoué, V., de La Pintièrre, C.T., Daniel, P., Rioux-Leclercq, N., Turlin, B., Mönkkönen, H., Mönkkönen, J., Boudjema, K., et al. (2010). Aminobisphosphonate-pretreated dendritic cells trigger successful Vgamma9Vdelta2 T cell amplification for immunotherapy in advanced cancer patients. *Cancer Immunol. Immunother.* *59*, 1611–1619.
- Caron, E., Vincent, K., Fortier, M.H., Laverdure, J.P., Bramoullé, A., Hardy, M.P., Voisin, G., Roux, P.P., Lemieux, S., Thibault, P., and Perreault, C. (2011). The MHC I immunopeptidome conveys to the cell surface an integrative view of cellular regulation. *Mol. Syst. Biol.* *7*, 533.
- Casares, N., Pequignot, M.O., Tesniere, A., Ghiringhelli, F., Roux, S., Chaput, N., Schmitt, E., Hamai, A., Hervas-Stubbs, S., Obeid, M., et al. (2005). Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. *J. Exp. Med.* *202*, 1691–1701.
- Catellani, S., Pierri, I., Gobbi, M., Poggi, A., and Zocchi, M.R. (2011). Imatinib treatment induces CD5+ B lymphocytes and IgM natural antibodies with anti-leukemic reactivity in patients with chronic myelogenous leukemia. *PLoS ONE* *6*, e18925.
- Chan, C.J., Andrews, D.M., McLaughlin, N.M., Yagita, H., Gilfillan, S., Colonna, M., and Smyth, M.J. (2010). DNAM-1/CD155 interactions promote cytokine and NK cell-mediated suppression of poorly immunogenic melanoma metastases. *J. Immunol.* *184*, 902–911.
- Chanan-Khan, A.A., Lonial, S., Weber, D., Borrello, I., Foà, R., Hellmann, A., Dimopoulos, M., Swern, A.S., and Knight, R. (2012). Lenalidomide in combination with dexamethasone improves survival and time-to-progression in patients ≥ 65 years old with relapsed or refractory multiple myeloma. *Int. J. Hematol.* *96*, 254–262.
- Chen, G., and Emens, L.A. (2013). Chemoimmunotherapy: reengineering tumor immunity. *Cancer Immunol. Immunother.* *62*, 203–216.
- Chen, C.I., Maecker, H.T., and Lee, P.P. (2008). Development and dynamics of robust T-cell responses to CML under imatinib treatment. *Blood* *111*, 5342–5349.
- Chen, J., Yuan, L., Fan, Q., Su, F., Chen, Y., and Hu, S. (2012). Adjuvant effect of docetaxel on the immune responses to influenza A H1N1 vaccine in mice. *BMC Immunol.* *13*, 36.
- Ciampricotti, M., Hau, C.S., Doornebal, C.W., Jonkers, J., and de Visser, K.E. (2012). Chemotherapy response of spontaneous mammary tumors is independent of the adaptive immune system. *Nat. Med.* *18*, 344–346, author reply 346.
- Corless, C.L., Barnett, C.M., and Heinrich, M.C. (2011). Gastrointestinal stromal tumours: origin and molecular oncology. *Nat. Rev. Cancer* *11*, 865–878.
- Coussens, L.M., Zitvogel, L., and Palucka, A.K. (2013). Neutralizing tumor-promoting chronic inflammation: a magic bullet? *Science* *339*, 286–291.
- Cullen, S.P., Henry, C.M., Kearney, C.J., Logue, S.E., Feoktistova, M., Tynan, G.A., Lavelle, E.C., Leverkus, M., and Martin, S.J. (2013). Fas/CD95-induced chemokines can serve as “find-me” signals for apoptotic cells. *Mol. Cell* *49*, 1034–1048.
- Delahaye, N.F., Rusakiewicz, S., Martins, I., Ménard, C., Roux, S., Lyonnet, L., Paul, P., Sarabi, M., Chaput, N., Semeraro, M., et al. (2011). Alternatively spliced Nkp30 isoforms affect the prognosis of gastrointestinal stromal tumors. *Nat. Med.* *17*, 700–707.
- Demaria, S., Volm, M.D., Shapiro, R.L., Yee, H.T., Oratz, R., Formenti, S.C., Muggia, F., and Symmans, W.F. (2001). Development of tumor-infiltrating lymphocytes in breast cancer after neoadjuvant paclitaxel chemotherapy. *Clin. Cancer Res.* *7*, 3025–3030.
- DeNardo, D.G., Brennan, D.J., Rexhepaj, E., Ruffell, B., Shiao, S.L., Madden, S.F., Gallagher, W.M., Wadhvani, N., Keil, S.D., Junaid, S.A., et al. (2011). Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. *Cancer Discov.* *1*, 54–67.
- Denkert, C., Loibl, S., Noske, A., Roller, M., Müller, B.M., Komor, M., Budczies, J., Darb-Esfahani, S., Kronenwett, R., Hanusch, C., et al. (2010). Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. *J. Clin. Oncol.* *28*, 105–113.
- Druker, B.J., Guilhot, F., O'Brien, S.G., Gathmann, I., Kantarjian, H., Gattnermann, N., Deininger, M.W., Silver, R.T., Goldman, J.M., Stone, R.M., et al.; IRIS Investigators. (2006). Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N. Engl. J. Med.* *355*, 2408–2417.
- Elliott, M.R., Chekeni, F.B., Trampont, P.C., Lazarowski, E.R., Kadl, A., Walk, S.F., Park, D., Woodson, R.I., Ostankovich, M., Sharma, P., et al. (2009).

Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. *Nature* **461**, 282–286.

Emens, L.A., Asquith, J.M., Leatherman, J.M., Kobrin, B.J., Petrik, S., Laiko, M., Levi, J., Daphtary, M.M., Biedrzycki, B., Wolff, A.C., et al. (2009). Timed sequential treatment with cyclophosphamide, doxorubicin, and an allogeneic granulocyte-macrophage colony-stimulating factor-secreting breast tumor vaccine: a chemotherapy dose-ranging factorial study of safety and immune activation. *J. Clin. Oncol.* **27**, 5911–5918.

Engelhardt, J.J., Boldajipour, B., Beemiller, P., Pandurangi, P., Sorensen, C., Werb, Z., Egeblad, M., and Krummel, M.F. (2012). Marginating dendritic cells of the tumor microenvironment cross-present tumor antigens and stably engage tumor-specific T cells. *Cancer Cell* **21**, 402–417.

Finn, O.J. (2008). Cancer immunology. *N. Engl. J. Med.* **358**, 2704–2715.

Finn, O.J. (2012). Immuno-oncology: understanding the function and dysfunction of the immune system in cancer. *Ann. Oncol.* **23**(Suppl 8), viii6–9.

Foy, K.C., Miller, M.J., Moldovan, N., Bozanovic, T., Carson Iii, W.E., and Kaurmaya, P.T. (2012). Immunotherapy with HER-2 and VEGF peptide mimics plus metronomic paclitaxel causes superior antineoplastic effects in transplantable and transgenic mouse models of human breast cancer. *Oncolimmunology* **1**, 1004–1016.

Frederick, D.T., Piris, A., Cogdill, A.P., Cooper, Z.A., Lezcano, C., Ferrone, C.R., Mitra, D., Boni, A., Newton, L.P., Liu, C., et al. (2013). BRAF inhibition is associated with enhanced melanoma antigen expression and a more favorable tumor microenvironment in patients with metastatic melanoma. *Clin. Cancer Res.* **19**, 1225–1231.

Fridman, W.H., Pagès, F., Sautès-Fridman, C., and Galon, J. (2012). The immune contexture in human tumours: impact on clinical outcome. *Nat. Rev. Cancer* **12**, 298–306.

Galluzzi, L., Kepp, O., and Kroemer, G. (2012a). Mitochondria: master regulators of danger signalling. *Nat. Rev. Mol. Cell Biol.* **13**, 780–788.

Galluzzi, L., Senovilla, L., Vacchelli, E., Eggermont, A., Fridman, W.H., Galon, J., Sautès-Fridman, C., Tartour, E., Zitvogel, L., and Kroemer, G. (2012b). Trial watch: Dendritic cell-based interventions for cancer therapy. *Oncolimmunology* **1**, 1111–1134.

Galluzzi, L., Senovilla, L., Zitvogel, L., and Kroemer, G. (2012c). The secret ally: immunostimulation by anticancer drugs. *Nat. Rev. Drug Discov.* **11**, 215–233.

Galluzzi, L., Vacchelli, E., Eggermont, A., Fridman, W.H., Galon, J., Sautès-Fridman, C., Tartour, E., Zitvogel, L., and Kroemer, G. (2012d). Trial Watch: Adoptive cell transfer immunotherapy. *Oncolimmunology* **1**, 306–315.

Galluzzi, L., Vitale, I., Abrams, J.M., Alnemri, E.S., Baehrecke, E.H., Blagosklonny, M.V., Dawson, T.M., Dawson, V.L., El-Deiry, W.S., Fulda, S., et al. (2012e). Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. *Cell Death Differ.* **19**, 107–120.

Galon, J., Costes, A., Sanchez-Cabo, F., Kirilovsky, A., Mlecnik, B., Lagorce-Pagès, C., Tosolini, M., Camus, M., Berger, A., Wind, P., et al. (2006). Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* **313**, 1960–1964.

Gardai, S.J., McPhillips, K.A., Frasch, S.C., Janssen, W.J., Starefeldt, A., Murphy-Ullrich, J.E., Bratton, D.L., Oldenborg, P.A., Michalak, M., and Henson, P.M. (2005). Cell-surface calreticulin initiates clearance of viable or apoptotic cells through trans-activation of LRP on the phagocyte. *Cell* **123**, 321–334.

Garg, A.D., Krysko, D.V., Verfaillie, T., Kaczmarek, A., Ferreira, G.B., Marysael, T., Rubio, N., Firczuk, M., Mathieu, C., Roebroek, A.J., et al. (2012). A novel pathway combining calreticulin exposure and ATP secretion in immunogenic cancer cell death. *EMBO J.* **31**, 1062–1079.

Ghiringhelli, F., Menard, C., Puig, P.E., Ladoire, S., Roux, S., Martin, F., Solary, E., Le Cesne, A., Zitvogel, L., and Chaffert, B. (2007). 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.* **56**, 641–648.

Ghiringhelli, F., Apetoh, L., Tesniere, A., Aymeric, L., Ma, Y., Ortiz, C., Vermaelen, K., Panaretakis, T., Mignot, G., Ullrich, E., et al. (2009). Activation of the NLRP3 inflammasome in dendritic cells induces IL-1 β dependent adaptive immunity against tumors. *Nat. Med.* **15**, 1170–1178.

Goibert, M., Treilleux, I., Bendriss-Vermare, N., Bachelot, T., Goddard-Leon, S., Arfi, V., Biota, C., Doffin, A.C., Durand, I., Olive, D., et al. (2009). Regulatory T cells recruited through CCL22/CCR4 are selectively activated in lymphoid infiltrates surrounding primary breast tumors and lead to an adverse clinical outcome. *Cancer Res.* **69**, 2000–2009.

Goel, S., Duda, D.G., Xu, L., Munn, L.L., Boucher, Y., Fukumura, D., and Jain, R.K. (2011). Normalization of the vasculature for treatment of cancer and other diseases. *Physiol. Rev.* **91**, 1071–1121.

Gonzalez-Aparicio, M., Alzuguren, P., Mauleon, I., Medina-Echeverez, J., Hervas-Stubbs, S., Mancheno, U., Berraondo, P., Cretaz, J., Gonzalez-Aseguinolaza, G., Prieto, J., and Hernandez-Alcoceba, R. (2011). Oxaliplatin in combination with liver-specific expression of interleukin 12 reduces the immunosuppressive microenvironment of tumours and eradicates metastatic colorectal cancer in mice. *Gut* **60**, 341–349.

Granados, D.P., Yahyaoui, W., Laumont, C.M., Daouda, T., Muratore-Schroeder, T.L., Côté, C., Laverdure, J.P., Lemieux, S., Thibault, P., and Perreault, C. (2012). MHC I-associated peptides preferentially derive from transcripts bearing miRNA response elements. *Blood* **119**, e181–e191.

Greuber, E.K., and Pendergast, A.M. (2012). Abl family kinases regulate Fc γ R-mediated phagocytosis in murine macrophages. *J. Immunol.* **189**, 5382–5392.

Halama, N., Michel, S., Kloor, M., Zoernig, I., Benner, A., Spille, A., Pommerencke, T., von Knebel, D.M., Folprecht, G., Luber, B., et al. (2011). Localization and density of immune cells in the invasive margin of human colorectal cancer liver metastases are prognostic for response to chemotherapy. *Cancer Res.* **71**, 5670–5677.

Hanahan, D., and Weinberg, R.A. (2011). Hallmarks of cancer: the next generation. *Cell* **144**, 646–674.

He, Y., Zha, J., Wang, Y., Liu, W., Yang, X., and Yu, P. (2013). Tissue damage-associated “danger signals” influence T-cell responses that promote the progression of preneoplasia to cancer. *Cancer Res.* **73**, 629–639.

Hellwig, C.T., and Rehm, M. (2012). TRAIL signaling and synergy mechanisms used in TRAIL-based combination therapies. *Mol. Cancer Ther.* **11**, 3–13.

Huang, Y., Goel, S., Duda, D.G., Fukumura, D., and Jain, R.K. (2013). Vascular normalization as an emerging strategy to enhance cancer immunotherapy. *Cancer Res.* **73**, 2943–2948.

Ignatiadis, M., Singhal, S.K., Desmedt, C., Haibe-Kains, B., Criscitiello, C., Andre, F., Loi, S., Piccart, M., Michiels, S., and Sotiriou, C. (2012). Gene modules and response to neoadjuvant chemotherapy in breast cancer subtypes: a pooled analysis. *J. Clin. Oncol.* **30**, 1996–2004.

Jackaman, C., Majewski, D., Fox, S.A., Nowak, A.K., and Nelson, D.J. (2012). Chemotherapy broadens the range of tumor antigens seen by cytotoxic CD8(+) T cells in vivo. *Cancer Immunol. Immunother.* **61**, 2343–2356.

Jain, R.K. (2005). Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science* **307**, 58–62.

Kan, S., Hazama, S., Maeda, K., Inoue, Y., Homma, S., Koido, S., Okamoto, M., and Oka, M. (2012). Suppressive effects of cyclophosphamide and gemcitabine on regulatory T-cell induction in vitro. *Anticancer Res.* **32**, 5363–5369.

Kaneno, R., Shurin, G.V., Tourkova, I.L., and Shurin, M.R. (2009). Chemomodulation of human dendritic cell function by antineoplastic agents in low non-cytotoxic concentrations. *J. Transl. Med.* **7**, 58.

Kazama, H., Ricci, J.E., Herndon, J.M., Hoppe, G., Green, D.R., and Ferguson, T.A. (2008). Induction of immunological tolerance by apoptotic cells requires caspase-dependent oxidation of high-mobility group box-1 protein. *Immunity* **29**, 21–32.

Knight, D.A., Ngloi, S.F., Li, M., Parmenter, T., Mok, S., Cass, A., Haynes, N.M., Kinross, K., Yagita, H., Koya, R.C., et al. (2013). Host immunity contributes to the anti-melanoma activity of BRAF inhibitors. *J. Clin. Invest.* **123**, 1371–1381.

Kodumudi, K.N., Woan, K., Gilvary, D.L., Sahakian, E., Wei, S., and Djou, J.Y. (2010). A novel chemomodulating property of docetaxel: suppression of myeloid-derived suppressor cells in tumor bearers. *Clin. Cancer Res.* **16**, 4583–4594.

Kodumudi, K.N., Weber, A., Sarnaik, A.A., and Pilon-Thomas, S. (2012). Blockade of myeloid-derived suppressor cells after induction of lymphopenia

- improves adoptive T cell therapy in a murine model of melanoma. *J. Immunol.* **189**, 5147–5154.
- Koya, R.C., Mok, S., Otte, N., Blacketer, K.J., Comin-Anduix, B., Tumeah, P.C., Minasyan, A., Graham, N.A., Graeber, T.G., Chodon, T., and Ribas, A. (2012). BRAF inhibitor vemurafenib improves the antitumor activity of adoptive cell immunotherapy. *Cancer Res.* **72**, 3928–3937.
- Krempski, J., Karyampudi, L., Behrens, M.D., Erskine, C.L., Hartmann, L., Dong, H., Goode, E.L., Kalli, K.R., and Knutson, K.L. (2011). Tumor-infiltrating programmed death receptor-1+ dendritic cells mediate immune suppression in ovarian cancer. *J. Immunol.* **186**, 6905–6913.
- Kroemer, G., Galluzzi, L., Kepp, O., and Zitvogel, L. (2013). Immunogenic cell death in cancer therapy. *Annu. Rev. Immunol.* **31**, 51–72.
- Krysko, D.V., and Vandenabeele, P. (2010). Clearance of dead cells: mechanisms, immune responses and implication in the development of diseases. *Apoptosis* **15**, 995–997.
- Ladoire, S., Mignot, G., Dabakuyo, S., Arnould, L., Apetoh, L., Rébé, C., Couderc, B., Martin, F., Bizollon, M.H., Vanoli, A., et al. (2011). In situ immune response after neoadjuvant chemotherapy for breast cancer predicts survival. *J. Pathol.* **224**, 389–400.
- Le, D.T., and Jaffee, E.M. (2012). Regulatory T-cell modulation using cyclophosphamide in vaccine approaches: a current perspective. *Cancer Res.* **72**, 3439–3444.
- Lesterhuis, W.J., de Vries, I.J., Aarntzen, E.A., de Boer, A., Scharenborg, N.M., van de Rakt, M., van Spronsen, D.J., Preijers, F.W., Figdor, C.G., Adema, G.J., and Punt, C.J. (2010). A pilot study on the immunogenicity of dendritic cell vaccination during adjuvant oxaliplatin/capecitabine chemotherapy in colon cancer patients. *Br. J. Cancer* **103**, 1415–1421.
- Lesterhuis, W.J., Punt, C.J., Hato, S.V., Eleveld-Trancikova, D., Jansen, B.J., Nierkens, S., Schreiber, G., de Boer, A., Van Herpen, C.M., Kaanders, J.H., et al. (2011). Platinum-based drugs disrupt STAT6-mediated suppression of immune responses against cancer in humans and mice. *J. Clin. Invest.* **121**, 3100–3108.
- Liu, W.M., Fowler, D.W., Smith, P., and Dalglish, A.G. (2010). Pre-treatment with chemotherapy can enhance the antigenicity and immunogenicity of tumours by promoting adaptive immune responses. *Br. J. Cancer* **102**, 115–123.
- Loi, S., Sirtaine, N., Piette, F., Salgado, R., Viale, G., Van Eeno, F., Rouas, G., Francis, P., Crown, J.P., Hiltre, E., et al. (2013a). Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. *J. Clin. Oncol.* **31**, 860–867.
- Loi, S., Pommey, S., Haibe-Kains, B., Beavis, P.A., Darcy, P.K., Smyth, M.J., and Stagg, J. (2013b). CD73 promotes anthracycline resistance and poor prognosis in triple negative breast cancer. *Proc. Natl. Acad. Sci. USA*. Published online June 17, 2013. <http://dx.doi.org/10.1073/pnas.1222251110>.
- Lynch, T.J., Bondarenko, I., Luft, A., Serwatowski, P., Barlesi, F., Chacko, R., Sebastian, M., Neal, J., Lu, H., Cuillerot, J.M., and Reck, M. (2012). Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non-small-cell lung cancer: results from a randomized, double-blind, multicenter phase II study. *J. Clin. Oncol.* **30**, 2046–2054.
- Ma, Y., Aymeric, L., Locher, C., Mattarollo, S.R., Delahaye, N.F., Pereira, P., Boucontet, L., Apetoh, L., Ghiringhelli, F., Casares, N., et al. (2011). Contribution of IL-17-producing gamma delta T cells to the efficacy of anticancer chemotherapy. *J. Exp. Med.* **208**, 491–503.
- Ma, Y., Adjemian, S., Mattarollo, S.R., Yamazaki, T., Aymeric, L., Yang, H., Portela Catani, J.P., Hannani, D., Duret, H., Steegh, K., et al. (2013). Anticancer chemotherapy-induced intratumoral recruitment and differentiation of antigen-presenting cells. *Immunity* **38**, 729–741.
- Maeda, K., Hazama, S., Tokuno, K., Kan, S., Maeda, Y., Watanabe, Y., Kamei, R., Shindo, Y., Maeda, N., Yoshimura, K., et al. (2011). Impact of chemotherapy for colorectal cancer on regulatory T-cells and tumor immunity. *Anticancer Res.* **31**, 4569–4574.
- Mahmoud, S.M., Paish, E.C., Powe, D.G., Macmillan, R.D., Grainge, M.J., Lee, A.H., Ellis, I.O., and Green, A.R. (2011). Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. *J. Clin. Oncol.* **29**, 1949–1955.
- Maio, M., Di Giacomo, A.M., Robert, C., and Eggermont, A.M. (2013). Update on the role of ipilimumab in melanoma and first data on new combination therapies. *Curr. Opin. Oncol.* **25**, 166–172.
- Martins, I., Kepp, O., Schlemmer, F., Adjemian, S., Tailler, M., Shen, S., Michaud, M., Menger, L., Gdoura, A., Tajeddine, N., et al. (2011). Restoration of the immunogenicity of cisplatin-induced cancer cell death by endoplasmic reticulum stress. *Oncogene* **30**, 1147–1158.
- Martins, I., Wang, Y., Michaud, M., Ma, Y., Sukkurwala, A.Q., Shen, S., Kepp, O., Metivier, D., Galluzzi, L., Perfettini, J.L., et al. (2013). Molecular mechanisms of ATP secretion during immunogenic cell death. *Cell Death Differ.* Published online July 12, 2013. <http://dx.doi.org/10.1038/cdd.2013.75>.
- Matsushita, H., Vesely, M.D., Koboldt, D.C., Rickert, C.G., Uppaluri, R., McGrini, V.J., Arthur, C.D., White, J.M., Chen, Y.S., Shea, L.K., et al. (2012). Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoeediting. *Nature* **482**, 400–404.
- Mattarollo, S.R., Loi, S., Duret, H., Ma, Y., Zitvogel, L., and Smyth, M.J. (2011). Pivotal role of innate and adaptive immunity in anthracycline chemotherapy of established tumors. *Cancer Res.* **71**, 4809–4820.
- Ménard, C., Blay, J.Y., Borg, C., Michiels, S., Ghiringhelli, F., Robert, C., Nonn, C., Chaput, N., Taïeb, J., Delahaye, N.F., et al. (2009). Natural killer cell IFN-gamma levels predict long-term survival with imatinib mesylate therapy in gastrointestinal stromal tumor-bearing patients. *Cancer Res.* **69**, 3563–3569.
- Menger, L., Vacchelli, E., Adjemian, S., Martins, I., Ma, Y., Shen, S., Yamazaki, T., Sukkurwala, A.Q., Michaud, M., Mignot, G., et al. (2012). Cardiac glycosides exert anticancer effects by inducing immunogenic cell death. *Sci. Transl. Med.* **4**, 43ra99.
- Michaud, M., Martins, I., Sukkurwala, A.Q., Adjemian, S., Ma, Y., Pellegatti, P., Shen, S., Kepp, O., Scoazec, M., Mignot, G., et al. (2011). Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. *Science* **334**, 1573–1577.
- Michaud, M., Sukkurwala, A.Q., Martins, I., Shen, S., Zitvogel, L., and Kroemer, G. (2012). Subversion of the chemotherapy-induced anticancer immune response by the ecto-ATPase CD39. *Oncol. Immunology* **1**, 393–395.
- Michels, T., Shurin, G.V., Naiditch, H., Sevko, A., Umansky, V., and Shurin, M.R. (2012). Paclitaxel promotes differentiation of myeloid-derived suppressor cells into dendritic cells in vitro in a TLR4-independent manner. *J. Immunotoxicol.* **9**, 292–300.
- Mlecik, B., Tosolini, M., Kirilovsky, A., Berger, A., Bindea, G., Meatchi, T., Bruneval, P., Trajanoski, Z., Fridman, W.H., Pagès, F., and Galon, J. (2011). Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. *J. Clin. Oncol.* **29**, 610–618.
- Morselli, E., Galluzzi, L., Kepp, O., Vicencio, J.M., Criollo, A., Maiuri, M.C., and Kroemer, G. (2009). Anti- and pro-tumor functions of autophagy. *Biochim. Biophys. Acta* **1793**, 1524–1532.
- Moschetta, M., Pretto, F., Berndt, A., Galler, K., Richter, P., Bassi, A., Oliva, P., Micotti, E., Valbusa, G., Schwager, K., et al. (2012). Paclitaxel enhances therapeutic efficacy of the F8-IL2 immunocytokine to EDA-fibronectin-positive metastatic human melanoma xenografts. *Cancer Res.* **72**, 1814–1824.
- Mustjoki, S., Richter, J., Barbany, G., Ehrencrona, H., Fioretos, T., Gedde-Dahl, T., Gjertsen, B.T., Hovland, R., Hernesniemi, S., Josefsen, D., et al. (2013). Impact of malignant stem cell burden on therapy outcome in newly diagnosed chronic myeloid leukemia patients. *Leukemia*. Published online January 18, 2013. <http://dx.doi.org/10.1038/leu.2013.19>.
- Nussbaumer, O., Gruenbacher, G., Gander, H., and Thurnher, M. (2011). DC-like cell-dependent activation of human natural killer cells by the bisphosphonate zoledronic acid is regulated by $\gamma\delta$ T lymphocytes. *Blood* **118**, 2743–2751.
- Obeid, M., Tesniere, A., Ghiringhelli, F., Fimia, G.M., Apetoh, L., Perfettini, J.L., Castedo, M., Mignot, G., Panaretakis, T., Casares, N., et al. (2007). Calreticulin exposure dictates the immunogenicity of cancer cell death. *Nat. Med.* **13**, 54–61.
- Ocana, A., Pandiella, A., Siu, L.L., and Tannock, I.F. (2011). Preclinical development of molecular-targeted agents for cancer. *Nat. Rev. Clin. Oncol.* **8**, 200–209.
- Panaretakis, T., Kepp, O., Brockmeier, U., Tesniere, A., Bjorklund, A.C., Chapman, D.C., Durchschlag, M., Joza, N., Pierron, G., van Endert, P., et al.

(2009). Mechanisms of pre-apoptotic calreticulin exposure in immunogenic cell death. *EMBO J.* **28**, 578–590.

Pasche, N., Wulfhard, S., Pretto, F., Carugati, E., and Neri, D. (2012). The antibody-based delivery of interleukin-12 to the tumor neovasculature eradicates murine models of cancer in combination with paclitaxel. *Clin. Cancer Res.* **18**, 4092–4103.

Poole, C.J., Earl, H.M., Hiller, L., Dunn, J.A., Bathers, S., Grieve, R.J., Spooner, D.A., Agrawal, R.K., Fernando, I.N., Brunt, A.M., et al.; NEAT Investigators and the SCTBG. (2006). Epirubicin and cyclophosphamide, methotrexate, and fluorouracil as adjuvant therapy for early breast cancer. *N. Engl. J. Med.* **355**, 1851–1862.

Pui, C.H., Campana, D., Pei, D., Bowman, W.P., Sandlund, J.T., Kaste, S.C., Ribeiro, R.C., Rubnitz, J.E., Raimondi, S.C., Onciu, M., et al. (2009). Treating childhood acute lymphoblastic leukemia without cranial irradiation. *N. Engl. J. Med.* **360**, 2730–2741.

Pulte, D., Furman, R.R., Broekman, M.J., Drosopoulos, J.H., Ballard, H.S., Olson, K.E., Kizer, J.R., and Marcus, A.J. (2011). CD39 expression on T lymphocytes correlates with severity of disease in patients with chronic lymphocytic leukemia. *Clin. Lymphoma Myeloma Leuk.* **11**, 367–372.

Ramakrishnan, R., Assudani, D., Nagaraj, S., Hunter, T., Cho, H.I., Antonia, S., Altiock, S., Celis, E., and Gabrilovich, D.I. (2010). Chemotherapy enhances tumor cell susceptibility to CTL-mediated killing during cancer immunotherapy in mice. *J. Clin. Invest.* **120**, 1111–1124.

Ramakrishnan, R., Huang, C., Cho, H.I., Lloyd, M., Johnson, J., Ren, X., Altiock, S., Sullivan, D., Weber, J., Celis, E., and Gabrilovich, D.I. (2012). Autophagy induced by conventional chemotherapy mediates tumor cell sensitivity to immunotherapy. *Cancer Res.* **72**, 5483–5493.

Raulet, D.H., Gasser, S., Gowen, B.G., Deng, W., and Jung, H. (2013). Regulation of ligands for the NKG2D activating receptor. *Annu. Rev. Immunol.* **31**, 413–441.

Ravichandran, K.S. (2011). Beginnings of a good apoptotic meal: the find-me and eat-me signaling pathways. *Immunity* **35**, 445–455.

Reits, E.A., Hodge, J.W., Herberts, C.A., Groothuis, T.A., Chakraborty, M., Wansley, E.K., Camphausen, K., Luiten, R.M., de Ru, A.H., Neijssen, J., et al. (2006). Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. *J. Exp. Med.* **203**, 1259–1271.

Rousseau, B., Loulergue, P., Mir, O., Krivine, A., Kotti, S., Viel, E., Simon, T., de Gramont, A., Goldwasser, F., Launay, O., and Tournigand, C. (2012). Immunogenicity and safety of the influenza A H1N1v 2009 vaccine in cancer patients treated with cytotoxic chemotherapy and/or targeted therapy: the VACANCE study. *Ann. Oncol.* **23**, 450–457.

Ruffell, B., Au, A., Rugo, H.S., Esserman, L.J., Hwang, E.S., and Coussens, L.M. (2012). Leukocyte composition of human breast cancer. *Proc. Natl. Acad. Sci. USA* **109**, 2796–2801.

Rusakiewicz, S., Semeraro, M., Sarabi, M., Desbois, M., Locher, C., Mendez, R., Vimond, N., Concha, A., Garrido, F., Isambert, N., et al. (2013). Immune infiltrates are prognostic factors in localized gastrointestinal stromal tumors. *Cancer Res.* **73**, 3499–3510.

Salmena, L., Poliseno, L., Tay, Y., Kats, L., and Pandolfi, P.P. (2011). A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell* **146**, 353–358.

Schiavoni, G., Sistigu, A., Valentini, M., Mattei, F., Sestili, P., Spadaro, F., Sanchez, M., Lorenzi, S., D'Urso, M.T., Belardelli, F., et al. (2011). Cyclophosphamide synergizes with type I interferons through systemic dendritic cell reactivation and induction of immunogenic tumor apoptosis. *Cancer Res.* **71**, 768–778.

Schmidt, M., Hellwig, B., Hammad, S., Othman, A., Lohr, M., Chen, Z., Boehm, D., Gebhard, S., Petry, I., Lebrecht, A., et al. (2012). A comprehensive analysis of human gene expression profiles identifies stromal immunoglobulin κ C as a compatible prognostic marker in human solid tumors. *Clin. Cancer Res.* **18**, 2695–2703.

Schreiber, R.D., Old, L.J., and Smyth, M.J. (2011). Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science* **331**, 1565–1570.

Senovilla, L., Vitale, I., Martins, I., Tailler, M., Pailleret, C., Michaud, M., Galluzzi, L., Adjemian, S., Kepp, O., Niso-Santano, M., et al. (2012). An immunosurveillance mechanism controls cancer cell ploidy. *Science* **337**, 1678–1684.

Senovilla, L., Vitale, I., Martins, I., Kepp, O., Galluzzi, L., Zitvogel, L., Castedo, M., and Kroemer, G. (2013). An anticancer therapy-elicited immunosurveillance system that eliminates tetraploid cells. *Oncology* **2**, e22409.

Sevko, A., Michels, T., Vrohligs, M., Umansky, L., Beckhove, P., Kato, M., Shurin, G.V., Shurin, M.R., and Umansky, V. (2013). Antitumor effect of paclitaxel is mediated by inhibition of myeloid-derived suppressor cells and chronic inflammation in the spontaneous melanoma model. *J. Immunol.* **190**, 2464–2471.

Sharma, A., Bode, B., Wenger, R.H., Lehmann, K., Sartori, A.A., Moch, H., Knuth, A., Boehmer, L., and Broek, M. (2011). γ -Radiation promotes immunological recognition of cancer cells through increased expression of cancer-testis antigens in vitro and in vivo. *PLoS ONE* **6**, e28217.

Shurin, G.V., Tourkova, I.L., Kaneno, R., and Shurin, M.R. (2009). Chemotherapeutic agents in noncytotoxic concentrations increase antigen presentation by dendritic cells via an IL-12-dependent mechanism. *J. Immunol.* **183**, 137–144.

Soeda, A., Morita-Hoshi, Y., Makiyama, H., Morizane, C., Ueno, H., Ikeda, M., Okusaka, T., Yamagata, S., Takahashi, N., Hyodo, I., et al. (2009). Regular dose of gemcitabine induces an increase in CD14⁺ monocytes and CD11c⁺ dendritic cells in patients with advanced pancreatic cancer. *Jpn. J. Clin. Oncol.* **39**, 797–806.

Soriani, A., Zingoni, A., Cerboni, C., Iannitto, M.L., Ricciardi, M.R., Di Gialleonardo, V., Cippitelli, M., Fiorda, C., Petrucci, M.T., Guarini, A., et al. (2009). ATM-ATR-dependent up-regulation of DNAM-1 and NKG2D ligands on multiple myeloma cells by therapeutic agents results in enhanced NK-cell susceptibility and is associated with a senescent phenotype. *Blood* **113**, 3503–3511.

Spisek, R., Charalambous, A., Mazumder, A., Vesole, D.H., Jagannath, S., and Dhodapkar, M.V. (2007). Bortezomib enhances dendritic cell (DC)-mediated induction of immunity to human myeloma via exposure of cell surface heat shock protein 90 on dying tumor cells: therapeutic implications. *Blood* **109**, 4839–4845.

Stagg, J., Divisekera, U., Duret, H., Sparwasser, T., Teng, M.W., Darcy, P.K., and Smyth, M.J. (2011). CD73-deficient mice have increased antitumor immunity and are resistant to experimental metastasis. *Cancer Res.* **71**, 2892–2900.

Sukkurwala, A.Q., Martins, I., Wang, Y., Schlemmer, F., Ruckenstein, C., Durchschlag, M., Michaud, M., Senovilla, L., Sistigu, A., Ma, Y., et al. (2013). Immunogenic calreticulin exposure occurs through a phylogenetically conserved stress pathway involving the chemokine CXCL8. *Cell Death Differ.* Published online June 21, 2013. <http://dx.doi.org/10.1038/cdd.2013.73>.

Sumimoto, H., Imabayashi, F., Iwata, T., and Kawakami, Y. (2006). The BRAF-MAPK signaling pathway is essential for cancer-immune evasion in human melanoma cells. *J. Exp. Med.* **203**, 1651–1656.

Suzuki, E., Kapoor, V., Jassar, A.S., Kaiser, L.R., and Albelda, S.M. (2005). Gemcitabine selectively eliminates splenic Gr-1⁺/CD11b⁺ myeloid suppressor cells in tumor-bearing animals and enhances antitumor immune activity. *Clin. Cancer Res.* **11**, 6713–6721.

Suzuki, E., Sun, J., Kapoor, V., Jassar, A.S., and Albelda, S.M. (2007). Gemcitabine has significant immunomodulatory activity in murine tumor models independent of its cytotoxic effects. *Cancer Biol. Ther.* **6**, 880–885.

Suzuki, Y., Mimura, K., Yoshimoto, Y., Watanabe, M., Ohkubo, Y., Izawa, S., Murata, K., Fujii, H., Nakano, T., and Kono, K. (2012). Immunogenic tumor cell death induced by chemoradiotherapy in patients with esophageal squamous cell carcinoma. *Cancer Res.* **72**, 3967–3976.

Taieb, J., Chaput, N., Ménard, C., Apetoh, L., Ullrich, E., Bonmort, M., Péquignot, M., Casares, N., Terme, M., Flament, C., et al. (2006). A novel dendritic cell subset involved in tumor immunosurveillance. *Nat. Med.* **12**, 214–219.

Tanaka, H., Matsushima, H., Mizumoto, N., and Takashima, A. (2009). Classification of chemotherapeutic agents based on their differential in vitro effects on dendritic cells. *Cancer Res.* **69**, 6978–6986.

Tesniere, A., Schlemmer, F., Boige, V., Kepp, O., Martins, I., Ghiringhelli, F., Aymeric, L., Michaud, M., Apetoh, L., Barault, L., et al. (2010). Immunogenic death of colon cancer cells treated with oxaliplatin. *Oncogene* **29**, 482–491.

- Thomas-Schoemann, A., Batteux, F., Mongaret, C., Nicco, C., Chéreau, C., Annereau, M., Dauphin, A., Goldwasser, F., Weill, B., Lemare, F., and Alexandre, J. (2012). Arsenic trioxide exerts antitumor activity through regulatory T cell depletion mediated by oxidative stress in a murine model of colon cancer. *J. Immunol.* *189*, 5171–5177.
- Tjon, A.S., Sint Nicolaas, J., Kwekkeboom, J., de Man, R.A., Kazemier, G., Tilanus, H.W., Hansen, B.E., van der Laan, L.J., Tha-In, T., and Metselaar, H.J. (2010). Increased incidence of early de novo cancer in liver graft recipients treated with cyclosporine: an association with C2 monitoring and recipient age. *Liver Transpl.* *16*, 837–846.
- Tsavaris, N., Kosmas, C., Vadiaka, M., Kanelopoulos, P., and Boulamatsis, D. (2002). Immune changes in patients with advanced breast cancer undergoing chemotherapy with taxanes. *Br. J. Cancer* *87*, 21–27.
- van de Ven, R., Reurs, A.W., Wijnands, P.G., van Wetering, S., Kruisbeek, A.M., Hooijberg, E., Scheffer, G.L., Scheper, R.J., and de Gruijl, T.D. (2012). Exposure of CD34+ precursors to cytostatic anthraquinone-derivatives induces rapid dendritic cell differentiation: implications for cancer immunotherapy. *Cancer Immunol. Immunother.* *61*, 181–191.
- van der Most, R.G., Currie, A.J., Cleaver, A.L., Salmons, J., Nowak, A.K., Mahendran, S., Larma, I., Prosser, A., Robinson, B.W., Smyth, M.J., et al. (2009). Cyclophosphamide chemotherapy sensitizes tumor cells to TRAIL-dependent CD8 T cell-mediated immune attack resulting in suppression of tumor growth. *PLoS ONE* *4*, e6982.
- Vandenabeele, P., Galluzzi, L., Vanden Berghe, T., and Kroemer, G. (2010). Molecular mechanisms of necroptosis: an ordered cellular explosion. *Nat. Rev. Mol. Cell Biol.* *11*, 700–714.
- Vanneman, M., and Dranoff, G. (2012). Combining immunotherapy and targeted therapies in cancer treatment. *Nat. Rev. Cancer* *12*, 237–251.
- Venereau, E., Casalgrandi, M., Schiraldi, M., Antoine, D.J., Cattaneo, A., De Marchis, F., Liu, J., Antonelli, A., Preti, A., Raeli, L., et al. (2012). Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine release. *J. Exp. Med.* *209*, 1519–1528.
- Vesely, M.D., Kershaw, M.H., Schreiber, R.D., and Smyth, M.J. (2011). Natural innate and adaptive immunity to cancer. *Annu. Rev. Immunol.* *29*, 235–271.
- Viaud, S., Flament, C., Zoubir, M., Pautier, P., LeCesne, A., Ribrag, V., Soria, J.C., Marty, V., Vielh, P., Robert, C., et al. (2011). Cyclophosphamide induces differentiation of Th17 cells in cancer patients. *Cancer Res.* *71*, 661–665.
- Vincent, J., Mignot, G., Chalmin, F., Ladoire, S., Bruchard, M., Chevriaux, A., Martin, F., Apetoh, L., Rébé, C., and Ghiringhelli, F. (2010). 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. *Cancer Res.* *70*, 3052–3061.
- von Boehmer, L., Draenert, A., Jungraithmayr, W., Inci, I., Niklaus, S., Boehler, A., Hofer, M., Stahel, R., Soltermann, A., van den Broek, M., et al. (2012). Immunosuppression and lung cancer of donor origin after bilateral lung transplantation. *Lung Cancer* *76*, 118–122.
- Waitz, R., Solomon, S.B., Petre, E.N., Trumble, A.E., Fassò, M., Norton, L., and Allison, J.P. (2012). Potent induction of tumor immunity by combining tumor cryoablation with anti-CTLA-4 therapy. *Cancer Res.* *72*, 430–439.
- Wan, S., Pestka, S., Jubin, R.G., Lyu, Y.L., Tsai, Y.C., and Liu, L.F. (2012). Chemotherapeutics and radiation stimulate MHC class I expression through elevated interferon-beta signaling in breast cancer cells. *PLoS ONE* *7*, e32542.
- West, N.R., Milne, K., Truong, P.T., Macpherson, N., Nelson, B.H., and Watson, P.H. (2011). Tumor-infiltrating lymphocytes predict response to anthracycline-based chemotherapy in estrogen receptor-negative breast cancer. *Breast Cancer Res.* *13*, R126.
- Westervelt, P., Pollock, J.L., Oldfather, K.M., Walter, M.J., Ma, M.K., Williams, A., DiPersio, J.F., and Ley, T.J. (2002). Adaptive immunity cooperates with liposomal all-trans-retinoic acid (ATRA) to facilitate long-term molecular remissions in mice with acute promyelocytic leukemia. *Proc. Natl. Acad. Sci. USA* *99*, 9468–9473.
- White, E. (2012). Deconvoluting the context-dependent role for autophagy in cancer. *Nat. Rev. Cancer* *12*, 401–410.
- Wilmott, J.S., Long, G.V., Howle, J.R., Haydu, L.E., Sharma, R.N., Thompson, J.F., Kefford, R.F., Hersey, P., and Scolyer, R.A. (2012). Selective BRAF inhibitors induce marked T-cell infiltration into human metastatic melanoma. *Clin. Cancer Res.* *18*, 1386–1394.
- Wu, X.R., He, X.S., Chen, Y.F., Yuan, R.X., Zeng, Y., Lian, L., Zou, Y.F., Lan, N., Wu, X.J., and Lan, P. (2012). High expression of CD73 as a poor prognostic biomarker in human colorectal cancer. *J. Surg. Oncol.* *106*, 130–137.
- Yamazaki, T., Hannani, D., Poirier-Colame, V., Ladoire, S., Locher, C., Prada, N., Chaput, N., Andre, F., Feudenberg, M., Galanos, C., et al. (2013). Defective immunogenic cell death of HMGB1-deficient cancers: compensatory therapy with synthetic TLR4 agonists. *Cell Death Differ.* Published online June 28, 2013. <http://dx.doi.org/10.1038/cdd.2013.72>.
- Yang, Y., Liu, C., Peng, W., Lizée, G., Overwijk, W.W., Liu, Y., Woodman, S.E., and Hwu, P. (2012). Antitumor T-cell responses contribute to the effects of dasatinib on c-KIT mutant murine mastocytoma and are potentiated by anti-OX40. *Blood* *120*, 4533–4543.
- Yuan, L., Wu, L., Chen, J., Wu, Q., and Hu, S. (2010). Paclitaxel acts as an adjuvant to promote both Th1 and Th2 immune responses induced by ovalbumin in mice. *Vaccine* *28*, 4402–4410.
- Zitvogel, L., Apetoh, L., Ghiringhelli, F., and Kroemer, G. (2008). Immunological aspects of cancer chemotherapy. *Nat. Rev. Immunol.* *8*, 59–73.
- Zitvogel, L., Kepp, O., and Kroemer, G. (2010). Decoding cell death signals in inflammation and immunity. *Cell* *140*, 798–804.
- Zitvogel, L., Kepp, O., and Kroemer, G. (2011). Immune parameters affecting the efficacy of chemotherapeutic regimens. *Nat. Rev. Clin. Oncol.* *8*, 151–160.