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Molecular and Translational Classifications of DAMPs in Immunogenic Cell Death

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Abbreviations: DAMP, damage-associated molecular pattern; DC, dendritic cell; ER, endoplasmic reticulum; GEMM, genetically engineered murine model; HSP, heat shock protein; Hyp, hypericin; ICD, immunogenic cell death; NDV, Newcastle disease virotherapy; PDT, photodynamic therapy; ROS, reactive oxygen species.

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The immunogenicity of malignant cells has recently been acknowledged as a critical determinant of efficacy in cancer therapy. Thus, besides developing direct immunostimulatory regimens, including dendritic cell-based vaccines, checkpoint-blocking therapies, and adoptive T-cell transfer, researchers have started to focus on the overall immunobiology of neoplastic cells. It is now clear that cancer cells can succumb to some anticancer therapies by undergoing a peculiar form of cell death that is characterized by an increased immunogenic potential, owing to the emission of the so-called "damage-associated molecular patterns" (DAMPs). The emission of DAMPs and other immunostimulatory factors by cells succumbing to immunogenic cell death (ICD) favors the establishment of a productive interface with the immune system. This results in the elicitation of tumor-targeting immune responses associated with the elimination of residual, treatment-resistant cancer cells, as well as with the establishment of immunological memory. Although ICD has been characterized with increased precision since its discovery, several questions remain to be addressed. Here, we summarize and tabulate the main molecular, immunological, preclinical, and clinical aspects of ICD, in an attempt to capture the essence of this phenomenon, and identify future challenges for this rapidly expanding field of investigation.

Keywords: anti-tumor immunity, immunogenicity, immunotherapy, molecular medicine, oncoimmunology, patient prognosis, translational medicine

INTRODUCTION AND HISTORICAL BACKGROUND

Augmenting the immunogenicity of cancer cells to improve the efficacy of cancer therapy is a paradigm that has gained significant momentum over the past 5 years (1–5). Researchers have realized that besides therapeutically exploiting innate or adaptive immune cells directly (e.g., through dendritic cell (DC)-based vaccines or adoptive T-cell transfer) and/or improving the effector functions of T cells (through checkpoint-blocking therapies), cancer cells also need to be made immunogenic (1, 4, 6, 7). This has diverted attention toward studying the interface between stressed or dying cancer cells and the immune system, in the hope of efficiently exploiting it for therapeutic purposes (1).

Early indications regarding immune system-driven tumor control emerged in the eighteenth century, when feverish infections in cancer patients were circumstantially associated with tumor remission (8). The first evidence that immunotherapy can be applied to achieve tumor regression emerged from the work of William Coley, who in the 1890s achieved tumor regression in some sarcoma/lymphoma patients upon the intra-tumoral injection of streptococcal cultures (provided by Robert Koch) (8, 9). In the following 43 years, Coley injected nearly 900 (mostly sarcoma) patients with his bacterial preparation (achieving a cure rate >10%), which later became known as "Coley's toxin" (8, 10). However, the Coley's toxin came under intense scrutiny owing to an elevated toxicity and some difficulties in reproducing remission rates (8). Eventually, the first experimental evidence that virus-unrelated tumors can indeed be recognized by the host immune system emerged in the 1940s, and by the 1960s, coupled with the discovery of T cells, it was proposed that the human immune system may also react against tumors (11). The ability

of anticancer therapies to enhance the immunogenic potential of malignant cells gained some appreciation by the 1970s (12–14). It was recognized that if specific treatments are applied (e.g., radiotherapy, the bacillus Calmette–Guerin, or some chemotherapeutics), the immunogenicity of malignant cells increases enough to induce durable anti-tumor immunity (12–14). By the 1980s, researchers started to report more specific observations regarding the therapeutic impact of cancer cell immunogenicity, e.g., the ability of curative hyperthermia to cause the (heat-shock based) generation of circumstantial anti-tumor immunity (15), the fact that the immunogenicity of cancer cells influences patient prognosis after radiotherapy (16), and the increase in tumor immunogenicity due to hydrostatic pressure (17). However, these early studies (especially those published before the 1980s) had several issues linked to a lack in consensus. For instance, due to early controversies on the existence of tumor-associated antigens (TAAs) (11), the target of tumor-specific immune responses was unclear, and the mechanism of action of some therapies came under scrutiny. Moreover, such therapies could operate by directly modulating immune effector cells rather than improving the immunogenic potential of tumors (18). In particular, the death of cancer cells exposed to therapy was never suspected to drive anti-tumor immunity, since it was considered to be a relatively "silent" process in terms of immunogenicity (19). Moreover, the classical "self/non-self" theory was unable to explain the possibility that dying cancer cells could elicit an immune response (20).

By the early 1990s, the molecular characterization of mice and human TAAs clarified the entities targeted by anti-tumor immune responses (11). Similarly, the so-called "danger theory" started to emerge, challenging the classical model of "self/non-self" immune recognition, especially in a diseased or damaged tissue (20, 21). This model proposed that the immune recognition is not restricted to "non-self" entities, but rather discriminates between "dangerous" and "safe" entities, irrespective of source (20–22). Indeed, "dangerous" entities include pathogens as well as injured, infected, diseased and necrotic tissues, or cells undergoing non-physiological cell death which emit danger signals (or alarmins) with pro-inflammatory activity (21, 22). These danger signals are now collectively referred to as "damage-associated molecular patterns" (DAMPs) (23). DAMPs are endogenous molecules that are concealed intracellularly in normal conditions, but are exposed or released upon stress, injury, cell death, thereby becoming able to bind cognate receptors on immune cells (3, 24–27). **Table 1** summarizes the most prominent DAMPs characterized to date and their mode of emission, the cell death pathway they are associated with, and their known cognate receptors. It is important to consider that not all DAMPs may act as immunogenic danger signals. Several DAMPs exist that are crucial for the maintenance of tissue homeostasis, and the avoidance of auto-immune responses, as they exert immunosuppressive effects, including phosphatidylserine (PS), annexin A1 (ANXA1), death domain 1α (DD1α), B-cell CLL/lymphoma 2 (BCL2) and some extracellular matrix-derived molecules (**Table 1**). Accordingly, the blockade of these anti-inflammatory DAMPs accentuates the immunogenic potential of dying cells, or renders immunogenic otherwise tolerogenic forms of cell death (28, 29). Moreover, some danger signals are not always involved in the immunogenicity of cell death, but act as "bystanders." This is the case for heat shock protein 90 kDa alpha (cytosolic), class A member 1 (HSP90AA1, best known as HSP90) exposed on the cell surface after melphalan treatment (30). Last (but not least), several DAMPs may be subjected to post-translational modifications (e.g., oxidation, reduction, citrullination) that may potentially neutralize, increase, or change their immunogenic properties (31, 32) – a process that is still incompletely understood.

Despite these advances, the overall role of regulated cell death (RCD) (97) in augmenting cancer immunogenicity remained obscure. Initial observations involving the immunogenicity of cell death in the efficacy of cancer therapy were published between 1998 and 2004, when it was proposed that the non-apoptotic demise of malignant cells (within the context of the so-called "immunogenic death") could be associated with the emission of the danger signal heat shock 70 kDa protein 1A (HSPA1A, best known as HSP70) (**Table 1**), enhancing the immunogenic potential of dying cancer cells *in vivo* (98, 99). The dogmatic view that only necrotic or non-apoptotic (as postulated by the "immunogenic death" concept) cancer cells are characterized by an elevated immunogenic potential started to be questioned by a series of studies published between 2005 and 2007 (41, 70, 100, 101). These publications outlined that cancer cells undergoing apoptosis in response to specific anticancer therapies are immunogenic [a subroutine termed immunogenic cell death (ICD)], as long as they emit precise DAMPs in a spatiotemporally defined fashion (26, 102, 103). Cells succumbing to ICD are sufficient for the elicitation of durable anti-tumor immune responses (1, 26, 53, 102, 104). ICD is indeed paralleled by the redirection and emission of DAMPs, owing to the stimulation of distinct danger signaling pathways occurring in synchrony with cell death signaling (103). **Table 2** summarizes the main signaling pathways that play a role in the trafficking and emission of DAMPs. ICD-associated DAMPs and other immunostimulatory factors released by cells destined to undergo ICD favor the establishment of a productive interface between dying cancer cells and innate immune cells (like DCs or macrophages), thereby leading to the initiation of a therapeutically relevant adaptive immune response (**Figure 1**) (102, 105). In some contexts, DAMPs may regulate the function of specific innate immune cell subsets, e.g., following anthracycline treatment, extracellular adenosine triphosphate (ATP) assists in recruitment and differentiation of $CD11c^+Cd11b^+Ly6C^{high}$ cells into $CD11c^+CD86^+MHCII^+$ DCs (106); similarly, necrosis associated F-actin exposure activates an immune response by directing the dead cell debris to specifically $CD8\alpha^+$ DCs (59, 107). Indeed, DCs and other antigen-presenting cells exposed to cancer cells succumbing to ICD can then prime $CD4^+$ T cells (and polarize them into T_H1, T_H17, or T_H1/T_H17-like phenotype), $CD8^+$ cytotoxic T lymphocytes (CTLs) and γδ T lymphocytes against one or several TAAs (**Figure 1**) (102). Of note, residual cancer cells that survive ICD inducers can also show some enduring immunogenic characteristics that make them susceptible to immunological control by CTLs (108–110).

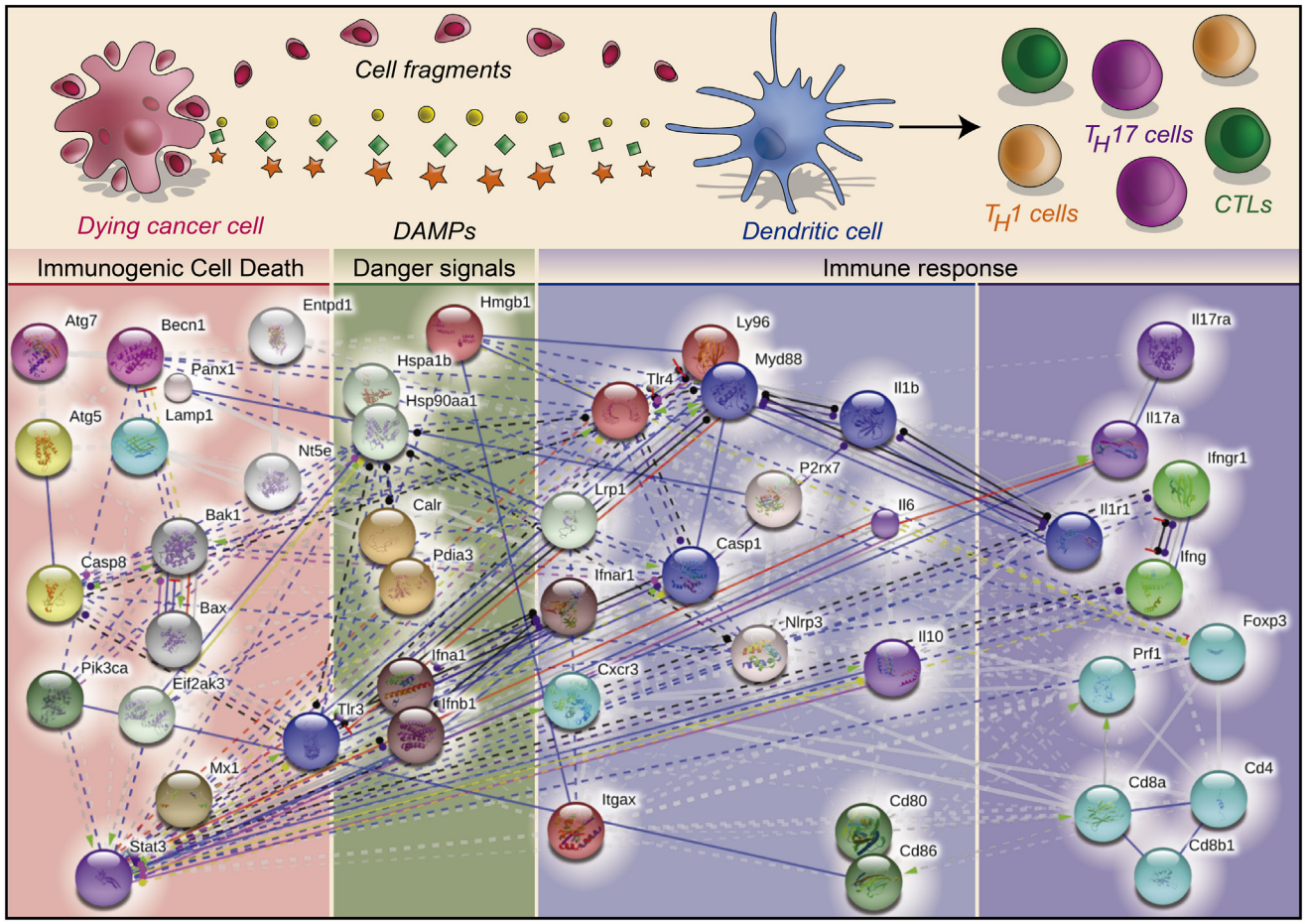

FIGURE 1 | The molecular complexity of immunogenic cell death in cancer. Cancer cells undergoing immunogenic cell death (ICD) emit danger signals for establishing a productive interface with components of the host immune system, including dendritic cells (DCs). DCs exposed to cancer cells succumbing to ICD "prime" the adaptive arm of the immune system, consisting of various effector T-cell populations, which in turn targets therapy-resistant cancer cells. Various molecules are critical for the execution of these processes. The molecular network of ICD-relevant proteins was build using the STRING modeling database (http://string-db.org/) (126).

IMMUNOGENIC CELL DEATH INDUCERS

Over the past few years, a number of single-agent ICD inducers have been discovered, encompassing conventional chemotherapeutics, targeted anticancer agents and various other biological and physicochemical therapies (18, 102, 104, 127). **Table 3** summarizes single-agent ICD inducers characterized so far, as per consensus guidelines (104), and the spectra of DAMPs and other immunostimulatory signals associated with them. For combinatorial therapeutic strategies capable of achieving ICD, readers may want to refer to other recent publications (18, 128, 129). It is clear that a general structure–function relationship capable of clustering all existing ICD inducers and predicting new ones does not exist (130), an issue that makes discovering new ICD-inducing therapies based on cheminformatic analyses challenging, if not impossible. A peculiar characteristic of most, if not all, ICD inducers is their ability to induce reactive oxygen species (ROS)-based/associated endoplasmic reticulum (ER) stress, as first delineated for anthracyclines (30, 34, 35, 42, 123, 131–133). This peculiarity was exploited for the targeted discovery of hypericin-based photodynamic therapy (Hyp-PDT) – a therapeutic modality that can trigger ICD through the induction of ROS that target the ER (35, 116, 134). Along with an ever more precise characterization of the links between ROS, ER stress, and ICD induction (135, 136), it became clear that the more "focused" ER stress is, the higher the probability of inducing ICD (3, 26, 53, 137). These observations paved way for a classification system based on how ICD inducers engage ER stress for cell death and danger signaling (3, 26, 53, 138). Based on this classification, Type I ICD inducers are defined as anticancer agents that act on non-ER proteins for the induction of cell death, but promote collateral ER stress for danger signaling, thereby operating on multiple targets (3, 26, 53), while Type II ICD inducers are anticancer agents that target the ER for both cell death induction and danger signaling (3, 26, 53). **Table 4** summarizes the classification of current ICD inducers into Type I and Type II, and their cell death/danger signaling targets. Such a classification suggest that while Type I ICD inducers can be discovered through various approaches (e.g., DAMP-based drug

screening platforms) (130, 139), putative Type II ICD inducers can be characterized rapidly on the basis of their ability to selectively or predominantly target the ER. Recent findings comforted the purpose and usefulness of this classification system, as two novel Type II ICD inducers [i.e., Pt^{II} N-heterocyclic carbene complex (140) and Newcastle disease virotherapy (NDV) (43)] were identified based on the notion that they induce predominant ROS-based ER stress (138). Nevertheless, as more ICD inducers and features are discovered, this classification system is expected to evolve or be substituted by a more refined one.

Since its discovery, a plethora of molecular and immunological components responsible for ICD have been discovered (**Figure 1**) (26, 102, 188). **Table 5** summarizes the molecular and immunological determinants of ICD characterized so far, as well as the models of ICD in which they operate (in a positive, negative or dispensable manner). Anthracyclines and oxaliplatin are the most common ICD inducers employed in experimental settings, followed by Hyp-PDT. According to current understanding, cancer cell-associated determinants of ICD can be subdivided into those that are common to all ICD inducers (i.e., "core" signaling components), and those that operate in an ICD inducer-dependent manner (i.e., "private" signaling components) (26, 189). Thus, eukaryotic translation initiation factor 2-alpha kinase 3 (EIF2AK3, best known as PERK) and the ER-to-Golgi secretory machinery are considered "core" signaling components on the cancer cell side (26, 102). Similarly, from the immune system side, a general role for (IFNγ-producing) $CD4^+$ and $CD8^+$ T cells has been confirmed for most, if not all, ICD inducers (**Table 5**). Interestingly, some components that are required for ICD induction by some agents (like autophagy for anthracyclines and oxaliplatin) (190) might be either dispensable for ICD induction by other agents, e.g., autophagy for NDV (43) and phosphorylation of eukaryotic translation initiation factor 2α (eIF2α), caspase-8 (CASP8) activation or cytosolic Ca^{2+} levels for Hyp-PDT (35); or even negatively regulate ICD in some settings, e.g., autophagy in case of Hyp-PDT (34) (**Table 5**). Thus, it will be important to expand our molecular knowledge of ICD to as many experimental settings as possible.

IMMUNOGENIC CELL DEATH FROM BENCH TO BEDSIDE

The relevance of ICD has been verified in a number of rodent models, with a variety of chemical and physicochemical ICD inducers (26, 102). **Table 6** summarizes the most prominent mouse or rat models used so far for the characterization and study of ICD. For the moment, ICD has been mostly investigated in heterotopic syngeneic subcutaneous models (195). Within such models, inter-species differences (mouse *versus* rats), inter-strain differences (among BALB/c, C57BL/6, C3H and KMF mice), and inter-cell line differences, as well as differences in therapeutic setups (prophylactic *versus* curative) have been amply accounted for (**Table 6**). Nevertheless, there is predominance in the use of cancer cells derived from carcinogen-induced tumors and transplanted subcutaneously (**Table 6**). In very few cases, ICD has been characterized in either orthotopic (for NDV) or spontaneous (for anthracyclines) tumor murine models (**Table 6**). This has been questioned as a prominent Achilles' heel of ICD research (195). While this criticism is valid, it has to be recognized that no rodent model is perfect at all immunological levels (196).

As a recent systematic review summarized (196), heterotopic murine models suffer from a number of caveats, including the inability to recapitulate the early interaction between transformed cells and the immune system and the incompatibility between the cancer type and the site-of-transplantation (196). Orthotopic murine models are useful as they overcome the cancer cell-tissue type incompatibility issue (196). While genetically engineered tumor murine models (GEMMs) overcome most of the issues mentioned above, they come with their own set of shortcomings, including a limited genetic mosaicism, a low tumor heterogeneity, a lack of well-defined immunogenic TAAs, the presence of unintended "passenger" genetic modifications, and a reduced mutational spectrum (196). Many of these parameters are critical for responses to immunotherapy/ICD. For instance, the lack of well-defined immunogenic TAAs was the reason why preliminary results obtained in spontaneously developing murine tumors disputed the very existence of TAAs (11). Similarly, a high mutational spectrum (which produces considerable amounts of neo-antigens) has been found to be mandatory for the clinical efficacy of checkpoint blockers (209). Last (but not least), laboratory rodent models in general are associated with some critical issues, including the fact that a high level of inbreeding (which produces a number of shortcomings e.g., homozygous recessive defects) reduces the general immunological fitness, responsiveness and diversity in these models (196, 210, 211). Moreover, numerous immunological differences between mouse and humans tend to affect the translational relevance of the findings obtained (26, 211, 212). Also, the time frames of tumor growth rates between rodent models and humans are relatively divergent (196, 213, 214). This further complicates clinical translation of immunotherapeutic paradigms since the level of immunosurveillance and immunoediting experienced by human tumors can be much higher than any rodent tumor model.

In summary, it would be ideal to test ICD across as many different rodent models as possible, in order to determine the features that can be exploited for therapeutic purposes in humans. Moreover, if ICD fails in a specific experimental model, active effort should be made to characterize the mechanisms behind such failure, since resistance phenotypes can have profound clinical implications. This emerges from various studies summarized in **Table 7**. Indeed, several ICD resistance mechanisms exist operating at both the cancer cell and the immune system level, which have been characterized in different experimental models. Several of these resistance mechanisms have also been identified in cancer patients, thereby justifying further studies along these lines **Table 7**.

A considerable amounts of clinical findings support the relevance of ICD or ICD-related signatures in (at least subsets of) cancer patients. As summarized in **Table 8**, various ICD-linked (specific) parameters have been associated with the prognosis of cancer patients treated with clinically relevant ICD inducers (like anthracyclines, oxaliplatin, paclitaxel, or radiotherapy). Moreover, it is becoming clear that ICD-related or ICD-derived (immunological) genetic signatures (e.g., a *MX1*-centered

metagene, a *CXCR3-PRF1-CASP1*-centered metagene, an *ASAH1*-centered metagene) can be positively associated with good prognosis in patients affected by various neoplasms, including breast, lung, and ovarian malignancies (141, 188, 220). These observations indicate that ICD or ICD-relevant parameters may have prognostic or predictive relevance in at least a subset of cancer patients. It will be important to characterize new and more specific ICD-associated parameters linked to patient prognosis as well as biomarkers that may predict improved disease outcome in cancer patient treated with ICD inducers. Of note, considering the current clinical experience with immunotherapies (209, 221), the patients with an increased likelihood to benefit from ICD inducers are probably those that display pre-existing (baseline) immune reactivity against cancer cells (220, 222, 223). This may depend on the ability of ICD to reboot and/or revive pre-existing TAA-directed immunity rather to prime *de novo* immune reactivity (5, 191, 224). In future, it would be crucial to characterize biomarkers that allow clinicians to delineate patients with reduced baseline immune reactivity against malignant cells so that proper combinatorial therapies involving ICD inducers can be implemented.

CONFRONTING THE CLINICAL REALITIES OF ANTI-TUMOR IMMUNITY

It is well-established that the response of cancer patients to immunotherapy relies on the activity of effector T cells [that employ their T-cell receptors (TCRs) for recognizing TAAs]. However, these TAA-targeting T cells may also constitute obstacles for effective anti-tumor immunity (234). As opposed to T lymphocytes recognizing pathogen-associated antigens (PAAs) (**Figure 2**), indeed, T cells directed against some TAAs (derived from non-mutated proteins that are source of self or near-to-self antigens) are developmentally subjected to negative selection in the thymus and peripheral lymphoid organs (234, 235) (**Figure 2**). As a result, T cells bearing TCRs with high affinity for self antigens (including some TAAs) are clonally deleted to avoid auto-immunity (234–237) (**Figure 2**). However, some "leakiness" in this process allows TAA-specific T cells possessing TCRs with low affinity to escape deletion (234, 236, 237) and persist, although at low precursor frequencies (238) (**Figure 2**). Unfortunately, as compared to PAA-specific T cells, which bear high-affinity TCRs (**Figure 2**), TAA-specific T cells exhibit limited effector and memory functions (234, 239). Coupled with the tendency of progressing tumors to generate a highly immunosuppressive microenvironment, this renders the insurgence of lifelong protective immunity nearly impossible (234). Of note, central and peripheral tolerance may not affect T cells reactive toward neo-tumor-specific antigens (neo-TSAs) e.g., tumor-specific neo-antigens that are generated *de novo* in the course of tumor progression because of mutational events (240, 241). However, the extent to which such neo-TSAs can elicit consistent "immunodominant" T cell reactivity is still a matter of investigation (240, 241). Nevertheless, in this context, inefficient T-cell stimulation can be overcome through the ICD-based improvement of effector T-cell functions (102). ICD can be further combined with checkpoint-blocking therapies, which

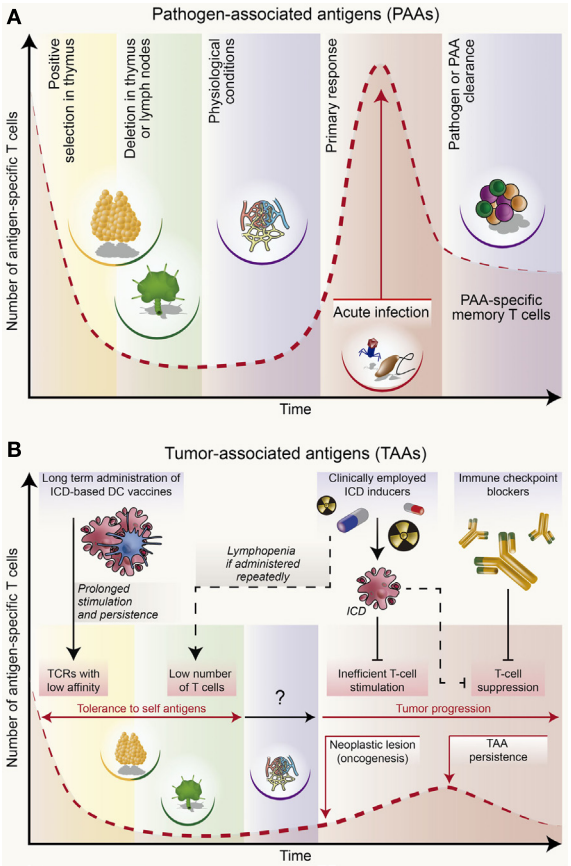

FIGURE 2 | Population dynamics of antigen-specific T cells during an immune response to infection or cancer. **(A)** T cells capable of putatively recognizing non-self, pathogen-associated antigens (PAAs) are not exposed to negative selection in the thymus or peripheral organs like lymph nodes. This allows for the constitutive presence of T lymphocytes bearing high-affinity T-cell receptor (TCR) in naïve conditions. Upon infection, these cells undergo robust expansion and acquire potent effector functions, hence driving an immune response that clears the pathogen and PAAs. Finally, PAA-specific T cells undergo contraction along with the establishment of immunological memory. To a limited extent, T cells reacting against PAAs expressed by virus-induced tumors may exhibit similar (although not identical) responses. **(B)** T cells that may recognize self or close-to-self antigens expressed by virus-unrelated malignancies undergo robust negative selection in the thymus and lymph nodes. Thus, all putative T lymphocytes bearing a high-affinity TCR against tumor-associated antigens (TAAs) are eliminated. However, some leakiness in this process allows for the persistence of TAA-specific T lymphocytes with low-affinity TCR, although at very low precursor frequencies. This is one of the reasons why in some individuals immunosurveillance at some stage fails to impede tumor progression. As malignant lesions progress, the amount of TAAs increases, causing a weak rise in TAA-specific T cells. However, tumor progression is generally coupled with the establishment of robust immunosuppressive networks that potently inhibit such TAA-targeting T cells. In this context, the administration of immunogenic cell death (ICD) according to a schedule that does not lead to lymphodepletion can favor the stimulation of TAA-targeting T cells and (re) instate immunosurveillance. Combining ICD inducers with checkpoint-blocking agents may further boost TAA-targeting immune responses. However, these treatments may not ensure the lifelong persistence of TAA-recognizing T cells, some of which are susceptible to elimination through tolerance mechanisms. Anticancer vaccines may counteract, at least to some extent, such loss. The figure was partly inspired from Baitsch et al. (234).

potently reverse immunosuppression (209, 242). However, the lifelong maintenance of anti-tumor T cells remains a particularly hard challenge.

In the clinical reality, anticancer agents are administered to patients in a limited number of cycles. Even if these therapeutic regimens may attain optimal efficacy in terms of ICD induction, they are unlikely to ensure the lifelong persistence of TAA-directed T cells with low-affinity TCR (234, 243). This probably reflects the contraction of TAA-targeting T cells occurring once the immunostimulatory stimulus provided by ICD ceases, owing to peripheral tolerance mechanisms (234). Clinically, it may not be feasible to administer ICD inducers repeatedly over time, since many of them can cause lymphopenia (which negatively affects disease outcome), or are associated with other side effects (244). It has been proposed that active immunization with ICD-based anticancer vaccines (which are associated with robust immunogenicity) given in a repetitive manner may achieve this goal (**Figure 2**) (234, 243, 245). Thus, it will be important to test whether the long-term administration of ICD-based anticancer vaccines can sustain the effector function of TAA-specific T cells bearing low-affinity TCRs, hence, ensuring lifelong disease-free survival. Of note, in the case of hematological malignancies, this issue could be overcome upon the adoptive transfer of CTLs expressing chimeric antigen receptors (CARs) (1). However, whether CAR-expressing CTLs generate protective immunological memory in the absence of considerable side effects remains to be determined. Moreover, the use of this therapeutic strategy against solid malignancies is relatively challenging owing to lack of well-defined "unique" TAAs (1, 246).

CONCLUSION

The model of ICD has been considerably refined since the initial identification of a cell death modality manifesting apoptotic features but able to induce an adaptive immune response. This model strives to integrate several phenomena observed throughout the second half of the twentieth century in one therapeutically relevant platform. However, as discussed above, several challenges still need to be addressed. First, comprehensive testing should be performed in advanced experimental settings like GEMMs or orthotopic tumor models. Second, ICD resistance mechanisms should be characterized with precision. Third, various issues linked to the successful translation of ICD to cancer therapy will have to be resolved, including (but not limited to) treatment schedules, dosages, and combinatorial strategies. This translational drive also needs to be coupled with effective strategies for the discovery of new and effective ICD inducers. Drug screening programs are often complicated by the possibility of false-positive (due to bystander presence of DAMPs) (30) or false-negative (due to limited number of biomarkers used for screening) hits. This issue can only be ironed out by discovering new and common regulators of ICD, and integrating them into existing screening platforms. Last, but not least, it will be important to identify new ICD-related/derived biomarkers that can be used to improve current protocols of patient stratification and clinical decision making. We are positive that all these objectives are at reach.

AUTHOR CONTRIBUTIONS

ADG did the literature study, data collection, as well as conceived and wrote the manuscript. PA provided senior supervision and guidance, conceived the paper, helped in writing, and critically revised the manuscript. LG improved and edited the manuscript. JMBSP helped with the preparation of figures. All authors participated in the critical reading of the manuscript (wherever applicable), approved content and conclusions, as well as helped in ensuring the accuracy of cited literature.

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TABLES

TABLE 1 | A list of prominent damage-associated molecular patterns (DAMPs) associated with cell death pathways or extracellular matrix.

DAMPs	Localization and mode-of-emission	Relevant cell death pathway	Receptors	Reference
Annexin A1	Surface exposed or actively/passively released?	Apoptosis	FPR-1 receptor	(33)
Adenosine triphosphate	Actively or passively released	ICD, apoptosis/secondary necrosis and necrosis	P_2Y_2 and P_2X_7	(34–37)
B-cell CLL/lymphoma 2	Passive release	Necrosis	TLR2	(38)
Biglycan	Extracellular matrix	–	TLR2, TLR4, P_2X_4, and P_2X_7	(39, 40)
Calreticulin	Mostly surface exposed; sometimes passively released	ICD	CD91	(35, 41–44)
Cardiolipin	Surface exposed?	Apoptosis	?	(45, 46)
Ceramide and sphingosine-1-phosphate	Surface exposed	Apoptosis	?	(47)
Covalent/cross-linked dimer of ribosomal protein S19	Passively released?	Apoptosis	CD88	(48–51)
Carbamoyl-phosphate synthase 1	?	?	?	(52)
Cyclophilin A	Passive release	Necrosis	CD147	(53)
Cytochrome *c*	Passively released?	Secondary necrosis and necrosis?	LPG?	(54, 55)
Death domain 1α	Surface exposed	Apoptosis	DD1α	(56)
Endothelial monocyte-activating polypeptide II	Passively released?	Apoptosis	CXCR3?	(50, 57, 58)
F-actin	Passive release	Necrosis	DNGR-1/Clec9a	(59)
Fibrinogen	Extracellular matrix	–	TLR4	(40)
Fibronectin extra domain A	Extracellular matrix	–	TLR4?	(40)
Fragments of human tyrosyl tRNA synthetase	Passively released?	Apoptosis	?	(50)
Genomic DNA, mRNA, snRNPs	Passive release	Necrosis	TLR3	(3, 60, 61)
GRP78/BiP	Passive release	Necrosis, apoptosis?	?	(31)
H_20_2	?	Apoptosis	?	(62)
Heat shock proteins (HSP70, HSP90, HSP60, HSP72, and GP96)	Surface exposure, active secretion, or passive release	ICD, apoptosis/secondary necrosis, necrosis	CD91, TLR2, TLR4, SREC-1 and FEEL-1	(63–67)
Heparan sulfate fragments	Extracellular matrix	–	TLR4	(40)
Hepatoma-derived growth factor	Passively released	Necrosis	?	(68)
Histones	Passively released	Necrosis	TLR-9	(69)
High-mobility group box 1	Mostly passively released; sometimes actively released	ICD, secondary necrosis and necrosis	TLR2, TLR4, RAGE and TIM3	(70–73)
High-mobility group nucleosome binding domain 1	Passive release	Necrosis	TLR4	(74)
Hyaluronan	Extracellular matrix	–	TLR2 and TLR4	(40)
IL-1α	Passive release	Necrosis	IL-1R	(75)
IL-33	Passive release	Necrosis	ST2	(3, 61)
IL-6	Passive release	Necrosis	IL-6R and GP130	(76)
Lysophosphatidylcholine	Passively released?	Apoptosis	G2A	(50, 77)
Mit DNA	Passively released	Necrosis	TLR-9	(78–80)
Monosodium urate or uric acid	Passively released	Necrosis	Purinergic receptors	(50, 81)
N-formylated peptides	Passively released	Necrosis	FPR-1	(78, 82–84)
Oxidation-associated molecular patterns (reactive protein carbonyls, per-oxidized phospholipids, oxidized low-density lipoprotein)	Passively released	Necrosis, Secondary necrosis	CD36, SR-A, TLR-2/4, CD14	(85–87)
Peroxiredoxin 1	Actively secreted or passively released	Apoptosis, necrosis	TLR4	(88)

(Continued)

TABLE 1 | Continued

DAMPs	Localization and mode-of-emission	Relevant cell death pathway	Receptors	Reference
Phosphatidylserine	Actively externalized on the surface	Apoptosis	TIM-1/-3/-4, BAI1, Stabilin-2, MFG-E8, C1q	(56, 89–93)
S100/calgranulin protein family members (S100A8, S100A9, S100A12/EN-RAGE)	Passively released	Necrosis	RAGE	(50, 94)
Tenascin-C	Extracellular matrix	–	TLR4?	(95)
Thrombospondin 1 and its heparin-binding domain	Passively released or surface associated	Apoptosis	$\alpha_v\beta_3$ integrin	(50, 96)
Versican	Extracellular matrix	–	TLR2, TLR6, and CD14	(40)

CD, cluster of differentiation; CLEC9A, C-type lectin domain family 9, member A; CPS-1, carbamoyl-phosphate synthase 1, mitochondrial; CXCR3, C-X-C motif receptor 3; FEEL-1/CLEVER-1, fasciclin EGF-like/common lymphatic endothelial and vascular endothelial receptor-1; FPR-1, formyl peptides receptor-1; G2A, G2 accumulation; HMGB1, high-mobility group box 1; HSP, heat shock proteins; ICD, immunogenic cell death; IL, interleukin; LPG, leucine-rich alpha-2-glycoprotein-1; MFG-E8, milk fat globule-egf factor 8 protein; Mit DNA, mitochondrial DNA; P2XR, P2X receptor; P2YR, P2Y receptor; RAGE, receptor for advanced glycation endproducts; SREC-1, scavenger receptor class f member 1; TFAM, mitochondrial transcription factor A; TIM, transmembrane immunoglobulin and mucin domain; TLR, toll-like receptor(s).

Glossary (5, 19, 97): (1) Necrosis: primary necrosis is a form of cell death that can occur in a regulated or accidental manner, characterized by cellular swelling and rapid breakdown of the plasma membrane; (2) Necroptosis: necroptosis is a form of regulated cell death (RCD) manifesting with necrotic morphology and controlled by a signaling cascade involving (among other proteins) RIPK1, RIPK3, and MLKL; (3) Apoptosis: apoptosis is a form of RCD largely dependent on caspases activity and morphologically characterized by cell shrinkage, membrane blebbing, formation of apoptotic bodies, chromatin condensation, and systematic DNA fragmentation; (4) Secondary Necrosis: Secondary necrosis is a terminal process experienced by late-apoptotic cells if they are not cleared by phagocytes in time, and is characterized by general spill-over of apoptotic cellular contents.

"?" Unclear or not determined yet.

TABLE 2 | Danger signaling pathways characterized as traffickers of DAMPs.

DAMPs	Role of ROS	Role of ER stress	Role of autophagy	Role of chaperone-mediated autophagy	Role of secretory pathway	Caspase activity	Role of lysosomes	Comments	Reference
Secreted ATP	+	+/0	+/0	0	+/0	+	+/0	Underlying pathway is highly inducer dependent	(34, 35, 111–113)
Released HMGB1	0	0	+	?	0	–	?	Mostly released passively on account of necrosis; only DT-EGF reported to cause active secretion so far	(73, 114, 115)
Secreted or surface HSP70	?	?	?	?	?	+	+	ABC transporters help in endolysosomal-secretion; HSP70 has also been reported to be secreted in an exosome surface-bound format	(116–122)
Surface CRT	+	+	–/0	+	+	+/0	?	LRP1/lipid rafts mediate surface tethering; components that positively regulate surface-CRT in an inducer-dependent fashion: ERp57, PI3K p110α, BAX/BAK, cytosolic ER-Ca^{2+}, BAP31; of note, anthracycline-induced pathway of surface CRT induction has been found to be conserved from yeast to mammals	(34, 35, 111, 112, 116, 123, 124)
Surface HSP90	+	+	–	?	+	+	?	–	(30, 125)

"+" denotes ability to positively regulate trafficking; "–" denotes ability to negatively regulate trafficking; "0" denotes confirmation of no role in regulation of trafficking and "?" denotes that the role in regulating the trafficking is unknown; "+/0" denotes positive or no role in regulation of trafficking in an inducer-dependent fashion; "–/0" denotes negative or no role in regulation of trafficking in an inducer-dependent fashion.

ATP, adenosine triphosphate; CRT, calreticulin; DT-EGF, epidermal growth factor receptor-targeted diphtheria toxin; ER, endoplasmic reticulum; HMGB1, high-mobility group box 1 protein; HSP, heat shock protein; LRP1, low-density lipoprotein receptor-related protein 1; ROS, reactive oxygen species.

TABLE 3 | A list of prominent single-agent immunogenic cell death (ICD) inducers in cancer and their specific associations with danger signaling and other immunostimulatory signaling.

ICD inducers	Associated ICD-relevant DAMPs		Other immunostimulatory activities or danger signals and other comments on immunomodulatory activity	Reference
	DAMP	Stage of cell death		
Anthracyclines (epirubicin, doxorubicin, idarubicin, mitoxantrone), oxaliplatin, UVC radiation and radiotherapy	Surface CRT Surface HSP70 Secreted ATP Released HMGB1	Pre-apoptotic Mid-apoptotic Early/mid-apoptotic Post-apoptotic	Activation of Type I IFN response comprising MX-1 centered signature, consisting of IFN-α/β and CXCL10; surface exposure of mannose-6-phopshate receptor, which enables better interface with CTLs and facilitates GZMB-mediated cell death; radiotherapy is known to increase expression levels of various antigens in number of cancer models as well as induce "abscopal effect" in both preclinical and clinical models; overall *CALR* levels were predictive of prolonged OS in radiotherapy-treated lung cancer patients	(26, 42, 102, 127, 141–144)
Anti-EGFR antibody – 7A7	Surface CRT Surface HSP70 Surface HSP90	Pre-apoptotic Early/mid-apoptotic Early/mid-apoptotic	–	(145)
Bleomycin	Surface CRT Secreted ATP Released HMGB1	Mid/post-apoptotic Mid/post-apoptotic Post-apoptotic	Induces ambivalent immune response, i.e., all valid ICD markers but also increased Treg differentiation and, thus, a good candidate for anti-Treg combinatorial therapy	(146)
Bortezomib	Surface HSP90 Surface CRT Surface HSP70	Early/mid-apoptotic Early/mid-apoptotic Early/mid-apoptotic	–	(26, 66, 100, 127)
Oncolytic Adenovirus	Surface CRT Released ATP Released HMGB1	?	Immunogenicity of these viruses can be further increased by producing transgenic versions producing CD40L or GM-CSF	(147, 148)
Clostridium difficile toxin B	Surface CRT Released ATP Released HMGB1 Released HSP70/90	Early/mid-apoptotic Post-apoptotic Post-apoptotic Post-apoptotic	–	(149)
Coxsackievirus B3 (CVB3)[#]	Surface CRT Secreted ATP Released HMGB1	Early-apoptotic Early/mid-apoptotic Post-apoptotic	–	(150, 151)
Cyclophosphamide	Surface CRT Released HMGB1	Pre-apoptotic Post-apoptotic	Facilitates an interface between gut microbiota (leaked due to gut perforation) and host immune system thereby allowing Th17 cells-dependent anti-tumor immune responses; cyclophosphamide's effects on anti-tumor immunity are strongly dose dependent. High doses of this chemotherapeutic can be immunosuppressive yet low or metronomic doses facilitate anti-tumor immunity through targeted depletion of Tregs/MDSCs. In ICD set-up, a low dose (100 mg/kg in mice) of cyclophosphamide was shown to exert anti-tumor immunity	(18, 152, 153)
High hydrostatic pressure	Surface CRT Surface HSP70 Surface HSP90 Secreted ATP Released HMGB1	Early/mid-apoptotic Early/mid-apoptotic Early/mid-apoptotic Mid/post-apoptotic Mid/post-apoptotic	–	(154–156)
Hypericin-based PDT	Surface CRT Surface HSP70 Surface HSP90 Secreted ATP Released HMGB1 Released HSP70/90 Released CRT	Pre-apoptotic Pre-apoptotic Pre-apoptotic Pre-apoptotic Post-apoptotic Post-apoptotic Post-apoptotic	High accumulation of OAMPs like protein carbonyls; down-regulates CD47; induces up-regulation of various molecules associated with Type I IFN response (*IRF7*, *IRF1*, *OASL, IL18, CXCL2, IL15, IL8*) but not IFN-α secretion	(26, 30, 34, 35, 112, 116, 157)
Microwave thermal ablation	Surface CRT Secreted ATP Released HMGB1	?	–	(158)
Newcastle disease virus (NDV)	Surface CRT Released HMGB1	Early/mid-necroptotic Post-necroptotic	Increases expression levels of PMEL17 antigen in glioma cells; NDV treatment has also been shown to induce "abscopal effect" in a murine melanoma model	(43, 159)
Paclitaxel	Surface CRT Released HMGB1	Early/mid-apoptotic Post-apoptotic	Overall *CALR* levels were predictive of prolonged OS or PFS in paclitaxel-treated ovarian cancer patients thereby establishing clinical validity of ICD in paclitaxel treatment set-up; paclitaxel has also been reported to enhance overall antigen levels	(42, 144, 160)

(Continued)

TABLE 3 | Continued

ICD inducers	Associated ICD-relevant DAMPs		Other immunostimulatory activities or danger signals and other comments on immunomodulatory activity	Reference
	DAMP	Stage of cell death		
Patupilone	Surface CRT	Early/mid-apoptotic	–	(128)
Photofrin-based PDT	Surface CRT Surface HSP70/60 Released HMGB1 Surface ceramide Surface S1P	Early/mid-apoptotic Early/mid-apoptotic Post-apoptotic Early/mid-apoptotic Early/mid-apoptotic	The only anticancer modality for which a comparison between DAMPs induced by *in vitro* versus *in vivo* treatment was carried out – however, none of ICD-related DAMPs were tested	(47, 161–164)
Pt^{II} N-heterocyclic carbene complex	Surface CRT Released ATP Released HMGB1	Pre-apoptotic Post-apoptotic Post-apoptotic	–	(140)
RIG-I-like helicases (RLH) ligand	Surface CRT Released HMGB1 Released HSP70	Early-apoptotic Post-apoptotic Post-apoptotic	Induces Type I IFN response	(165)
Septacidin	Surface CRT Secreted ATP Released HMGB1	Pre-apoptotic Early/mid-apoptotic Post-apoptotic	–	(139)
Shikonin	Surface CRT Surface HSP70	Early/mid-apoptotic Early/mid-apoptotic	Also, causes surface exposure of GRP78 a prominent inducer of pro-tumorigenic effects; enhances overall cancer antigen levels	(160)
Vorinostat	Surface CRT Secreted ATP Released HMGB1	Early/mid-apoptotic Post-apoptotic Post-apoptotic	–	(166)
Wogonin	Surface CRT Released ATP Released HMGB1	Early-apoptotic Post-apoptotic Post-apoptotic	Surface-Annexin A1 is also induced by wogonin. In an ICD set-up, the role of Annexin A1 is not clear since it is a noted anti-inflammatory factor	(167)

CRT or CALR, calreticulin; CTLs, cytotoxic T lymphocytes; DAMPs, damage-associated molecular patterns; EGFR, epidermal growth factor receptor; GZMB, granzyme B; HMGB1, high-mobility group box-1 protein; HSP, heat shock protein; ICD, immunogenic cell death; IFN, interferon; MDSC, myeloid-derived suppressor cells; OAMPs, oxidation-associated molecular patterns; OS, overall survival; PFS, progression-free survival.

Important note: It is worth noting that recently various promising candidate therapies have emerged that induce in vitro DAMPs relevant for ICD, e.g., Rose Bengal-based PDT (168), Docosahexaenoic acid (169), and Capsaicin (170, 171). Such agents may emerge as potent inducers of ICD in future, however, in order to establish them as inducers of ICD-like immunogenicity, it is imperative to confirm their (i.e., cancer cells treated with these agents) ability to stimulate T cells (in vitro or in vivo) and/or induce anti-cancer vaccination effect, in vivo, as per the consensus guidelines (104).

Glossary: In the current setting, it is crucial to differentiate between the meanings of the words, "immunogenic" and "immunogenicity" as they are not supposed to have interchangeable meanings. Immunogenic, derives from the word immunogen, which refers to any substance that can elicit an immune response; this includes, whole cells or organisms (eukaryotic or prokaryotic), specific cellular entities or specific proteins (e.g., antigens) (172). On the other hand, immunogenicity is a much more specific terms that is closer to antigenicity in operational sense, since it refers to the ability of a specific entity (e.g., an antigen or an epitope) to be recognized by the immune system through binding interactions with T or B cells, which may or may not result in an overt immunological response (4, 11).

"?" Unclear or not determined yet.

"#" Unconfirmed anti-tumour immune responses in adaptive immune system-competent.

TABLE 4 | Classification of ICD inducers into Type I and Type II based on their ER or non-ER-targeting *modus operandi*.

ICD inducer	Site of Cell-death inducing effects	Site of danger signaling induction	Reference
Type I inducers – agents that induce icd through a "collateral" er stress effect			
Anthracyclines (epirubicin, doxorubicin, idarubicin, mitoxantrone), oxaliplatin, UVC radiation and radiotherapy	Nucleus (DNA or the DNA replication machinery proteins)	ER, autophagy, pannexin channels, lysosomes	(36, 41, 70, 111, 130, 173, 174)
Anti-EGFR antibody – 7A7	Cell surface (epidermal growth factor receptor or EGFR)	ER	(145)
Bleomycin	Nucleus (causes DNA strand-breaks)	ER?	(146)
Bortezomib	Cytosol (26S proteasome or ERAD machinery; CIP2A/cancerous inhibitor of protein phosphatase 2A)	ER	(100, 175, 176)
Clostridium difficile toxin B	Cytoskeleton (causes cytoskeletal disruption by targeting RhoA, CDC42 and Rac1)	ER	(149, 177)
Cyclophosphamide	Nucleus (DNA)	ER	(152)
High hydrostatic pressure	Broad disrupting/denaturing effects on membranes, and proteins	ER (mitochondria?)	(154, 178)
Microwave thermal ablation	Hyperthermic ablation of cellular components	ER?	(158)
Paclitaxel, patupilone	Cytoskeleton (target microtubules thereby disrupting cytoskeletal functions)	ER	(42, 104, 179)
Photofrin-based PDT	Cellular membranes (ROS-based damage of membranes)	ER?	(180, 181)
RIG-I-like helicases (RLH) ligand	Cytosol (targets RIG-I-like helicases)	ER?	(165)
Septacidin	?	ER	(139)
Shikonin	Cytosol (tumor-specific pyruvate kinase-M2 protein)	ER	(160, 182)
Vorinostat	Nucleus/Cytosol (targets histone deacetylase)	ER?	(166)
Wogonin	Mitochondria (generates mitochondria-derived ROS)	ER	(167, 183)
Type II inducers – agents that induce icd through a "focused" er stress effect			
Hypericin-based PDT	ER (ROS-based damage at the ER membrane)	ER	(35, 63, 116, 181, 184, 185)
Oncolytic adenovirus	ER (ER membranes and lumen)	ER	(104, 147)
Oncolytic coxsackievirus B3 (CVB3)	ER (ER membranes and lumen)	ER	(150, 186)
Oncolytic Newcastle disease virus (NDV)	ER (ER membranes and lumen)	ER	(43, 159, 187)
Pt^{II} N-heterocyclic carbene complex	Predominantly targets ER (generates ER-directed ROS)	ER	(140)

EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; ICD, immunogenic cell death; PDT, photodynamic therapy; ROS, reactive oxygen species.
"?" Unclear or not determined yet.

TABLE 5 | A list of molecular and immunological components crucial for regulation of ICD.

Molecular or immunological components	Acting on the level of?	Role in regulating ICD or ICD-related determinants for various therapies/inducers			Confirmed by which experimental intervention?	Reference
		Positive regulation	Negative regulation	No role in regulation		
Actin cytoskeleton	Cancer cells	Anthracyclines, hypericin-PDT	–	–	Pharmacological inhibitors of actin polymerization	(35, 123)
ATG5, ATG7, or BECN1	Cancer cells	Anthracyclines, oxaliplatin	Hypericin-PDT	Newcastle disease virotherapy	ATG5, ATG7 or BECN1 si/shRNA, ATG5 KO MEFs, or transgenic mice model of spontaneous melanoma with *Atg7*$^{-/-}$ phenotype or pharmacological inhibitors of macroautophagy	(34, 43, 112)
BAX/BAK	Cancer cells	Anthracyclines, hypericin-PDT	–	–	BAX/BAK KO MEFs or Bax/Bak si/shRNA	(35, 123)
Calreticulin	Cancer cells	Anthracyclines, radiotherapy, oxaliplatin, hypericin-PDT	–	–	CRT si/shRNA	(35, 41, 116, 123)
Caspase 1	Host immune system	Anthracyclines and/or oxaliplatin	–	–	*Casp1*$^{-/-}$ mice	(36)
Caspase-8	Cancer cells	Anthracyclines	–	Hypericin-PDT	Caspase-8 si/shRNA or HeLa cancer cells expressing CrmA (a caspase-8 inhibitory protein)	(35, 123)
$CD4^+/CD8^+$ T cells	Host immune system	Anthracyclines and/or oxaliplatin, hypericin-PDT, high hydrostatic pressure, bortezomib, vorinostat, photofrin-PDT, Newcastle disease virotherapy, cyclophosphamide	–	–	Antibody-based depletion; *Ex vivo* co-culture experiments	(34, 43, 100, 102, 152, 161, 162, 166, 191)
CXCL10	Host immune system	Anthracyclines and/or oxaliplatin	–	–	Recombinant protein	(102, 141)
CXCR3	Host immune system	Anthracyclines and/or oxaliplatin	–	–	*Cxcr3*$^{-/-}$ mice or antibody-based blockade	(141)
eIF2α-P	Cancer cells	Anthracyclines	–	Hypericin-PDT	MEFs expressing non-phosphorylable version of eIF2α-P, salubrinal or pharmacological inhibitors of GADD34	(35, 123)
ER-Ca^{2+}	Cancer cells	Anthracyclines	–	Hypericin-PDT	BAPTA, a Ca^{2+} chelator or Reticulon-1C overexpression;	(35)
ERp57	Cancer cells	Anthracyclines	–	Hypericin-PDT	ERp57 si/shRNA or ERp57 KO MEFs	(35, 116)
ER-to-Golgi transport	Cancer cells	Anthracyclines, hypericin-PDT	–	–	Brefeldin A, a secretory pathway inhibitor	(35, 123)
HMGB1	Cancer cells	Anthracyclines	–	–	HMGB1 si/shRNA	(70)
HSP90	Cancer cells	Bortezomib	–	–	Pharmacological HSP90 inhibitors	(66, 67, 100)
HSP70	Cancer cells	Shikonin	–	–	Antibody-mediated protein depletion	(192)
IFN-α/β or IFN-α-receptor	Cancer cells	Anthracyclines, cyclophosphamide, and/or oxaliplatin	–	–	Antibody-based blockade or recombinant proteins (wherever applicable)	(141, 152)
IFN-γ and IFN-γ-receptor	Host immune system	Anthracyclines and/or oxaliplatin	–	–	*Ifng*$^{-/-}$ or *Ifngr1*$^{-/-}$ mice	(70, 102)
IL17A or IL17A-receptor	Host immune system	Anthracyclines and/or oxaliplatin	–	–	*Il17a*$^{-/-}$ or *Il17ra*$^{-/-}$ mice	(36, 193)
IL1-receptor	Host immune system	Anthracyclines and/or oxaliplatin	–	–	*Il1r1*$^{-/-}$ mice	(36)
IL-1β	Host immune system	Anthracyclines and/or oxaliplatin	–	–	Antibody-based blockade	(36)
Lipid rafts	Cancer cells	Mitoxantrone	–	Hypericin-PDT	MBC, a cholesterol-chelator that disrupts lipid rafts	(35)

(Continued)

TABLE 5 | Continued

Molecular or immunological components	Acting on the level of?	Role in regulating ICD or ICD-related determinants for various therapies/inducers			Confirmed by which experimental intervention?	Reference
		Positive regulation	Negative regulation	No role in regulation		
LRP1	Cancer cells	Mitoxantrone, hypericin-PDT	–	–	LRP1 shRNA, LRP1 KO MEFs, LRP1 KO CHO cells and LRP1 overexpression in CHO cells	(35)
LY96 and MyD88 (TLR-adaptors)	Host immune system	Anthracyclines and/or oxaliplatin	–	–	*Ly96*$^{-/-}$ or *Myd88*$^{-/-}$ mice	(102)
NLRP3	Host immune system	Anthracyclines and/or oxaliplatin	–	–	*Nlrp3*$^{-/-}$ mice	(36)
P2 × 7 receptor	Host immune system	Anthracyclines and/or oxaliplatin	–	–	*P2rx7*$^{-/-}$ mice	(36)
Perforin	Host immune system	Anthracyclines and/or oxaliplatin	–	–	*Prf1*$^{-/-}$ mice	(36, 70, 102)
PERK	Cancer cells	Anthracyclines, hypericin-PDT, wogonin	–	–	PERK si/shRNA, PERK KO MEFs	(35, 123, 167)
PI3K p110α	Cancer cells	Anthracyclines, hypericin-PDT, wogonin	–	–	PI3K p110α shRNA or wortmannin, a pharmacological inhibitor	(35, 167)
Rag2	Host immune system	Anthracyclines and/or oxaliplatin, vorinostat, cyclophosphamide, photofrin-PDT, Newcastle disease virotherapy	–	–	*Rag2*$^{-/-}$ mice	(43, 70, 102, 152, 161, 162, 166)
STAT3	Cancer cells	Anthracyclines and/or oxaliplatin	–	–	*Stat3*$^{-/-}$ cancer cells	(194)
TLR3	Cancer cells	Anthracyclines and/or oxaliplatin	–	–	TLR3 si/shRNA or *Tlr3*$^{-/-}$ cancer cells	(141)
TLR4	Host immune system	Anthracyclines and/or oxaliplatin	–	–	*Tlr4*$^{-/-}$ mice	(70, 102)
TNF or TNF-receptor	Host immune system	Anthracyclines and/or oxaliplatin	–	–	*Tnf*$^{-/-}$ or *Tnfr1*$^{-/-}$ mice	(102)
LAMP2A	Cancer cells?	Mitoxantrone and hypericin-PDT	–	–	LAMP2A KO MEFs	(112)

ATG, autophagy-related protein; BECN1, beclin-1; CD, cluster of differentiation; CRT, calreticulin; CXCL, C-X-C ligand; CXCR, C-X-C motif receptor; eIF2, eukaryotic initiation factor 2; ER, endoplasmic reticulum; ERp57, endoplasmic reticulum protein 57; HMGB1, high-mobility group box 1; HSP, heat shock protein; Hyp-PDT, hypericin-based photodynamic therapy; ICD, immunogenic cell death; IFN, interferon; IL, interleukin; KO MEFs, knock-out murine embryonic fibroblasts; LAMP, lysosome-associated membrane glycoprotein; LRP1, low-density lipoprotein receptor-related protein 1; MBC, methyl-β-cyclodextrin; NLRP3, NOD-like receptor family, pyrin domain containing 3; PERK, protein kinase RNA-like endoplasmic reticulum kinase; PI3K, phosphoinositide 3-kinase; PRF, perforin; TLR, toll-like receptor; TNF, tumor necrosis factor.

TABLE 6 | A list of prominent preclinical mice or rat models used for analysis of ICD.

ICD inducer	Mice tumor models utilized for positive ICD characterization or ICD "restoration/rescue" analysis			
	Heterotopic subcutaneous mice or rat models	Orthotopic mice models	Spontaneous tumor mice models	Carcinogen-induced tumor models
Anthracyclines	CT26 cells in BALB/c mice – prophylactic immunization model (41, 70, 111, 123, 197) and curative tumor model (41, 70, 111, 197); MCA205 cells in C57BL/6 mice – prophylactic immunization and curative tumor model (36, 70, 111, 130); MCA-2/-4 cells in C57BL/6 mice – curative tumor model (36); D122 cells in C57BL/6 mice – prophylactic immunization model (145); AY27 cells in Fischer 344 rats – prophylactic immunization model (42)	–	MMTV-*Neu*T breast cancer mice model – curative set-up (198); *Braf*$^{Ca/+}$; *Pten*$^{fl/fl}$-melanoma mice model – curative set-up (199)	–
Anti-EGFR antibody (7A7)	D122 cells in C57BL/6 mice – curative tumor model and prophylactic immunization model (145)	–	–	–
Bleomycin	CT26 cells in BALB/c mice – curative tumor model (146)	–	–	–

(Continued)

TABLE 6 | Continued

ICD inducer	Mice tumor models utilized for positive ICD characterization or ICD "restoration/rescue" analysis			
	Heterotopic subcutaneous mice or rat models	**Orthotopic mice models**	**Spontaneous tumor mice models**	**Carcinogen-induced tumor models**
Bortezomib	67NR cells in BALB/c mice – prophylactic immunization model with use of stimulated DCs (200); B16 cells in C57BL/6 mice – curative tumor model, combination treatment with AdVMART1/DC and bortezomib is significantly better than bortezomib alone (201); HM-1 cells in C57BL/6 x C3/He F_1 origin mice – prophylactic immunization model (202)	–	–	–
CD40L-encoding Oncolytic Adenovirus	MB49 cells in C57BL/6 mice – curative tumor model (147)	–	–	–
Clostridium difficile toxin B	CT26 cells in BALB/c mice – prophylactic immunization model (149)	–	–	–
Coxsackievirus B3	A549 and EBC-1 cells in nude BALB/c mice – curative tumor model (150)	–	–	–
Cyclophosphamide	EG7 cells in C57BL/6 mice (152); AB1-HA cells in BALB/c mice – curative tumor model followed by resistance to challenge with live cells (203)	–	–	–
Hypericin-based PDT	CT26 cells in BALB/c mice – prophylactic immunization model (35); – curative tumor model (184); AY27 cells in Fischer 344 rats – prophylactic immunization model (42); B78 cells in C57BL/6 mice – prophylactic immunization model (30)	–	–	–
Microwave thermal ablation	K7M2 cells in BALB/c mice or UMR106 cells in SD rats – prophylactic immunization model (158)	–	–	–
Newcastle disease virus (NDV)	B16 cells in C57BL/6 mice – curative tumor model (159)	GL261 cells in C57BL/6 mice – curative tumor model (43)	–	–
Oxaliplatin	CT26 cells in BALB/c mice – prophylactic immunization model (123, 197); – curative tumor model (197); EL4 cells in C57BL/6 mice – curative tumor model (36); EG7 cells in C57BL/6 mice – curative tumor model (36); EG7 cells in C3H mice – prophylactic immunization model (70)	–	–	–
Photofrin-based PDT	EMT6 cells in BALB/c mice – curative tumor model (161); SCCVII cells in C3H/HeN mice – curative tumor model (162, 163)	–	–	–
Radiotherapy	CT26 cells in BALB/c – prophylactic immunization model (204); 410.4 cells in BALB/c mice – prophylactic immunization model (205); EG7 cells in C57BL/6 mice and SCC VII cells in C3H mice – prophylactic immunization model (206); B16F10 cells in C57BL/6 mice – prophylactic immunization model with the use of irradiated cancer cells, as well as DCs stimulated with irradiated cancer cells (207)	–	–	–
RIG-I-like helicases (RLH) ligand	Panc02 cells in C57BL/6 mice – prophylactic immunization and curative tumor model (165)	–	–	–
Septacidin	MCA205 cells in BALB/c mice – prophylactic set-up (139);	–	–	–
Shikonin	B16 cells in C57BL/6 mice – prophylactic immunization model (160); P388 cells in KMF mice – curative tumor model (208)	4T1 cells in BALB/c mice – curative tumor model (192);	–	–
UVC irradiation	CT26 cells in BALB/c mice – prophylactic immunization model (204); EG7 cells in C57BL/6 mice – curative tumor model (152)	–	–	–
Vorinostat	MC38 or Eμ-myc 4242/299 lymphoma in C57BL/6 mice – curative tumor set-up (166)	–	–	–
High hydrostatic pressure	No mice or rat based preclinical data available to support their ICD-functions			
Pt^{II} N-heterocyclic carbene complex				

DC, dendritic cell; ICD, immunogenic cell death; PDT, photodynamic therapy.

TABLE 7 | Existence of intrinsic or naturally occurring resistance to ICD in experimental cancer models.

ICD inducer(s)	Experimental set-up where resistance was observed	Reason behind Resistance	Rescued by?	Clinical applicability verified?	Reference
***In vivo* preclinical setting (cancer cell or host immune system-level resistance)**					
Anthracyclines or anthracycline plus oxaliplatin	C3H mice with naturally occurring *tlr4* mutation	Host immune system-level resistance**:** defective *TLR4* in C3H mice causes failure of HMGB1-mediated immunity thereby leading to resistance to anti-cancer vaccination effect associated with anthracyclines treatment	Adoptive transfer of TLR4-expressing DCs loaded with dying tumor cells	Yes; breast cancer, colon cancer, and lung cancer patients carrying TLR4 gene mutation that ablates its ability to bind its ligands is associated with worse prognosis post-treatment	(215)
Doxorubicin	AT-3 or 4T1.2 breast cancer cells in C57BL/6 or BALB/c mice, respectively	Cancer cell-level resistance: CD73 overexpression confers chemo-resistance to doxorubicin by suppressing anti-tumor immunity through A2A adenosine receptors	Blockade of CD73	Yes; in triple-negative breast cancer patients, high CD73 in anthracycline-treatment set-up associated with lower rate of complete responses	(216)
Mitoxantrone and Hypericin-PDT	AY27 rat bladder cancer cells in Fischer 344 rats	Cancer cell-level resistance: low endogenous CRT levels, resulted in severely reduced surface-CRT upon treatment with mitoxantrone or Hyp-PDT; this in turn compromised immunogenic phagocytic clearance and anti-cancer vaccination effect	Exogenous addition of recombinant CRT	Yes; high tumoral *CALR* levels correlated with high expression of phagocytosis-associated genes and predicted for prolonged survival after RT or PTX treatment of lung or ovarian cancer patients respectively	(42)
Oxaliplatin	Autochthonous transgenic adenocarcinoma of the mouse prostate (TRAMP) model of metastatic prostate cancer	Host immune system-level resistance**:** immunosuppressive B cells expressing IgA, IL10 and PD-L1 cause resistance to anti-tumorigenic effects of oxaliplatin	Genetic or pharmacological depletion of B cells	Not directly, but possible validity is supported by human patient data showing that IL-10 expressing IgA+ cells are abundant in therapy-resistant prostate cancer and are negative prognostic indicators	(217)
***In vitro* preclinical setting (cancer cell-level resistance)**					
Anthracycline	SH-SY5Y neuroblastoma cell line	Anthracycline treatment of these cells failed to induce surface-CRT due to reduced capacity to efflux ER-Ca^{2+} into cytosol	Overexpression of reticulon-1C	–	(132)
Doxorubicin	HT29-dx and HT29 iNOS-cells (human colon cancer cells)	Doxorubicin failed to induce NO synthesis, which resulted in reduced toxicity, reduced surface-CRT and subsequently compromised immunogenic phagocytic clearance and DC stimulation	Addition of sodium nitroprusside or a NO donor	–	(218)
Doxorubicin	MDR+ human cancer cells (HT29-dx, A549-dx and MCF-7-dx)	Increased MDR levels caused increased P-glycoprotein expression which caused resistance to doxorubicin-induced ICD by affecting immunogenic phagocytic removal	Addition of zoledronic acid	Not directly	(219)

CD, cluster of differentiation; CRT or CALR, calreticulin; DC, dendritic cells; ER, endoplasmic reticulum; HMGB1, high-mobility group box-1 protein; HSP, heat shock protein; Hyp-PDT, hypericin-photodynamic therapy; ICD, immunogenic cell death; IL, interleukin; MDR, multiple drug-resistance; NO, nitric oxide; NOS, nitric oxide synthase; PD-L1, programed cell death protein ligand 1; PTX, paclitaxel; RT, radiotherapy; TLR, toll-like receptor.

TABLE 8 | A list of clinical observations supporting the existence of ICD in cancer patients.

ICD inducer	Standard-of-care therapy or regularly applied palliative therapy in clinic?	ICD-related characteristics regulating clinical patient prognosis or treatment-responsiveness
Anthracyclines	Yes	*P2RX7* loss-of-function mutation that compromises ICD also negatively affects MFS in breast cancer patients treated with adjuvant anthracyclines (36); breast cancer patients possessing a wild-type *TLR4* benefited more from the anthracyclines than those who possessed a mutated *TLR4* that compromises ICD (70); an *MX1*-centered Type I IFN signature in anthracycline-treated breast cancer patients predicts for improved disease outcome (141); combined positivity for cytoplasmic LC3B+ puncta and nuclear HMGB1 is a positive predictor of improved survival following adjuvant anthracycline-based chemotherapy (225)
High hydrostatic pressure	No; but HHP-based anticancer DC vaccines are currently being applied in clinical trials against prostate cancer and ovarian cancer (155)	No data are available
Hypericin-based PDT	No; but few clinical trials have been carried out for non-melanoma skin cancer (226), cutaneous T-cell lymphoma (227), mesothelioma (228), and basal or squamous cell carcinoma (229)	No data are available
Oncolytic adenoviruses	No; but oncolytic adenoviruses are currently being applied in various clinical trials in cancer patients	Serum HMGB1 levels and the temporal change in their levels during treatment was identified as a prognostic and predictive biomarker in cancer patients (230)
Oxaliplatin	Yes	Similar to anthracyclines, cancer patients possessing wild-type *TLR4* exhibited prolonged PFS and OS in comparison to patients bearing the loss-of-function allele of *TLR4* (197)
Paclitaxel	Yes	High tumoral *CALR* levels in paclitaxel-treated ovarian cancer patients associated with prolonged OS/PFS as well as increased expression levels of various phagocytosis-associated genes (42)
Photofrin-based PDT	Yes; FDA-approved for application in esophageal and lung cancer (231)	No data available
Radiotherapy	Yes	In patients of eosophageal squamous cell carcinoma (ESCC) receiving chemo-radiotherapy significant increase in serum HMGB1-levels and increased intra-tumoral staining of HMGB1 correlated with better patient survival (232); high tumoral *CALR* levels in radiotherapy-treated lung cancer patients associated with prolonged OS as well as increased expression levels of various phagocytosis-associated genes (42)
Shikonin	No; but shikonin is currently being applied in an observational clinical study of breast cancer patients (NCT01287468)	No data are available
UVC irradiation	No; but UV treatment is sometimes applied for the preparation of clinical cell-based anticancer vaccines (233)	No data are available
Bortezomib, Anti-EGFR antibody (7A7), bleomycin, cyclophosphamide, microwave thermal ablation, vorinostat	Yes	No data are available
Coxsackievirus B3; *Clostridium difficile* toxin B; Microwave thermal ablation; Newcastle disease virus (NDV); RIG-I-like helicases (RLH) ligand; Septacidin; Pt^{II} N-heterocyclic carbene complex; Patupilone	No	No data are available

CRT or CALR, calreticulin; HMGB1, high-mobility group box-1 protein; Hyp-PDT, hypericin-photodynamic therapy; ICD, immunogenic cell death; IFN, interferon; OS, overall survival; PFS, progression-free survival; TLR, toll-like receptor.