CELL DEATH AND IMMUNITY

# Immunogenic cell death in cancer and infectious disease

*Lorenzo Galluzzi[1–6], Aitziber Buqué[2–6], Oliver Kepp[2–5,7], Laurence Zitvogel[6,8–10] and Guido Kroemer[2–5,7,11,12]*

Abstract | Immunogenicity depends on two key factors: antigenicity and adjuvanticity. The presence of exogenous or mutated antigens explains why infected cells and malignant cells can initiate an adaptive immune response provided that the cells also emit adjuvant signals as a consequence of cellular stress and death. Several infectious pathogens have devised strategies to control cell death and limit the emission of danger signals from dying cells, thereby avoiding immune recognition. Similarly, cancer cells often escape immunosurveillance owing to defects in the molecular machinery that underlies the release of endogenous adjuvants. Here, we review current knowledge on the mechanisms that underlie the activation of immune responses against dying cells and their pathophysiological relevance.

Microorganism-associated molecular patterns
(MAMPs). Conserved microbial components that, upon detection by the host, can favour the establishment of immunological tolerance or promote a state of accrued resistance to infection.

Damage-associated molecular patterns
(DAMPs). Endogenous molecules that are normally invisible to the host immune system but, once emitted by stressed or dying cells, initiate danger signalling.

[1]*Department of Radiation Oncology, Weill Cornell Medical College, 10065 New York, New York, USA.*
[2]*Equipe 11 labellisée Ligue contre le Cancer, Centre de Recherche des Cordeliers, 75006 Paris, France.*

*Correspondence to L.G. or G.K.*
*deadoc@vodafone.it; kroemer@orange.fr*

doi:10.1038/nri.2016.107
Published online 17 Oct 2016

The daily demise of several billions of normal cells from the human body goes virtually unrecognized by the immune system. This is important as the preservation of whole-body homeostasis involves the continuous turnover of multiple cellular compartments, and the activation of an immune response against dead cell-associated antigens would have catastrophic consequences[1,2]. Conversely, the death of only a few cells infected by an infectious pathogen can trigger a robust antigen-specific immune response. In this context, successful immune responses not only clear invading pathogens from the body, but also result in the establishment of long-term immunological memory[2]. What are the differences between these two instances of cellular demise? For decades, the 'self/non-self' model has been used as the sole framework to differentiate between homeostatic (that is, self and non-antigenic) and pathogen-driven (that is, non-self and antigenic) forms of cell death. However, work from the late 1990s unveiled the limitations of this model by demonstrating that endogenous entities can also initiate an immune response, at least under specific circumstances[3,4]. Such a paradigm shift revolutionized immunology as it pointed to the existence of at least one factor other than antigenicity that explains why some, but not all, forms of cell death are immunogenic.

Even before pathogens elicit adaptive immunity, specific microorganism-associated molecular patterns (MAMPs) are detected by sensors that are expressed by a wide variety of cells, including monocytes, macrophages, dendritic cells (DCs) and other components of the innate immune system[5]. Such MAMPs operate as natural adjuvants, and their interaction with pattern recognition receptors (PRRs) not only establishes a first line of defence against infection but also generates the ideal conditions for the initiation of antigen-specific immune responses[5,6]. MAMPs do not invariably exert net immunostimulatory effects, and in some instances they are actively involved in the establishment of immunological tolerance to microorganisms and symbiosis[7]. However, the mechanisms that determine whether specific MAMPs or combinations thereof mediate immunostimulatory versus immunosuppressive effects remain to be clarified[7]. Irrespective of this unknown, the activation of adaptive immunity against endogenous entities also relies on PRR signalling. In this latter scenario, PRRs are activated by damage-associated molecular patterns (DAMPs). Similar to their microbial counterparts, DAMPs produced by dying cells can act as adjuvants and communicate a state of danger to the organism[8]. However, these DAMPs are unable to initiate an adaptive immune response unless dying cells display an increased antigenicity — that is, they possess antigenic epitopes that have not previously elicited central or peripheral tolerance. Such neo-epitopes may be encoded by microbial genes, as well as by host genes that mutate in the course of oncogenesis and tumour progression[2]. Taken together, these observations indicate that the immunogenicity of cell death relies on a combination of antigenicity (provided by neo-epitopes) and adjuvanticity (conferred by specific MAMPs or DAMPs).

Corroborating the importance of both antigenicity and adjuvanticity for the engagement of antigen-specific immune responses by dying cells, human malignancies with a high mutational load show a superior response to

Checkpoint blockers
Clinically used monoclonal antibodies that instate (or reinstate) anticancer immunosurveillance by inhibiting immunosuppressive receptors like cytotoxic T lymphocyte associated protein 4 (CTLA4) or programmed cell death 1 (PDCD1; also known as PD1).

Unfolded protein response
(UPR). Stress-responsive mechanism that increases the ability of the endoplasmic reticulum to cope with an increased load of unfolded polypeptides.

Box 1 | **Methods to test the immunogenicity of cell death**

Several biochemical correlates of immunogenic cell death (ICD) have been identified, including (but not limited to) the exposure of calreticulin (CALR) on the surface of dying cells, and the release of large amounts of ATP and high-mobility group box 1 (HMGB1) into the extracellular milieu[10]. However, none of these processes (taken alone or in combination) can predict the immunogenicity of cell death with absolute certainty, suggesting that there are unknown ICD-related damage-associated molecular patterns (DAMPs) that remain elusive[14]. Indeed, mouse cancer cells exposed to cardiac glycosides show marked CALR exposure, ATP secretion and HMGB1 release, but they fail to elicit antigen-specific responses associated with immunological memory after inoculation in immunocompetent mice unless they are also exposed to overtly cytotoxic chemotherapeutics[129]. Along similar lines, structural, chemical or physical properties of the agents under consideration cannot be used to predict their ability to induce bona fide ICD, as exemplified by the cisplatin–oxaliplatin contradiction[42] (see also main text).

Taken together, these observations indicate that the ability of a specific agent to drive bona fide ICD must be assessed *in vivo*, in appropriate mouse models of oncogenesis[14]. Since ICD-inducing agents mediate antineoplastic effects that mostly depend on the engagement of adaptive immunity, the efficacy of these interventions is highly compromised (if not fully abrogated) in immunodeficient mice[14]. However, the same applies to various agents that have off-target immunostimulatory effects, as they act on components of the innate or adaptive immune system, but fail to kill cancer cells in an immunogenic manner (for instance, gemcitabine)[124]. Thus, the gold-standard approach to test the ability of a specific agent to induce bona fide ICD relies on vaccination assays based on, first, the inoculation of neoplastic cells killed *in vitro* by a putative ICD inducer into syngeneic, immunocompetent mice and, second, the challenge of vaccinated mice with living cancer cells of the same type (1 week later)[14]. In this scenario, the percentage of tumour-free mice provides a robust estimate of the immunogenicity of cell death[14]. One of the main limitations of this system is that human cancer cells cannot be directly investigated for their ability to trigger an adaptive immune response (owing to cross-species-specific immunological incompatibility)[14]. Although *in vitro* DC maturation assays may be used to partially circumvent this obstacle, humanized mice that provide a fully syngeneic model of oncogenesis are urgently awaited to obtain profound insights into the immunogenicity of cell death in the human system.

immunotherapy with checkpoint blockers[9] than tumours with a relatively low number of somatic mutations, and such a response mostly (if not entirely) depends on adaptive immunity. Cancer cells with innate or experimentally enforced defects in pathways that are required for cell death-associated DAMP release, including autophagy and the unfolded protein response (UPR), fail to die in an immunogenic manner in response to stimuli that would otherwise cause bona fide immunogenic cell death (ICD)[10,11]. Moreover, artificially boosting the availability of specific DAMPs efficiently converts non-immunogenic forms of cell demise into instances of ICD[12].

Thus, in the presence of increased antigenicity, adjuvanticity must be limited for cell death to be overlooked by the immune system, and both pathogens and malignant cells evolve under such a selective pressure. This implies that DAMPs occupy a privileged position in the mechanisms that determine the immunogenicity of cell death, irrespective of whether cells succumb to exogenous or endogenous cues. Here, we discuss current knowledge on the mechanisms by which cell death is perceived as immunogenic, focusing on the stress response pathways that underlie DAMP emission by dying cells, the receptors and cells of the host that detect DAMPs, and the pathophysiological implications of these processes.

## Forms of ICD

For a long time, cell death has been misleadingly classified in a dichotomic manner. Thus, although apoptosis (defined on the basis of morphological features) was considered to be a physiological, regulated and non-immunogenic (or even tolerogenic) instance of cell death, necrosis (also associated with specific morphological traits) was viewed as a pathological, incontrollable and immunogenic variant of cellular demise[13]. Now it has become evident that such clear-cut differences do not exist. Thus, cells may succumb to the activation of a genetically encoded molecular machinery (that is, in a regulated manner) while exhibiting a necrotic morphotype; regulated forms of necrosis participate in development and tissue homeostasis, and apoptotic cells can trigger an antigen-specific immune response[13]. One of the consequences of this conceptual revolution is that the gold-standard approach to determine whether cell death may be immunogenic no longer involves morphological or biochemical assessments on dying cells, but rather relies on vaccination experiments in which murine dying cells are injected into immunocompetent syngeneic mice[14] (BOX 1). No less than four types of ICD have been discovered so far, each of which relies on the emission and detection of a specific panel of DAMPs (FIG. 1; TABLE 1).

Author addresses

[1]Department of Radiation Oncology, Weill Cornell Medical College, 10065 New York, New York, USA.
[2]Equipe 11 labellisée Ligue contre le Cancer, Centre de Recherche des Cordeliers, 75006 Paris, France.
[3]INSERM, U1138, 75006 Paris, France.
[4]Université Paris Descartes/Paris V, Sorbonne Paris Cité, 75006 Paris, France.
[5]Université Pierre et Marie Curie/Paris VI, 75006 Paris, France.
[6]Gustave Roussy Comprehensive Cancer Institute, 94805 Villejuif, France.
[7]Metabolomics and Cell Biology Platforms, Gustave Roussy Comprehensive Cancer Institute, 94805 Villejuif, France.
[8]INSERM, U1015, 94805 Villejuif, France.
[9]Center of Clinical Investigations in Biotherapies of Cancer (CICBT) 1428, 94805 Villejuif, France.
[10]Université Paris Sud/Paris XI, 94270 Le Kremlin-Bicêtre, France.
[11]Karolinska Institute, Department of Women's and Children's Health, Karolinska University Hospital, 17176 Stockholm, Sweden.
[12]Pôle de Biologie, Hopitâl Européen George Pompidou, AP-HP, 75015 Paris, France.

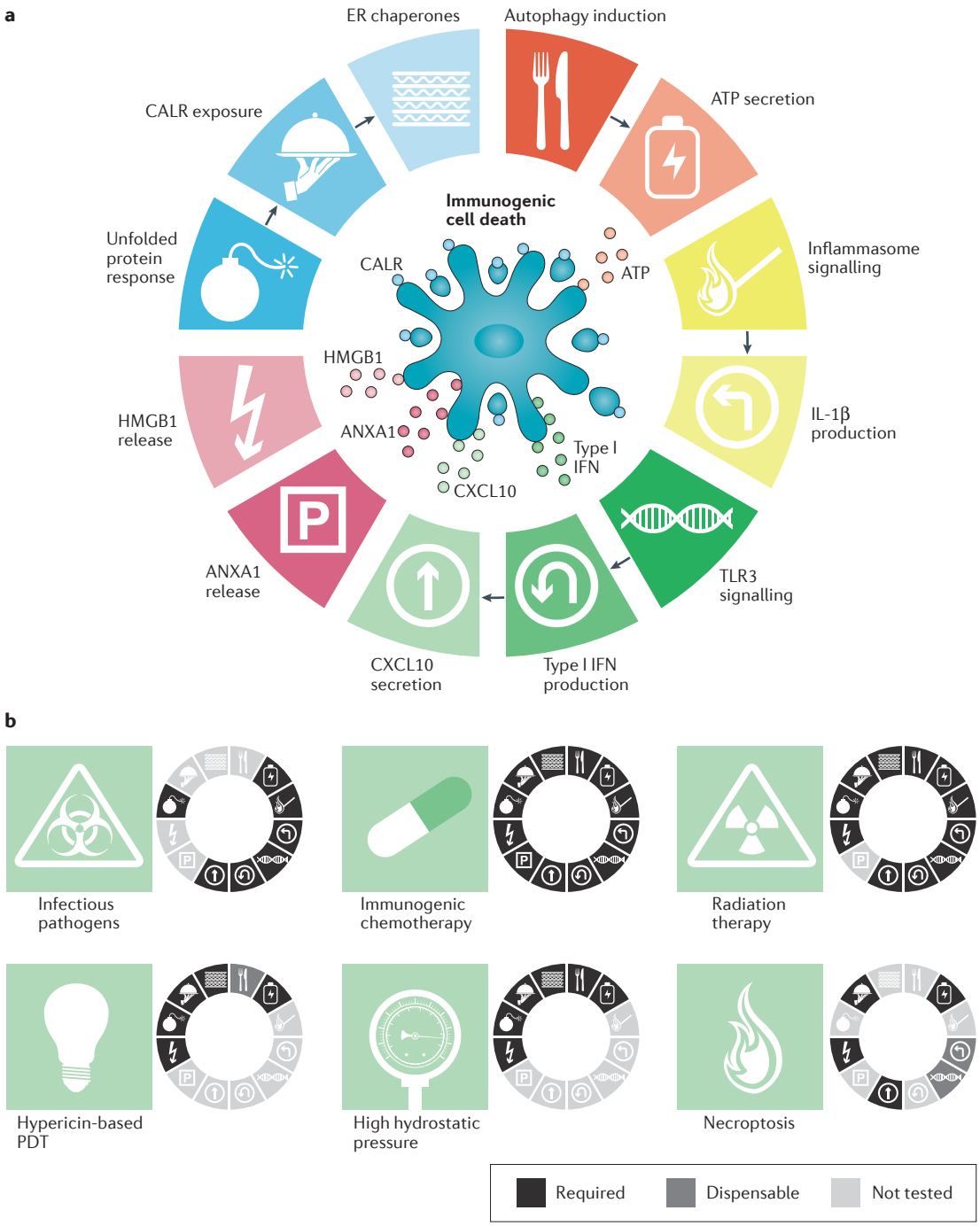


Figure 1 | **Differential requirements for the immunogenicity of cell death. a** | Several processes have been linked to the immunogenicity of cell death, including the unfolded protein response and consequent exposure of calreticulin (CALR) and other endoplasmic reticulum (ER) chaperones on the cell surface; the activation of autophagy and consequent secretion of ATP; the release of interleukin-1β (IL-1β) upon inflammasome signalling; the activation of Toll-like receptor 3 (TLR3), resulting in a type I interferon (IFN) response that stimulates the production of CXC-chemokine ligand 10 (CXCL10); as well as the release of high-mobility group box 1 (HMGB1) and annexin A1 (ANXA1). **b** | Distinct variants of immunogenic cell death depend on (or are associated with) the emission of different sets of danger signals from dying cells. PDT, photodynamic therapy.

***ICD driven by pathogens.*** Cell death constitutes one of the most ancient mechanisms of defence against invasion by pathogens. Although the delayed death of infected cells eventually favours pathogen spreading, both viruses and obligate intracellular bacteria such as *Salmonella enterica* require living and metabolically active cells to replicate, at least in the first phases of infection[15,16]. Early after (or even before) infection, cells can sense multiple MAMPs by means of specific PRRs, each of which operates to favour the disposal of the pathogen and to communicate the

Table 1 | **Danger signalling in immunogenic cell death**

| Danger signal | PRR | Function(s) | Note(s) | Refs |
|---|---|---|---|---|
| ANXA1 | FPR1 | Guides the final approach of APCs to dying cells | Syngeneic tumours growing in *Fpr1*$^{-/-}$ mice are normally infiltrated by myeloid cells in response to immunogenic chemotherapeutics | 39 |
| ATP | • P2RX7<br>• P2RY2 | Favours the recruitment of APCs and their activation | The secretion of ATP by cancer cells undergoing chemotherapy-driven ICD, but not ICD triggered by hypericin-based PDT, relies on autophagy | 36,74 |
| CALR | LRP1 | Promotes the uptake of dead cell-associated antigens | CALR exposure seems to be required for the immunogenicity of dying cancer cells, but its role in the immunogenicity of cells succumbing to infection has not yet been tested | 33,35, 46–48, 55,57,69, 84,87,88 |
| Cellular RNA | TLR3 | Promotes the synthesis of pro-inflammatory factors including type I IFNs | TLR3 signalling in cancer cells undergoing ICD appears to stem from the detection of cellular RNA in the endosomal compartment | 37 |
| CpG DNA | TLR9 | Promotes the synthesis of pro-inflammatory factors including type I IFNs | TLR9 is involved in the detection of unmethylated CpG-rich DNA, which is generally bacterial, within the endosomal compartment | 17 |
| CXCL10 | CXCR3 | Stimulates T cell recruitment | CXCL10 is released by cancer cells succumbing to anthracycline-driven ICD as a consequence of autocrine and/or paracrine type I IFN signalling | 37 |
| dsDNA | CDSs | Promotes the synthesis of pro-inflammatory factors including type I IFNs | Most CDSs promote type I IFN synthesis by activating STING | 19 |
| dsRNA | TLR3 | Promotes the synthesis of pro-inflammatory factors including type I IFNs | TLR3 is involved in the detection of viral dsRNA in the endosomal compartment | 17 |
| PDIA3 | ? | Promotes the uptake of dead cell-associated antigens | The exposure of PDIA3 with CALR is obligatory for the immunogenicity of some, but not all, forms of ICD | 34,46,72 |
| Flagellin | TLR5 | Promotes the synthesis of pro-inflammatory factors including type I IFNs | Flagellin is an abundant component of bacterial flagella | 17 |
| HMGB1 | • AGER<br>• TLR2<br>• TLR4 | Promotes the synthesis of pro-inflammatory factors including type I IFNs | HMGB1 has multiple immunostimulatory functions upon binding to various PRRs, but its role in ICD seems to depend on TLR4 | 35,47,55, 57,99 |
| HSP70 | LRP1 | Stimulates the uptake of dead cell-associated antigens | Although the exposure of HSP70 has been shown to accompany several instances of ICD, its requirement in the process has not been tested | 35,46,47, 61,87 |
| HSP90 | LRP1 | Stimulates the uptake of dead cell-associated antigens | Although the exposure of HSP90 has been shown to accompany several instances of ICD, its requirement in the process has not been tested | 35,47,87 |
| Lipopolysaccharide | TLR4 | Promotes the synthesis of pro-inflammatory factors including type I IFNs | Lipopolysaccharide is an abundant component of the outer wall of all Gram-negative bacteria | 17 |
| ssRNA | TLR7 | Promotes the synthesis of pro-inflammatory factors including type I IFNs | TLR7 is involved in the detection of viral ssRNA in the endosomal compartment | 17 |
| Type I IFNs | IFNAR | Promote CXCL10 secretion by cancer cells and exert immunostimulatory effects | Type I IFNs support chemotherapy-driven ICD mostly via autocrine or paracrine circuitries, and they exert immunostimulatory effects by triggering IFNAR signalling in immune cells | 23,37 |
| Viral RNA | RLRs | Promotes the synthesis of pro-inflammatory factors including type I IFNs | Distinct RLRs recognize specific viral RNA species but operate via a common signalling pathway dependent on MAVS | 20 |

AGER, advanced glycosylation end product-specific receptor; ANXA1, annexin A1; APC, antigen-presenting cell; CALR, calreticulin; CDS, cytosolic DNA sensor; CXCL10, CXC-chemokine ligand 10; CXCR3, CXC-chemokine receptor 3; ds, double-stranded; FPR1, formyl peptide receptor 1; HMGB1, high-mobility group box 1; HSP70, heat shock protein 70 kDa; HSP90, heat shock protein 90 kDa; ICD, immunogenic cell death; IFN, interferon; IFNAR, interferon α/β-receptor; LRP1, LDL receptor related protein 1; MAVS, mitochondrial antiviral signalling protein; PDIA3, protein disulfide isomerase family A member 3; PDT, photodynamic therapy; P2RX7, purinergic receptor P2X7; P2RY2, purinergic receptor P2Y2; PRR, pattern recognition receptor; RLR, RIG-I-like receptor; ss, single-stranded; STING, stimulator of interferon genes; TLR, Toll-like receptor.

incipient danger to neighbouring cells. Thus, microbial components as diverse as lipopolysaccharide, lipoteichoic acid, flagellin, unmethylated CpG-containing oligodeoxynucleotides, and single- or double-stranded RNA are rapidly detected by dedicated Toll-like receptors (TLRs, which are located on the plasma membrane or within endosomal compartments), cytosolic DNA sensors, RIG-I-like receptors or NOD-like receptors (which are mostly cytoplasmic) to elicit an intracellular and microenvironmental danger response[17–20]. Intracellular danger signalling involves the activation of autophagy and the UPR[21,22], whereas the microenvironmental danger response relies on the PRR-driven secretion of pro-inflammatory cytokines, including (but not limited to) tumour necrosis factor (TNF) and type I interferons (IFNs)[23]. In addition, in specific cell types, including macrophages, the extracellular battle against infectious challenges also depends on inflammasome activation within dying cells, culminating in the secretion of mature interleukin-1β (IL-1β) and IL-18 (REF. 24).

Beyond its fundamental contribution to the preservation of cellular homeostasis in physiological conditions[25], autophagy has a major role in the control of invading viral or bacterial pathogens[26,27]. Accordingly, defects in the molecular machinery for autophagy increase the susceptibility of mice to infection by several viruses and bacteria[26,27]. The UPR is coupled to the inactivating phosphorylation of eukaryotic translation initiation factor 2A (eIF2A), which results in prominent antiviral effects owing to the inhibition of CAP-dependent protein synthesis[22]. Thus, replacing endogenous eIF2A with a non-phosphorylatable variant ($eIF2A^{S51A}$) abrogates the control of viral infection in mice[28]. Besides exerting antiviral and antibacterial effects at the intracellular level, autophagy and the phosphorylation of eIF2A are connected to the release of DAMPs by mouse and human cancer cells succumbing to chemotherapy- or irradiation-driven ICD[10] (see below). However, to what extent autophagy and the UPR contribute to the immunogenicity of cell death triggered by viruses and intracellular bacteria has not yet been determined. Regardless of this unknown, when infected cells die, their corpses are rapidly taken up by professional antigen-presenting cells (APCs)[23]. Owing to the presence of viral or bacterial components, these corpses also contain an abundant repertoire of non-self antigenic epitopes (which by definition are not subjected to central or peripheral tolerance). In these conditions, cellular corpses and their debris are rapidly processed by DCs and presented on MHC class I and class II molecules to antigen-specific $CD8^+$ and $CD4^+$ T cells, respectively, resulting in the elicitation of potent responses associated with immunological memory, especially in the context of robust PRR signalling[29,30] (FIG. 1).

***ICD elicited by chemotherapeutics.*** Mouse cancer cells exposed to some chemotherapeutics that are currently used in the clinic, including doxorubicin, mitoxantrone, oxaliplatin and bortezomib, undergo bona fide ICD, as demonstrated by vaccination experiments in mice[31,32]. In mice, chemotherapy-driven ICD relies on the eIF2A phosphorylation-dependent exposure of endoplasmic reticulum (ER) chaperones such as calreticulin (CALR)[33], protein disulfide isomerase family A member 3 (PDIA3; also known as ERp57)[34], heat shock protein 70 kDa (HSP70; also known as HSPA1A)[35] and heat shock protein 90 kDa (HSP90; also known as HSP90AA1)[35] on the plasma membrane of dying cancer cells. Moreover, it involves the autophagy-mediated secretion of ATP[36], the activation of a cancer cell-intrinsic type I IFN response and consequent secretion of CXC-chemokine ligand 10 (CXCL10)[37], as well as the release of high-mobility group box 1 (HMGB1)[38] and annexin A1 (ANXA1)[39]. Many of these manifestations of ICD have also been observed in human cancer cells succumbing to immunogenic chemotherapy[10,11,35], although human cells cannot be characterized in vaccination assays. A panel of experimental interventions that interfere with the ability of mouse cancer cells to release these DAMPs as they succumb to ICD-promoting chemotherapeutics abrogate their capacity to vaccinate mice against a subsequent challenge with living cells of the same type; these include small-interfering RNA (siRNA)-mediated downregulation of CALR[33] or HMGB1 (REF. 38), short-hairpin RNA (shRNA)-mediated knockout of essential components of the autophagic machinery such as autophagy related 5 (ATG5) or ATG7 (REF. 36), the overexpression of the extracellular ATP-degrading enzyme ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1; also known as CD39)[36,40], as well as the inactivation of both copies of *Ifnar1* (which encodes interferon α/β-receptor subunit 1) or *Anxa1* (REFS 37,39) (FIGS 1,2).

Along similar lines, chemotherapeutic agents that are intrinsically unable to promote the release of one or more of these DAMPs (or the activation of the underlying stress responses) *a priori* fail to promote ICD. As an example, cisplatin differs from its derivative oxaliplatin in its ability to trigger the UPR and the consequent translocation of CALR to the outer leaflet of the plasma membrane of dying cells[41]. Accordingly (and in spite of an otherwise similar activity profile), cisplatin-treated mouse cancer cells fail to vaccinate syngeneic hosts in conditions in which oxaliplatin-treated cells efficiently do so[41]. In this setting, the exogenous co-provision of a UPR inducer such as thapsigargin or tunicamycin efficiently restores the immunogenicity of cisplatin-elicited cell death, demonstrating that defects in the intracellular mechanisms that underlie the emission of ICD-associated DAMPs can be corrected for therapeutic purposes[42]. Such an intrinsic discrepancy between cisplatin and oxaliplatin also suggests that the ICD-promoting capacity of one specific intervention cannot be predicted based on structural or biochemical features but must be evaluated in vaccination assays[14] (BOX 1).

Importantly, the degree of antigenicity displayed by mouse and human cancer cells varies to a significant extent, reflecting the elevated heterogeneity of mutational load that is observed even across cancers of the same type[9]. It is therefore tempting to speculate that — besides affecting the response of patients with melanoma, non-small cell lung carcinoma or colorectal cancer to immunotherapy with checkpoint blockers[43–45]

Cytosolic DNA sensors
Intracellular PRRs including Z-DNA binding protein 1 (ZBP1; also known as DAI) that are involved in the detection of cytosolic double-stranded DNA.

RIG-I-like receptors
Intracellular PRRs resembling DEAD box protein 58 (DDX58; also known as RIG-I) that are involved in the detection of cytosolic double-stranded RNA.

NOD-like receptors
Intracellular PRRs involved in the detection of a wide panel of MAMPs of both bacterial and viral origin.

Inflammasome
Large supramolecular platform responsible for the activation of caspase 1 and consequent proteolytic maturation of IL-1β and IL-18.

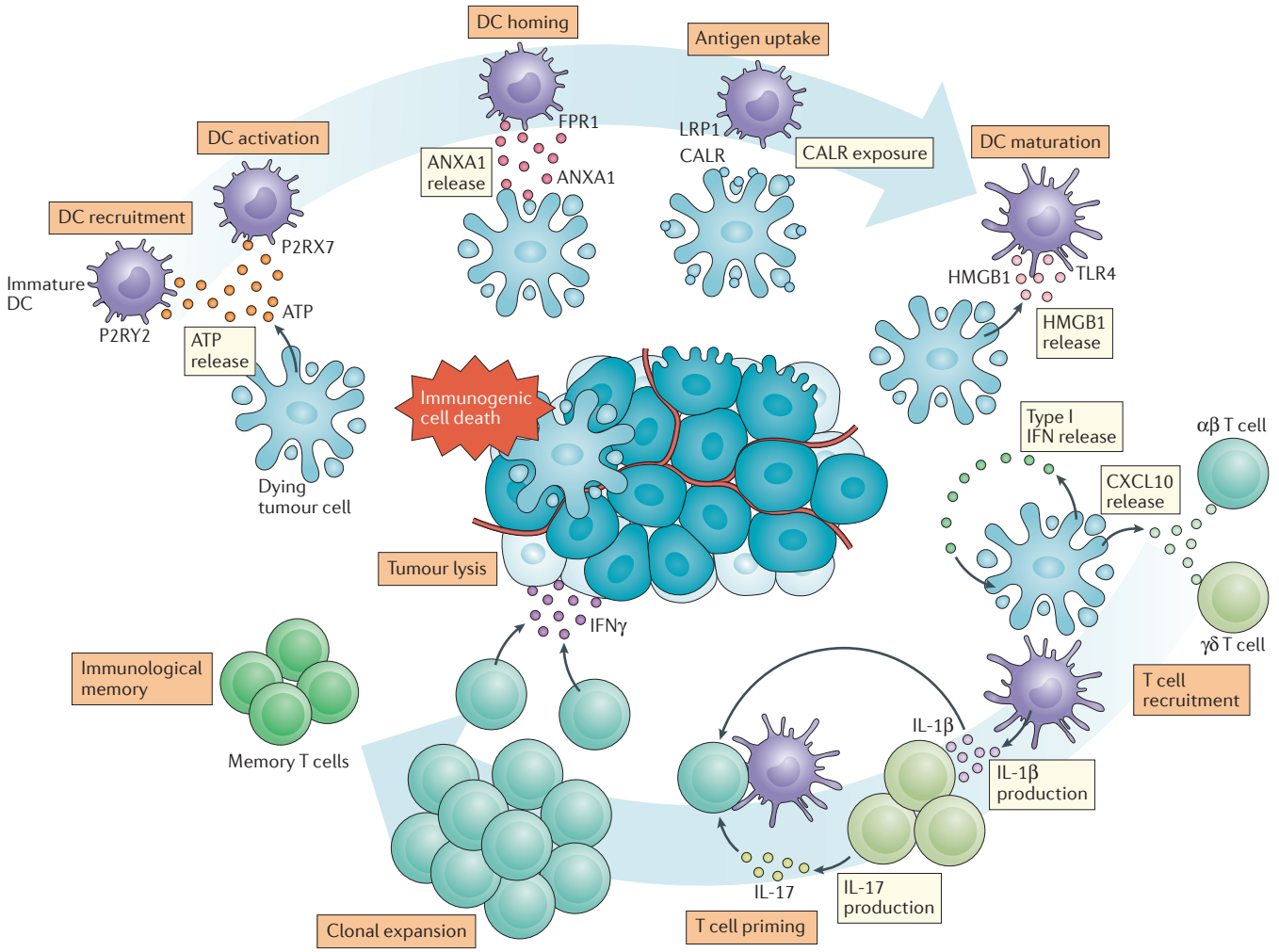


Figure 2 | **Mechanisms of chemotherapy-driven ICD.** In response to inducers of immunogenic cell death (ICD), such as doxorubicin or oxaliplatin, malignant cells expose calreticulin (CALR) and other endoplasmic reticulum chaperones on their surface, secrete ATP, initiate a cell-intrinsic type I interferon (IFN) response culminating in the production of CXC-chemokine ligand 10 (CXCL10), and release high-mobility group box 1 (HMGB1) and annexin A1 (ANXA1). Upon binding to cognate receptors on the surface of myeloid or lymphoid cells, these damage-associated molecular patterns favour the uptake of cell corpses and debris thereof by antigen-presenting cells, including dendritic cells (DCs) in the context of robust immunostimulatory signals, eventually leading to the priming of an adaptive immune response involving both αβ and γδ T cells. In addition to being associated with the establishment of immunological memory, such a response has the potential to eradicate malignant cells that survive chemotherapy via an IFNγ-dependent mechanism. CXCR3, CXC-chemokine receptor 3; FPR1, formyl peptide receptor 1; IFNAR1; interferon α/β-receptor subunit 1; IL, interleukin; LRP1, LDL receptor related protein 1; P2RX7, purinergic receptor P2X7; P2RY2, purinergic receptor P2Y2; TLR4, Toll-like receptor 4.

Photodynamic therapy
(PDT). A treatment for pre-malignant and malignant skin conditions, based on the administration of a drug that operates as a photosensitizer followed by exposure to a particular type of light.

Accidental necrosis
A form of cell death that cannot be modulated by pharmacological or genetic interventions, invariably manifesting with necrotic morphological features.

(which fully relies on the activation or reactivation of a tumour-targeting immune response) — mutational burden may influence the propensity of cancer cells undergoing ICD to engage adaptive immunity. However, to the best of our knowledge, all malignant cells challenged so far with bona fide ICD inducers efficiently triggered protective immune responses in vaccination tests[10]. Thus, it is possible that even the lowest level of mutational load associated with oncogenesis generates sufficient antigenicity to support immunogenicity. Indeed, neoplastic cells express several neo-antigens that (at least in the initial phases of malignant transformation and tumour progression) constitute modified variants of self that are not subject to central and peripheral tolerance[9] (BOX 2).

***ICD activated by physical cues.*** So far, three distinct physical interventions have been shown to trigger bona fide ICD of mouse cancer cells, as documented in vaccination assays: irradiation, hypericin-based photodynamic therapy (PDT) and high hydrostatic pressure[46–48]. Importantly, the induction of ICD by these interventions does not reflect the mere disassembly of the plasma membrane and consequent spillage of cytoplasmic content into the extracellular milieu[49]. Indeed, mouse cancer cells undergoing accidental necrosis in response to freeze–thawing or boiling are unable to activate DCs *in vitro*[50] and fail to elicit protective immunity upon inoculation of syngeneic mice[31,51]. The molecular mechanisms that account for the immunogenicity of

Box 2 | **Neo-antigens, tumour-associated antigens and immunosuppression**

As tumours arise and progress, they accumulate an increasing load of somatic mutations, resulting in the synthesis of several neo-antigens that (at least initially) are not subjected to tolerance[9]. Thus, tumours *de facto* evolve as potentially antigenic entities, and this has considerable therapeutic implications. Indeed, the propensity of patients with cancer to respond to checkpoint blockers has been robustly linked to tumour mutational load[9]. However, neo-antigens are not only patient-specific but also tumour-specific, implying that they are difficult to harness for therapeutic purposes.

Malignant cells also express wild-type proteins that are not (or much less so) expressed by their normal counterparts, including (but not limited to) proteins that are only expressed during embryonic development and proteins that are specifically synthesized in immunologically privileged tissues (such as the testes)[130]. An entire branch of immunotherapy has focused on the development of therapeutic interventions that would specifically target one or several of such tumour-associated antigens (TAAs), with negligible clinical success[130]. Several reasons underlie such a failure, including: first, the fact that TAAs are covered by central or peripheral tolerance mechanisms (although often to incomplete degrees); second, the difficulties in identifying a single or a few TAAs that are absolutely required for the survival of cancer cells but dispensable for their normal counterparts; third, the obstacles posed by the development of a clinically implementable approach for the elicitation of TAA-specific immune responses in patients; and, fourth, the immunosuppressive networks established by progressing neoplasms[130].

Indeed, malignant lesions escape immunosurveillance only as they acquire the capacity to manipulate the local and systemic microenvironment to annihilate innate and adaptive immune responses[121,122]. The mainstays of tumour-induced immunosuppression are: the inhibition of damage-associated molecular pattern (DAMP) signalling (see also main text); the marked infiltration of neoplastic lesions by immunosuppressive cells, including $CD4^+CD25^+FOXP3^+$ regulatory T cells, M2 macrophages and immature dendritic cells (DCs), which generally occurs at the expense of immunostimulatory or effector cells such as mature DCs, T helper 1 ($T_H1$) cells, $CD8^+$ T cells, natural killer (NK) cells and M1 macrophages; the abundant production within the tumour microenvironment of cytokines including interleukin-10 (IL-10) and transforming growth factor-β1 (TGFβ1); and the disruption of normal haematopoiesis, generally associated with an increased release in the circulation of rather immature myeloid cells with robust immunosuppressive activity, the so-called myeloid-derived suppressor cells (MDSCs)[121,122]. Counteracting one or multiple mechanisms whereby progressing tumours escape immunosurveillance (generally in combination with other therapeutic modalities) currently stands out as one of the most promising approaches for the treatment of several malignancies.

neoplastic cells exposed to irradiation, hypericin-based PDT and high hydrostatic pressure have been characterized less extensively than the pathways underlying chemotherapy-driven ICD. Nevertheless, it has been demonstrated that some modules of the machinery promoting ICD are universally required for the immunogenicity of dying cancer cells (at least in mice), whereas others are 'private' and contribute to ICD in a limited number of settings (see below) (FIG. 1).

The immunogenic potential of irradiation was initially recognized in the context of vaccination mediated by cancer cell lysates[52]. DCs loaded with irradiated malignant cells were found to elicit robust immune responses both in mice[53] and in patients with cancer[54]. Soon thereafter, vaccination experiments unequivocally demonstrated that γ-irradiation as well as UVC light could kill mouse cancer cells in a manner that — in immunocompetent hosts — elicits an adaptive immune response associated with the establishment of protective immunological memory[48,55]. More recently, α-irradiation with $^{213}Bi$ particles has been added to the list of bona fide ICD inducers, as per vaccination experiments with mouse MC38 colorectal carcinoma cells and syngeneic hosts[56]. The ability of irradiation to trigger ICD relies on UPR-dependent exposure of CALR[48,55,57] and ATP secretion driven by autophagy[57,58], in addition to type I IFN signalling[59,60]. Moreover, ICD induction by radiation therapy is accompanied by the exposure of HSP70 on the surface of malignant cells[61], TLR3 signalling[62], HMGB1 release[57,61,63] and IL-1β release upon inflammasome activation[64]; however, the requirement of these processes for the engagement of adaptive immunity has not yet been tested formally. Importantly, ICD driven by irradiation and the consequent activation of a tumour-specific $CD8^+$ T cell-dependent immune response are responsible for the abscopal effect[65], especially when radiation therapy is combined with a checkpoint blocker like ipilimumab[66–68]. In this context, it should be noted that dose and schedule dramatically affect the immunogenicity of irradiation-driven cell death. Thus, whereas single-dose radiation therapy seems unable to induce innate immunity against dying cancer cells, fractionated radiotherapy results in optimal immunostimulatory effects (at least in mice)[68]. This observation lends further support to the notion that immune responses to cell death critically rely on the timely release of endogenous adjuvant (in the context of increased antigenicity). It is tempting to speculate that radiation therapy may also boost the antigenicity of cancer cells, at least to some extent, but this conjecture remains to be experimentally addressed (FIG. 1).

Similar to chemotherapy- and irradiation-driven ICD, the immunogenic demise of human and mouse cells exposed to hypericin-based PDT or high hydrostatic pressure is accompanied by the exposure of ER chaperones including CALR, HSP70 and HSP90 on the plasma membrane[46,47,69], ATP secretion[47,69] and HMGB1 release[47,70]. Accordingly, the exposure of DCs to human cancer cells succumbing to hypericin-based PDT or high hydrostatic pressure induces not only the upregulation of various DC activation markers, including CD80, CD83, CD86 and MHC class II molecules but also the secretion of several pro-inflammatory cytokines, such as IL-1β, IL-12 and TNF, resulting in the priming of tumour-specific $CD8^+$ T cells[47,69]. Moreover, mouse cancer cells responding to hypericin-based PDT or high hydrostatic pressure efficiently protect syngeneic, immunocompetent mice against a subsequent challenge with living cells of the same type[69,71] (FIG. 1).

The molecular mechanisms that underlie danger signalling in cancer cells that are exposed to chemotherapy, irradiation, hypericin-based PDT or high hydrostatic pressure are more heterogeneous than it might appear at first glance. Indeed, whereas CALR exposure on malignant cells succumbing to chemotherapy-driven ICD is accompanied by (and mechanistically depends on) the phosphorylation of eIF2A, the activation of caspase 8 and the co-exposure of PDIA3, neoplastic cells responding to hypericin-based PDT externalize CALR independently of eIF2A phosphorylation, caspase 8 activation and PDIA3 (REFS 34,46,72,73). Similarly, a proficient autophagic response is an absolute requirement for ATP secretion triggered by anthracyclines, oxaliplatin and irradiation[36,58,73]. Conversely, autophagy not only seems to be irrelevant for the secretion of ATP elicited

Abscopal effect
Immunological response to radiation therapy whereby the irradiation of a malignant lesion results in the regression or stabilization of a distant, non-irradiated lesion.

by hypericin-based PDT in human cancer cells and mouse embryonic fibroblasts but also appears to inhibit the immunogenicity of this intervention owing to its capacity to limit oxidative damage, the UPR and consequent CALR exposure (which in this setting is initiated by reactive oxygen species)[74]. These observations indicate that not all danger signals may be universally required for the engagement of adaptive immunity in all scenarios. Moreover, they suggest that (at least in some cases) some intracellular mechanisms may functionally compensate for each other to ensure danger signalling. To what extent (if any) hypericin-based PDT and high hydrostatic pressures alter the antigenicity of neoplastic cells remains to be determined (FIG. 1).

***Necroptotic ICD.*** Necroptosis is a form of regulated cell death that is precipitated by the receptor-interacting serine/threonine kinase 3 (RIPK3)-catalysed phosphorylation of the pseudokinase mixed lineage kinase domain-like (MLKL), which results in the rapid formation of MLKL oligomers that irreversibly permeabilize the plasma membrane[75,76]. In some (but not all) variants of necroptosis, RIPK3 is activated by a signalling cascade that emanates from TNF receptor superfamily member 1A (TNFRSF1A; also known as TNFR1) and depends on the RIPK3 homologue RIPK1, implying that it can be delayed by the chemical RIPK1 inhibitor necrostatin 1 (REF. 13). Although necroptosis was soon recognized to be a highly pro-inflammatory form of cell death[77], its ability to engage the adaptive arm of the immune system and to evoke an antigen-specific immune response has not been investigated until recently. Thus, mouse lung carcinoma TC-1 and EL4 lymphoma cells (which naturally express high levels of RIPK3) exposed to necroptosis-inducing conditions (that is, TNF plus Z-VAD-fmk and a SMAC mimetic) die as they expose CALR on the plasma membrane, secrete ATP and release HMGB1 — three manifestations of ICD that, in this setting, are abrogated by knocking out *Ripk3* or *Mlkl*[78]. Accordingly, necroptotic TC-1 cells, but not TC-1 cells undergoing accidental necrosis upon freeze–thawing, efficiently vaccinate syngeneic C57BL/6 mice against a subsequent challenge with living cells of the same type[78]. TC-1 cells exposed to mitoxantrone also manifest markers of necroptosis, such as RIPK3 aggregation and the phosphorylation of MLKL, and die according to a kinetic that can be altered by the absence of *Ripk3* or *Mlkl*[78]. Most importantly, necroptosis-deficient *Ripk3*$^{-/-}$ or *Mlkl*$^{-/-}$ TC-1 cells succumbing to mitoxantrone fail to elicit protective immunity in C57BL/6 mice in conditions in which wild-type cells efficiently do so — a defect that is linked to reduced ATP secretion and limited HMGB1 release[78]. Along similar lines, *Ripk3*$^{-/-}$ as well as *Mlkl*$^{-/-}$ TC-1 cells growing in C57BL/6 mice are less sensitive to mitoxantrone-based chemotherapy than their wild-type counterparts as they are poorly infiltrated by CD11c$^{+}$CD86$^{+}$ APCs and CD8$^{+}$ T cells[78]. The local administration of an inhibitor of extracellular ATPases plus a synthetic TLR4 ligand restores the infiltration of necroptosis-deficient tumours by APCs and CD8$^{+}$ T cells, hence re-establishing normal sensitivity to mitoxantrone-based chemotherapy[78].

Comparable results have been obtained using mouse CT26 colorectal carcinoma cells (which do not naturally express high levels of RIPK3) engineered to express a variant of RIPK3 that can be activated by chemical-driven dimerization[51]. CT26 cells artificially driven into necroptosis secrete ATP, release HMGB1 and are rapidly phagocytosed by bone-derived mononuclear cells, which results in the upregulation of several activation markers including CD80, CD86 and MHC class II molecules[51]. Moreover, necroptotic CT26 cells (but not CT26 cells undergoing accidental necrosis in response to freeze–thawing) efficiently triggered an adaptive immune response driven by IFNγ-secreting CD8$^{+}$ T cells and accompanied by robust immunological memory in syngeneic BALB/c mice[51]. No signs of UPR were detected in CT26 cells undergoing necroptosis upon RIPK3 dimerization[51], which supports the notion that the adjuvanticity of ICD may be ensured by mechanistically distinct but functionally complementary pathways.

Further corroborating the hypothesis that necroptosis may constitute a functionally distinct variant of ICD, at least in some settings, the administration of Z-VAD-fmk (which favours necroptotic cell death by inhibiting caspases)[79] has been found to considerably ameliorate the therapeutic effect of irradiation-based multimodal therapy (fractionated irradiation plus dacarbazine plus hyperthermia) in immunocompetent mice bearing syngeneic B16 melanomas, an effect that depended on TLR signalling and was abrogated by the co-administration of the ATP-degrading enzyme apyrase[63] (see below). Z-VAD-fmk augmented the percentage of B16 cells dying with a necrotic morphology in response to multimodal therapy, resulting in increased HMGB1 release. This was associated with a superior adjuvanticity *in vitro*, as monitored by the expression of CD86, MHC class II molecules and TNF by APCs exposed to supernatants from dying B16 cells. *In vivo*, the presence of Z-VAD-fmk promoted tumour and lymph node infiltration by DCs and CD8$^{+}$ T cells secreting IFNγ[63]. Together, these observations suggest that different variants of ICD may synergize in the induction of potent adaptive immune responses, at least in the presence of adequate antigenicity (FIG. 1).

## Danger signalling in ICD

Once emitted by dying cells, DAMPs orchestrate antigen-specific immune responses by acting on both innate and adaptive components of the immune system[11,80]. Importantly, not all DAMPs are immunostimulatory, and immunosuppressive DAMPs like adenosine (a byproduct of extracellular ATP degradation) and prostaglandin E2 (an eicosanoid) seem to have a key function in the maintenance of tolerance in the course of physiological instances of cell death[81,82]. Irrespective of their importance for immunological homeostasis, immunosuppressive DAMPs are not discussed in further detail here.

***CALR and ER chaperones.*** CALR, PDIA3, HSP70, HSP90 and possibly other ER chaperones that are exposed on the membrane of cells undergoing ICD act as 'eat-me' signals, hence promoting the uptake of cell

Z-VAD-fmk
A chemical agent that inhibits a wide panel of proteolytic enzymes, including several caspases and calpains.

SMAC mimetic
A chemical agent that resembles second mitochondria-derived activator of caspase (SMAC; also known as DIABLO) in its ability to inhibit various members of the inhibitor of apoptosis (IAP) protein family.

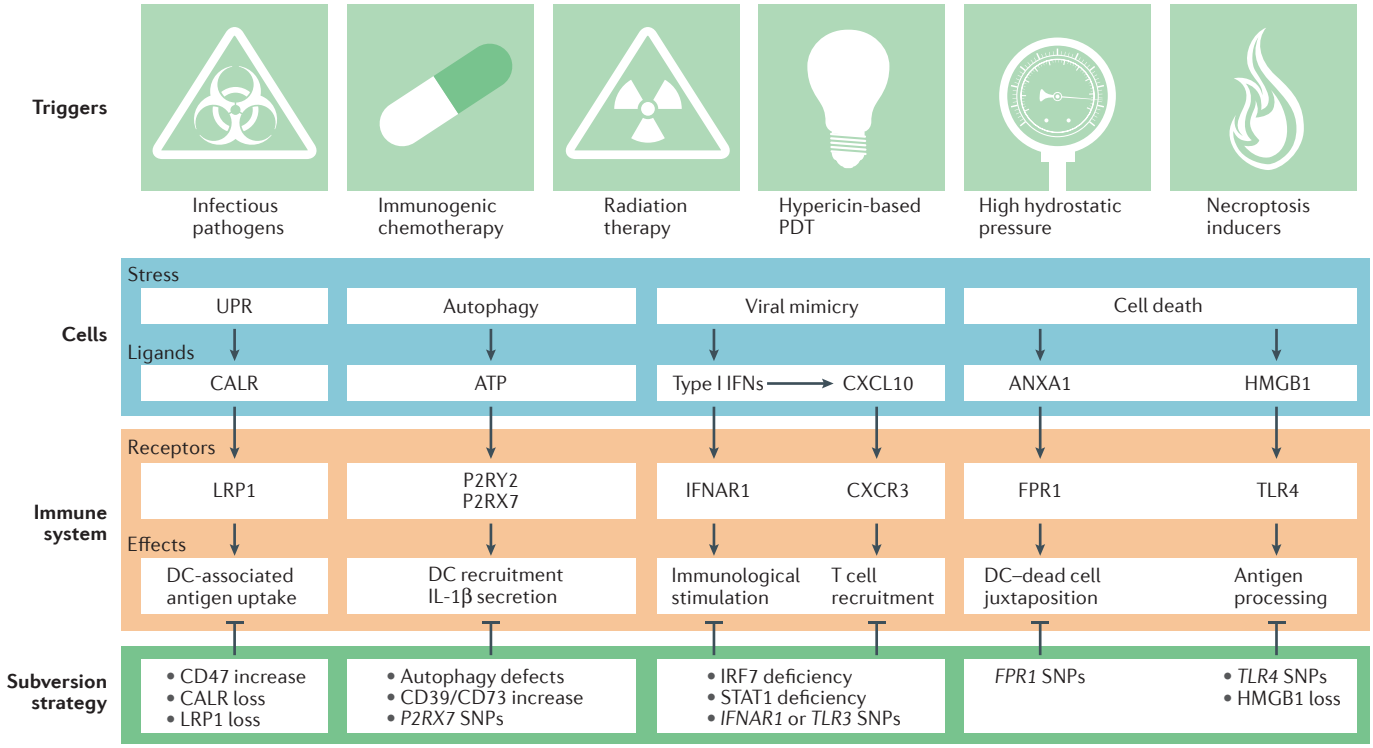


Figure 3 | **Subversion of danger signalling.** As they escape immunosurveillance, infectious pathogens and malignant cells acquire the ability to suppress danger signalling or the consequences thereof. In addition, clinical data obtained in patients with cancer indicate that multiple defects in the perception of damage-associated molecular patterns (DAMPs) by the host are associated with worsened disease outcome. ANXA1, annexin A1; CALR, calreticulin; CXCL10, CXC-chemokine ligand 10; CXCR3, CXC-chemokine receptor 3; DC, dendritic cell; FPR1, formyl peptide receptor 1; HMGB1, high-mobility group box 1; IFN, interferon; IFNAR1; interferon α/β-receptor subunit 1; IL-1β, interleukin-1β; IRF7, interferon regulatory factor 7; LRP1, LDL receptor related protein 1; PDT, photodynamic therapy; P2RX7, purinergic receptor P2X7; P2RY2, purinergic receptor P2Y2; SNP, single nucleotide polymorphism; STAT1, signal transducer and activator of transcription 1; TLR, Toll-like receptor; UPR, unfolded protein response.

corpses and debris by APCs[83]. The main docking site for CALR on the surface of cancer cells is provided by LDL receptor related protein 1 (LRP1; also known as CD91)[69]. In line with this notion, the immunogenicity of cancer cell death driven by hypericin-based PDT can be reduced by shRNA-dependent downregulation of LRP1 (REF. 69). LRP1 is also the main ER chaperone receptor expressed by human and mouse myeloid cells[84,85], and mouse macrophages lacking LRP1 exhibit limited phagocytic potential[84,86]. Moreover, the activation of DCs by human BC3 primary effusion lymphoma cells succumbing to bortezomib-driven ICD or human T24 bladder cancer cells treated with the putative ICD inducer capsaicin can be inhibited by a LRP1-targeting monoclonal antibody[87] or by LRP1 silencing through RNA interference[88], respectively. However, formal evidence indicating that LRP1 expression in the myeloid compartment is absolutely required for the initiation of adaptive immune responses by ICD is still missing. According to some investigators, ER chaperones are 'sticky' proteins and do not need receptors to bind membranes. Supporting this possibility, CALR-deficient cancer cells can be efficiently coated by CALR upon short incubation with the recombinant protein, which restores their uptake by phagocytes and hence reverses their intrinsically limited capacity to undergo productive ICD[33,89]. Robust expression of the CALR antagonist CD47 (which operates as a 'don't eat-me signal') has been linked to dismal prognosis in individuals affected by various cancers, including acute myeloid leukaemia, oesophageal carcinoma and ovarian cancer[90–92]. Conversely, high CALR levels in neoplastic cells (correlating with eIF2A phosphorylation) have recently been associated with improved clinical outcome in two independent cohorts of non-small-cell lung carcinoma patients[93]. Moreover, the monocytes of eight individuals with advanced melanoma progressing in an unusually slow manner were found to express elevated amounts of LRP1 compared with monocytes from eight patients who progressed normally[94], which suggests that danger signalling through the CALR–LRP1 axis may influence the outcome of patients with cancer in settings in which immunosurveillance (be it natural or therapy-elicited) is involved (FIG. 3; TABLE 2).

***Extracellular ATP.*** Extracellular ATP mediates robust chemotactic and adjuvant-like effects by interacting with purinergic receptor P2Y2 (P2RY2) and purinergic receptor P2X7 (P2RX7), respectively, on APCs and their precursors[40,95]. Thus, the immunogenicity of cell death is abrogated not only when ATP fails to accumulate in

Table 2 | **Evasion of danger signalling by pathogens and cancer cells***

| Danger signal | Strategy‡ | Setting‡ | Notes | Refs |
|---|---|---|---|---|
| ***Infectious pathogens*** | | | | |
| UPR and ER chaperone signalling | Inhibition of eIF2A phosphorylation | Multiple viral pathogens | Several viruses encode inhibitors of eIF2A kinases or proteins that promote eIF2A dephosphorylation | 115,116 |
| | CALR interactors | Multiple viral pathogens | Dozens of viral proteins interact with, and hence modulate the activity of, various ER chaperones — including CALR | 115,116 |
| Autophagy | BECN1 inhibition | HSV-1 | The neurovirulence factor ICP34.5 has prominent autophagy-blocking activity, hence inhibiting adaptive immunity | 114 |
| | Autophagosome formation inhibition | *Salmonella enterica* | Mutations in ATG16L1 and NOD2 limit pathogen clearance, and are associated with increased incidence of Crohn disease | 16 |
| Inflammasome signalling | Inflammasome assembly blockage | • Myxoma virus<br>• Shope fibroma virus | At least two distinct viral PYCARD-binding proteins have been described | 117 |
| | Caspase 1 inhibition | Multiple viral and bacterial pathogens | Influenza A virus, baculovirus, vaccinia virus, myxoma virus, *Yersinia spp.*, *Streptococcus pneumoniae* and other microbes express inhibitors of caspase 1 | 117 |
| Type I IFN signalling | *Ifnar1*$^{-/-}$ genotype | Multiple viral and bacterial pathogens | The absence of *Ifnar1* renders mice highly susceptible to infection, and this absence is sometimes required for the establishment of mouse models of infectious disease | 106–109 |
| | MHC class I downregulation | HPV-38 | E6 and E7 from HPV-38 interfere with type I IFN-driven MHC upregulation | 119 |
| Cell death | BCL-2-like protein expression | Multiple viral pathogens | More than 10 distinct viruses encode structural BCL-2-like proteins | 15 |
| | Caspase 3 or caspase 8 inhibition | Multiple viral pathogens | Baculovirus, cytomegalovirus and vaccinia virus encode three distinct inhibitors of caspases | 15 |
| | Necroptosis inhibition | • Murine cytomegalovirus<br>• HSV-1<br>• HSV-2 | ICP6, ICP10 and vIRA robustly inhibit necrosome formation in human cells | 118 |
| ***Cancer cells*** | | | | |
| UPR and ER chaperone signalling | Improved ER homeostasis | Patients affected by multiple tumours | High levels of GRP78 correlated with worsened disease outcome | 123 |
| | CALR loss | Patients with NSCLC | CALR levels of expression in malignant cells correlated with the phosphorylation of eIF2A and influenced disease outcome | 93 |
| | Limited HSP exposure | Patients with NHL | Limited HSP90 exposure was associated with no clinical responses to autologous cancer cell-based vaccination | 123 |
| | CD47 upregulation | Patients affected by multiple tumours | Low CD47 levels on neoplastic cells correlated with improved disease outcome | 90–92 |
| | LRP1 downregulation | Patients with melanoma | High LRP1 levels on monocytes were associated with slow progression | 94 |
| Autophagy and ATP signalling | Overexpression of BCL-2-like proteins | Patients affected by multiple tumours | Several cancers are characterized by the overexpression of BCL-2-like proteins, which potently inhibit autophagy | 125 |
| | BECN1 downregulation | Breast cancer patients | Decreased *BECN1* mRNA levels were associated with poor prognosis | 25 |
| | CD39 and/or CD73 overexpression | Patients affected by multiple tumours | High CD39 and/or CD73 levels on malignant or immune cells correlated with worsened disease outcome | 123 |
| | *P2RX7* SNPs | Patients with breast cancer | Loss-of-function *P2RX7* mutation was associated with shortened time-to-metastasis | 95 |
| RNA signalling | *TLR3* SNPs | Patients affected by multiple tumours | *TLR3* mutational status influenced disease outcome | 123 |
| | TLR3 downregulation | Patients affected by multiple tumours | High TLR3 mRNA or protein levels were associated with improved disease outcome | 123 |
| | TRIF downregulation | Patients with hepatocellular carcinoma | Robust TRIF expression correlated with increased overall survival | 123 |

Table 2 (cont.) | **Evasion of danger signalling by pathogens and cancer cells***

| Danger signal | Strategy‡ | Setting‡ | Notes | Refs |
|---|---|---|---|---|
| *Cancer cells (cont.)* | | | | |
| Type I IFN signalling | *IFNAR1* SNPs | Patients with glioma | Loss-of-function *IFNAR1* mutation was associated with worsened disease outcome | 123 |
| | IRF7 downregulation | Patients with breast cancer | Low IRF7 levels have been linked to decreased metastasis-free survival | 110 |
| | STAT1 deficiency | Patients with breast cancer | Approximately 33% of breast cancer biopsies displayed undetectable or extremely reduced STAT1 levels | 111 |
| ANXA1 signalling | *FRP1* SNPs | Patients with breast cancer | Loss-of-function *FPR1* mutation was associated with shortened time-to-metastasis and decreased overall survival | 39 |
| HMGB1 signalling | HMGB1 loss | Patients with breast cancer | Loss of nuclear HMGB1 positively correlated with tumour size | 104 |
| | *TLR4* SNPs | Patients with breast cancer | Loss-of-function *TLR4* mutation was associated with shortened time-to-metastasis | 38 |
| Cell death | *TP53* mutations | Patients affected by multiple tumours | Mutations in *TP53* are found in >50% of all human cancers, and are associated with increased resistance to cell death | 125 |
| | Altered expression of BCL-2 family members | Patients affected by multiple tumours | Many cancers overexpress anti-apoptotic BCL-2-like proteins or inactivate their pro-apoptotic counterparts | 125 |

ANXA1, annexin A1; ATG16L1, autophagy related 16 like 1; BCL-2, B cell lymphoma 2; BECN1, beclin 1; CALR, calreticulin; eIF2A, eukaryotic translation initiation factor 2A; ER, endoplasmic reticulum; FPR1, formyl peptide receptor 1; GRP78, 78 kDa glucose-regulated protein; HMGB1, high-mobility group box 1; HPV-38, human papillomavirus type 38; HSP, heat shock protein; HSV, herpes simplex virus; IFN, interferon; IFNAR1, interferon-α/β-receptor subunit 1; IRF, interferon regulatory factor; LRP1, LDL receptor related protein 1; NOD2, nucleotide binding oligomerization domain containing 2; NSCLC, non-small-cell lung carcinoma; NHL, non-Hodgkin lymphoma; P2RX7, purinergic receptor P2X7; PYCARD, PYD and CARD containing; SNP, single nucleotide polymorphism; STAT1, signal transducer and activator of transcription 1; TLR, Toll-like receptor; TRIF, TIR-domain-containing adaptor protein inducing IFNβ; UPR, unfolded protein response. *Active subversion or passively favourable situations. ‡Examples of which.

the microenvironment of dying cells[36,40,96] (see above), but also when *P2ry2* or *P2rx7* are absent from the myeloid compartment of the host[40,95]. In mouse DCs, purinergic signalling via P2RX7 promotes inflammasome activation coupled to the release of IL-1β[95], which is required for the initial lymphoid response to ICD driven by IL-17-producing γδ T cells[97]. Accordingly, *Nlrp3*$^{-/-}$ mice (which lack an essential component of the inflammasome)[98], *Il17a*$^{-/-}$ or *Il17ra*$^{-/-}$ mice (both of which are defective in the IL-17 system), as well as mice receiving an IL-1β-neutralizing antibody are unable to mount adaptive immune responses against syngeneic cancer cells succumbing to chemotherapy-driven ICD[95]. A loss-of-function polymorphism in *P2RX7* has also been shown to negatively affect disease outcome in a cohort of patients with breast carcinoma treated with anthracycline-based chemotherapy[95], suggesting that danger signalling has translational relevance in the context of cancer therapy (FIG. 3; TABLE 2).

***HMGB1.*** The molecular mechanisms that underlie the release of HMGB1 from cells undergoing ICD remain to be elucidated. Regardless of this unknown, extracellular HMGB1 mediates robust adjuvant-like effects by binding to various distinct PRRs, including TLR2, TLR4 and advanced glycosylation end product-specific receptor (AGER; also known as RAGE)[99]. However, whereas *Tlr2*$^{-/-}$ and *Ager*$^{-/-}$ mice can be normally vaccinated by syngeneic cancer cells that undergo ICD in response to chemotherapy, the same does not hold true for *Tlr4*$^{-/-}$ mice or *Myd88*$^{-/-}$ mice (which lack myeloid differentiation primary-response protein 88, a key adaptor for TLR signalling)[38]. Thus, danger signalling through the HMGB1–TLR4–MYD88 axis appears to be required for adaptive immune responses against mouse cancer cells succumbing to ICD. Further supporting the translational relevance of ICD, loss-of-function polymorphisms in *TLR4* have been associated with unfavourable disease outcome in cohorts of patients with breast carcinoma treated with anthracyclines[38], patients with head and neck squamous cell carcinoma undergoing systemic chemotherapy[100], and subjects with melanoma receiving a DC-based vaccine[101] or other treatment modalities[102]. Moreover, the loss of HMGB1 from malignant cells has been noted to negatively affect prognosis in patients with breast cancer treated with anthracycline-based adjuvant chemotherapy[103,104]. Of note, HMGB1 has also been proposed to mediate immunosuppressive functions on binding to hepatitis A virus cellular receptor 2 (HAVCR2; also known as TIM3), at least in mice[105]. The significance of this signal transduction cascade for cancer therapy, however, remains to be elucidated (FIG. 3; TABLE 2).

***Type I IFNs.*** Type I IFNs are secreted by virtually all cells upon viral or bacterial infection, reflecting the detection of multiple MAMPs by PRRs[23]. Upon binding to IFNAR1–IFNAR2 heterodimers, type I IFNs not only increase the resistance of neighbouring cells to infection, but also stimulate the activation of macrophages, DCs and natural killer (NK) cells, hence alerting them of pathogens[23]. Consistent with this notion, *Ifnar1*$^{-/-}$ mice are much more susceptible than their wild-type counterparts to several viruses, including respiratory

syncytial virus, Zika virus and mouse hepatitis virus, as well as to intracellular bacteria such as *S. enterica*[106–109]. Type I IFNs are also produced by mouse neoplastic cells succumbing to chemotherapy-driven ICD, most likely reflecting TLR3 activation by cancer cell-derived RNA[37]. In this setting, however, the adjuvant effects of type I IFNs mainly stem from the activation of a cancer cell-autonomous signal transduction cascade resulting in CXCL10 secretion[37]. Thus, the efficacy of anthracycline-based chemotherapy in mice is reduced in the presence of an IFNAR1-neutralizing antibody, or when neoplastic cells (but not the host) lack *Ifnar1*, *Ifnar2* or *Tlr3*. In all these models, therapeutic efficacy can be partially restored by the co-administration of recombinant CXCL10 (REF. 37). Accordingly, neutralizing the main CXCL10 receptor, namely, CXC-chemokine receptor 3 (CXCR3), with a monoclonal antibody also impairs the efficacy of anthracycline-based chemotherapy *in vivo*[37]. Along similar lines, *Ifnar1*$^{-/-}$ mouse cancer cells killed by doxorubicin cannot vaccinate syngeneic hosts in conditions in which wild-type cells efficiently do so. Moreover, the rate of tumour-free mice in this setting is not affected by the absence of *Ifnar1* in the host but is significantly reduced if vaccination is performed with wild-type mouse cells exposed to doxorubicin in the presence of monoclonal antibodies neutralizing type I IFNs or IFNAR1 (REF. 37). Although CXCL10 is known to mediate chemotactic effects on T cells, it remains to be determined whether this is the mechanism that underlies its adjuvant activity in the context of chemotherapy-driven ICD. Irrespective of this, the expression of a type I IFN-related metagene has been shown to predict the pathological complete response of patients with breast carcinoma to anthracycline-based chemotherapy[37]. Reduced levels of interferon regulatory factor 7 (IRF7), one of the transducers involved in type I IFN signalling, have been linked to decreased metastasis-free survival in a large cohort of over 800 women with breast carcinoma[110]. Similarly, approximately one third of 161 human breast cancers displayed undetectable or very low levels of signal transducer and activator of transcription 1 (STAT1; another component of the type I IFN signalling pathway)[111]. Moreover, high levels of TLR3 in neoplastic lesions have been associated with improved disease outcome in breast carcinoma patients treated with a TLR3 agonist plus radiation therapy[112]. These observations lend further support to the idea that danger signalling is clinically relevant in the context of anticancer chemo- and radiotherapy (FIG. 3; TABLE 2).

***ANXA1.*** ANXA1 belongs to a superfamily of proteins that bind acidic phospholipids in a $Ca^{2+}$-dependent manner, and it is expressed at moderate-to-high levels by both myeloid and lymphoid cells as well as by many epithelial cell types[113]. Initially, ANXA1 was characterized for its key role in the resolution of inflammatory responses, which mainly reflects the ability of secreted or surface-exposed ANXA1 to elicit autocrine, paracrine or juxtacrine signalling via formyl peptide receptor 2 (FPR2)[113]. More recently, our group demonstrated that the therapeutic effect of anthracyclines or oxaliplatin in mice is lost when cancer cells lack *Anxa1* and when the host immune system lacks *Fpr1* (which encodes another ANXA1 receptor)[39]. Mouse *Fpr1*$^{-/-}$ APCs infiltrate malignant lesions treated with anthracyclines or oxaliplatin as well as their wild-type counterparts do, but they display a selective defect in the final approach to dying cancer cells, and hence cannot initiate an adaptive immune response[39]. A loss-of-function polymorphism in *FPR1* has been associated with decreased time-to-metastasis and overall survival in a cohort of patients with breast carcinoma receiving anthracycline-based chemotherapy, as well as in a cohort of patients with colorectal carcinoma treated with oxaliplatin[39]. Importantly, the impact of *FPR1* status could only be observed among patients with wild-type *TLR3* and *TLR4* (REF. 39), which suggests that these three PRRs operate in the same pathway linking chemotherapy-driven ICD to the elicitation of a clinically relevant, tumour-targeting immune response (FIG. 3; TABLE 2).

## Subversion of ICD

The concept of ICD has been developed in tumour models, in which adjuvanticity is solely dictated by DAMPs. This considerably simplified the molecular characterization of the phenomenon. However, the primordial function of the immune system is not anticancer immunosurveillance but rather the control of invading pathogens, suggesting that ICD may play a major role in the context of microbial infection. In favour of this hypothesis, it appears that pathogenic viruses and bacteria have developed multiple strategies to subvert the release or detection of DAMPs, presumably with the aim of escaping immune control.

Thus, several microorganisms including viruses and bacteria express functional orthologues of anti- or pro-apoptotic members of the BCL-2 protein family[15], autophagy inhibitors[16,114], proteins that favour the dephosphorylation of eIF2A[115,116], inflammasome-inhibiting factors[117], caspase blockers[15], proteins that prevent the assembly of the necrosome[118], and/or inhibitors of type I IFN signalling[119], which often (if not always) are required for overt pathogenicity (TABLE 2). The existence of such a multipronged armamentarium strongly suggests that the selective pressure on limiting the adjuvanticity of infection-driven cell death has been high during (at least some stages of) the host–pathogen co-evolution. Controlling immunogenicity by limiting adjuvanticity (which is mainly determined by the host) seems indeed more straightforward than doing so by limiting antigenicity (which is mainly determined by the genetics of the pathogen).

Similar considerations can be made for malignant cells. Indeed, according to the immunosurveillance model, overtly malignant lesions only develop once neoplastic cells fully escape immune recognition and elimination[120], and such a potentially lethal progression is intimately linked to the inhibition of various processes involved in DAMP emission and sensing, and/or the immunological consequences thereof[121–123] (TABLE 2). Like pathogens, advanced tumours often exhibit an elevated antigenicity[9], but they efficiently

BCL-2 protein family
A large group of proteins sharing one to four B cell lymphoma 2 (BCL-2) homology (BH) domains, which play a crucial role in the regulation of some variants of apoptotic cell death.

Necrosome
An amyloid-like supramolecular platform that precipitates necroptosis by allowing for the physical and functional interaction between RIPK1, RIPK3 and MLKL.

Antigen spreading
Immunological phenomenon whereby the antigenic targets of an adaptive immune response expand and diversify over time, presumably as a consequence of sustained cell death and DAMP signalling.

control immunogenicity by various mechanisms operating on (or downstream of) adjuvanticity[121–123] (BOX 2), which considerably impairs the efficiency of multiple chemo-, radio- and immunotherapeutic regimens[124]. Thus, cancer cells obtain an advantage not only from defective DAMP emission or sensing (see above), but also from the suppression of upstream adaptive mechanisms, including the UPR and autophagy, and from the subversion of the signal transduction cascades that precipitate cell death[125] (TABLE 2).

Together, these observations reinforce the notion that restoring the immunogenicity of cancer cells constitutes a prominent, clinically-relevant goal, and that the most promising target in this sense is adjuvanticity (or processes downstream thereof).

## Conclusions and perspectives

ICD stands out as a major initiator of adaptive immunity in the context of infectious and malignant diseases, both of which involve increased antigenicity and adjuvanticity. Adjuvanticity depends on the release or exposure of specific MAMPs or DAMPs, and both infectious pathogens and neoplastic cells have developed an arsenal of strategies to subvert danger signalling and hence avoid immune detection. Of note, danger signalling largely reflects the communication of a state of cellular stress to the organism. Thus, the stress-responsive processes that underlie danger signalling (including autophagy) operate to maintain whole-body homeostasis across the plasma membrane. Owing to its prominent cytoprotective activity, autophagy limits the intrinsic susceptibility of neoplastic cells to the cytotoxic effects of anticancer agents[25]. Accordingly, human and mouse autophagic-deficient malignant cells growing *in vitro* or in immunodeficient hosts are more sensitive than their wild-type counterparts to chemotherapy and irradiation[25,58]. However, cancers evolving in immunocompetent syngeneic mice are less sensitive to treatment if their autophagic proficiency is compromised by genetic interventions[58]. Similar considerations can be made for other mechanisms of cellular adaptation, such as the UPR[22]. This suggests not only that the functions of these processes (which are found in all eukaryotes) seem to have expanded from the preservation of cellular fitness to the maintenance of homeostasis at the organismal level, but also that protection of whole-body homeostasis generally dominates over the maintenance of cellular fitness.

Finally, it is tempting to speculate that (at least some) autoimmune disorders may originate from situations in which an unwarranted wave of cell death is mistakenly perceived as immunogenic. In this setting, robust DAMP signalling might drive an adaptive immune response even against innocuous antigens, possibly as a consequence of antigen spreading, and hence overcome tolerance to self. An intriguing finding in support of this notion is that various disorders with an autoimmune component, including rheumatoid arthritis[126], coeliac disease[127] and inflammatory bowel disease[128], are associated with increased circulating levels of CALR or CALR-specific antibodies. Thus, it will be interesting to determine whether artificial exacerbation of DAMP signalling in the context of cell death (but in the absence of accrued antigenicity) can drive autoimmune disorders, and — if so — whether the antigenic target of such a pathological response is under defective tolerance.

In summary, DAMP signalling occupies a hierarchically superior position in the preservation of whole-body immunological and inflammatory homeostasis, hence constituting a promising target for the development of therapeutic agents with oncological and non-oncological applications.


1. Fuchs, Y. & Steller, H. Programmed cell death in animal development and disease. *Cell* **147**, 742–758 (2011).
2. van Kempen, T. S., Wenink, M. H., Leijten, E. F., Radstake, T. R. & Boes, M. Perception of self: distinguishing autoimmunity from autoinflammation. *Nat. Rev. Rheumatol.* **11**, 483–492 (2015).
3. Matzinger, P. Tolerance, danger, and the extended family. *Annu. Rev. Immunol.* **12**, 991–1045 (1994).
4. Janeway, C. A. Jr. The immune system evolved to discriminate infectious nonself from noninfectious self. *Immunol. Today* **13**, 11–16 (1992).
5. Broz, P. & Monack, D. M. Newly described pattern recognition receptors team up against intracellular pathogens. *Nat. Rev. Immunol.* **13**, 551–565 (2013).
6. Cao, X. Self-regulation and cross-regulation of pattern-recognition receptor signalling in health and disease. *Nat. Rev. Immunol.* **16**, 35–50 (2016).
7. Chu, H. & Mazmanian, S. K. Innate immune recognition of the microbiota promotes host–microbial symbiosis. *Nat. Immunol.* **14**, 668–675 (2013).
8. Fuchs, Y. & Steller, H. Live to die another way: modes of programmed cell death and the signals emanating from dying cells. *Nat. Rev. Mol. Cell Biol.* **16**, 329–344 (2015).
9. Schumacher, T. N. & Schreiber, R. D. Neoantigens in cancer immunotherapy. *Science* **348**, 69–74 (2015).
10. Kroemer, G., Galluzzi, L., Kepp, O. & Zitvogel, L. Immunogenic cell death in cancer therapy. *Annu. Rev. Immunol.* **31**, 51–72 (2013).
11. Krysko, D. V. *et al.* Immunogenic cell death and DAMPs in cancer therapy. *Nat. Rev. Cancer* **12**, 860–875 (2012).
12. Bezu, L. *et al.* Combinatorial strategies for the induction of immunogenic cell death. *Front. Immunol.* **6**, 187 (2015).
13. Galluzzi, L. *et al.* Essential versus accessory aspects of cell death: recommendations of the NCCD 2015. *Cell Death Differ.* **22**, 58–73 (2015).
14. Kepp, O. *et al.* Consensus guidelines for the detection of immunogenic cell death. *Oncoimmunology* **3**, e955691 (2014).
15. Galluzzi, L., Brenner, C., Morselli, E., Touat, Z. & Kroemer, G. Viral control of mitochondrial apoptosis. *PLoS Pathog.* **4**, e1000018 (2008).
16. LaRock, D. L., Chaudhary, A. & Miller, S. I. *Salmonella*e interactions with host processes. *Nat. Rev. Microbiol.* **13**, 191–205 (2015).
17. Gay, N. J., Symmons, M. F., Gangloff, M. & Bryant, C. E. Assembly and localization of Toll-like receptor signalling complexes. *Nat. Rev. Immunol.* **14**, 546–558 (2014).
18. Kanneganti, T. D. Central roles of NLRs and inflammasomes in viral infection. *Nat. Rev. Immunol.* **10**, 688–698 (2010).
19. Barber, G. N. STING-dependent cytosolic DNA sensing pathways. *Trends Immunol.* **35**, 88–93 (2014).
20. Yoneyama, M., Onomoto, K., Jogi, M., Akaboshi, T. & Fujita, T. Viral RNA detection by RIG-I-like receptors. *Curr. Opin. Immunol.* **32**, 48–53 (2015).
21. Sica, V. *et al.* Organelle-specific initiation of autophagy. *Mol. Cell* **59**, 522–539 (2015).
22. Bettigole, S. E. & Glimcher, L. H. Endoplasmic reticulum stress in immunity. *Annu. Rev. Immunol.* **33**, 107–138 (2015).
23. McNab, F., Mayer-Barber, K., Sher, A., Wack, A. & O'Garra, A. Type I interferons in infectious disease. *Nat. Rev. Immunol.* **15**, 87–103 (2015).
24. Latz, E., Xiao, T. S. & Stutz, A. Activation and regulation of the inflammasomes. *Nat. Rev. Immunol.* **13**, 397–411 (2013).
25. Galluzzi, L. *et al.* Autophagy in malignant transformation and cancer progression. *EMBO J.* **34**, 856–880 (2015).
26. Travassos, L. H. *et al.* Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. *Nat. Immunol.* **11**, 55–62 (2010).
27. Liang, X. H. *et al.* Protection against fatal Sindbis virus encephalitis by beclin, a novel Bcl-2-interacting protein. *J. Virol.* **72**, 8586–8596 (1998).
28. Talloczy, Z. *et al.* Regulation of starvation- and virus-induced autophagy by the eIF2$\alpha$ kinase signaling pathway. *Proc. Natl Acad. Sci. USA* **99**, 190–195 (2002).
29. Roche, P. A. & Furuta, K. The ins and outs of MHC class II-mediated antigen processing and presentation. *Nat. Rev. Immunol.* **15**, 203–216 (2015).
30. Joffre, O. P., Segura, E., Savina, A. & Amigorena, S. Cross-presentation by dendritic cells. *Nat. Rev. Immunol.* **12**, 557–569 (2012).
31. Casares, N. *et al.* Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. *J. Exp. Med.* **202**, 1691–1701 (2005).
**This is the first formal demonstration that anthracycline-treated mouse cancer cells can induce an adaptive immune response upon inoculation of immunocompetent syngeneic hosts in the absence of any adjuvant.**
32. Dudek, A. M., Garg, A. D., Krysko, D. V., De Ruysscher, D. & Agostinis, P. Inducers of immunogenic cancer cell death. *Cytokine Growth Factor Rev.* **24**, 319–333 (2013).

33. Obeid, M. *et al.* Calreticulin exposure dictates the immunogenicity of cancer cell death. *Nat. Med.* **13**, 54–61 (2007).
34. Panaretakis, T. *et al.* The co-translocation of ERp57 and calreticulin determines the immunogenicity of cell death. *Cell Death Differ.* **15**, 1499–1509 (2008).
35. Fucikova, J. *et al.* Human tumor cells killed by anthracyclines induce a tumor-specific immune response. *Cancer Res.* **71**, 4821–4833 (2011).
36. Michaud, M. *et al.* Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. *Science* **334**, 1573–1577 (2011).
**This article provides robust evidence in support of the notion that autophagy is strictly required for mouse cancer cells succumbing to immunogenic chemotherapeutics to release amounts of ATP that are compatible with the engagement of adaptive immunity.**
37. Sistigu, A. *et al.* Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. *Nat. Med.* **20**, 1301–1309 (2014).
38. Apetoh, L. *et al.* Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat. Med.* **13**, 1050–1059 (2007).
39. Vacchelli, E. *et al.* Chemotherapy-induced antitumor immunity requires formyl peptide receptor 1. *Science* **350**, 972–978 (2015).
**This article demonstrates that chemotherapy-driven ICD depends on the interaction between ANXA1 released by dying cancer cells and FPR1 expressed by the host.**
40. Elliott, M. R. *et al.* Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. *Nature* **461**, 282–286 (2009).
**This is the first demonstration that extracellular nucleotides including ATP and UTP generate a steep chemotactic gradient around dying cells, which guide the recruitment of myeloid cells through P2RY2.**
41. Tesniere, A. *et al.* Immunogenic death of colon cancer cells treated with oxaliplatin. *Oncogene* **29**, 482–491 (2010).
42. Martins, I. *et al.* Restoration of the immunogenicity of cisplatin-induced cancer cell death by endoplasmic reticulum stress. *Oncogene* **30**, 1147–1158 (2011).
43. Le, D. T. *et al.* PD-1 blockade in tumors with mismatch-repair deficiency. *N. Engl. J. Med.* **372**, 2509–2520 (2015).
44. Gubin, M. M. *et al.* Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature* **515**, 577–581 (2014).
45. Van Allen, E. M. *et al.* Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science* **350**, 207–211 (2015).
46. Garg, A. D., Krysko, D. V., Vandenabeele, P. & Agostinis, P. Hypericin-based photodynamic therapy induces surface exposure of damage-associated molecular patterns like HSP70 and calreticulin. *Cancer Immunol. Immunother.* **61**, 215–221 (2012).
47. Fucikova, J. *et al.* High hydrostatic pressure induces immunogenic cell death in human tumor cells. *Int. J. Cancer* **135**, 1165–1177 (2014).
**This is the first comprehensive molecular characterization of high hydrostatic pressure-driven ICD in human cancer cells.**
48. Obeid, M. *et al.* Calreticulin exposure is required for the immunogenicity of γ-irradiation and UVC light-induced apoptosis. *Cell Death Differ.* **14**, 1848–1850 (2007).
49. Galluzzi, L., Bravo-San Pedro, J. M., Kepp, O. & Kroemer, G. Regulated cell death and adaptive stress responses. *Cell. Mol. Life Sci.* **73**, 2405–2410 (2016).
50. Goldszmid, R. S. *et al.* Dendritic cells charged with apoptotic tumor cells induce long-lived protective $CD4^+$ and $CD8^+$ T cell immunity against B16 melanoma. *J. Immunol.* **171**, 5940–5947 (2003).
51. Aaes, T. L. *et al.* Vaccination with necroptotic cancer cells induces efficient anti-tumor immunity. *Cell Rep.* **15**, 274–287 (2016).
52. Bloy, N. *et al.* Trial watch: dendritic cell-based anticancer therapy. *Oncoimmunology* **3**, e963424 (2014).
53. Strome, S. E. *et al.* Strategies for antigen loading of dendritic cells to enhance the antitumor immune response. *Cancer Res.* **62**, 1884–1889 (2002).
54. Kurokawa, T., Oelke, M. & Mackensen, A. Induction and clonal expansion of tumor-specific cytotoxic T lymphocytes from renal cell carcinoma patients after stimulation with autologous dendritic cells loaded with tumor cells. *Int. J. Cancer* **91**, 749–756 (2001).
55. Gameiro, S. R. *et al.* Radiation-induced immunogenic modulation of tumor enhances antigen processing and calreticulin exposure, resulting in enhanced T-cell killing. *Oncotarget* **5**, 403–416 (2014).
56. Gorin, J. B. *et al.* Antitumor immunity induced after α irradiation. *Neoplasia* **16**, 319–328 (2014).
57. Golden, E. B. *et al.* Radiation fosters dose-dependent and chemotherapy-induced immunogenic cell death. *Oncoimmunology* **3**, e28518 (2014).
58. Ko, A. *et al.* Autophagy inhibition radiosensitizes *in vitro*, yet reduces radioresponses *in vivo* due to deficient immunogenic signalling. *Cell Death Differ.* **21**, 92–99 (2014).
**This report discriminated for the first time between the cell-intrinsic and cell-extrinsic effects of autophagy in cancer cells responding to radiation therapy, unequivocally demonstrating that the latter dominate over the former in immunocompetent settings.**
59. Deng, L. *et al.* STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors. *Immunity* **41**, 843–852 (2014).
60. Lim, J. Y., Gerber, S. A., Murphy, S. P. & Lord, E. M. Type I interferons induced by radiation therapy mediate recruitment and effector function of $CD8^+$ T cells. *Cancer Immunol. Immunother.* **63**, 259–271 (2014).
61. Brusa, D., Migliore, E., Garetto, S., Simone, M. & Matera, L. Immunogenicity of 56°C and UVC-treated prostate cancer is associated with release of HSP70 and HMGB1 from necrotic cells. *Prostate* **69**, 1343–1352 (2009).
62. Bernard, J. J. *et al.* Ultraviolet radiation damages self noncoding RNA and is detected by TLR3. *Nat. Med.* **18**, 1286–1290 (2012).
63. Werthmöller, N., Frey, B., Wunderlich, R., Fietkau, R. & Gaipl, U. S. Modulation of radiochemoimmunotherapy-induced B16 melanoma cell death by the pan-caspase inhibitor zVAD-fmk induces anti-tumor immunity in a HMGB1-, nucleotide- and T-cell-dependent manner. *Cell Death Dis.* **6**, e1761 (2015).
64. Chen, L. C. *et al.* Tumour inflammasome-derived IL-1β recruits neutrophils and improves local recurrence-free survival in EBV-induced nasopharyngeal carcinoma. *EMBO Mol. Med.* **4**, 1276–1293 (2012).
65. Demaria, S. *et al.* Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. *Int. J. Radiat. Oncol. Biol. Phys.* **58**, 862–870 (2004).
**This report demonstrates that increasing the availability of DCs is sufficient to induce robust abscopal effects in response to irradiation.**
66. Golden, E. B., Demaria, S., Schiff, P. B., Chachoua, A. & Formenti, S. C. An abscopal response to radiation and ipilimumab in a patient with metastatic non-small cell lung cancer. *Cancer Immunol. Res.* **1**, 365–372 (2013).
67. Vanpouille-Box, C., Pilones, K. A., Wennerberg, E., Formenti, S. C. & Demaria, S. *In situ* vaccination by radiotherapy to improve responses to anti-CTLA-4 treatment. *Vaccine* **33**, 7415–7422 (2015).
68. Dewan, M. Z. *et al.* Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. *Clin. Cancer Res.* **15**, 5379–5388 (2009).
69. Garg, A. D. *et al.* A novel pathway combining calreticulin exposure and ATP secretion in immunogenic cancer cell death. *EMBO J.* **31**, 1062–1079 (2012).
**This report characterizes the molecular mechanisms underlying CALR exposure and ATP secretion by mouse cancer cells exposed to hypericin-based PDT.**
70. Korbelik, M., Zhang, W. & Merchant, S. Involvement of damage-associated molecular patterns in tumor response to photodynamic therapy: surface expression of calreticulin and high-mobility group box-1 release. *Cancer Immunol. Immunother.* **60**, 1431–1437 (2011).
71. Weiss, E. M. *et al.* High hydrostatic pressure treatment generates inactivated mammalian tumor cells with immunogeneic features. *J. Immunotoxicol.* **7**, 194–204 (2010).
72. Panaretakis, T. *et al.* Mechanisms of pre-apoptotic calreticulin exposure in immunogenic cell death. *EMBO J.* **28**, 578–590 (2009).
73. Galluzzi, L., Kepp, O. & Kroemer, G. Enlightening the impact of immunogenic cell death in photodynamic cancer therapy. *EMBO J.* **31**, 1055–1057 (2012).
74. Garg, A. D. *et al.* ROS-induced autophagy in cancer cells assists in evasion from determinants of immunogenic cell death. *Autophagy* **9**, 1292–1307 (2013).
**This paper shows that autophagy not only is dispensable for ATP release by cancer cells succumbing to hypericin-based PDT, but also limits CALR exposure by virtue of its antioxidant effects.**
75. Wang, H. *et al.* Mixed lineage kinase domain-like protein MLKL causes necrotic membrane disruption upon phosphorylation by RIP3. *Mol. Cell* **54**, 133–146 (2014).
76. Dondelinger, Y. *et al.* MLKL compromises plasma membrane integrity by binding to phosphatidylinositol phosphates. *Cell Rep.* **7**, 971–981 (2014).
77. Pasparakis, M. & Vandenabeele, P. Necroptosis and its role in inflammation. *Nature* **517**, 311–320 (2015).
78. Yang, H. *et al.* Contribution of RIP3 and MLKL to immunogenic cell death signaling in cancer chemotherapy. *Oncoimmunology* **5**, e1149673 (2016).
**References 51 and 78 demonstrate for the first time that necroptosis can be perceived as immunogenic and hence induce an adaptive immune response.**
79. Galluzzi, L., López-Soto, A., Kumar, S. & Kroemer, G. Caspases connect cell-death signaling to organismal homeostasis. *Immunity* **44**, 221–231 (2016).
80. Galluzzi, L., Kepp, O. & Kroemer, G. Mitochondria: master regulators of danger signalling. *Nat. Rev. Mol. Cell Biol.* **13**, 780–788 (2012).
81. Cekic, C. & Linden, J. Purinergic regulation of the immune system. *Nat. Rev. Immunol.* **16**, 177–192 (2016).
82. Hangai, S. *et al.* PGE2 induced in and released by dying cells functions as an inhibitory DAMP. *Proc. Natl Acad. Sci. USA* **113**, 3844–3849 (2016).
83. Poon, I. K., Lucas, C. D., Rossi, A. G. & Ravichandran, K. S. Apoptotic cell clearance: basic biology and therapeutic potential. *Nat. Rev. Immunol.* **14**, 166–180 (2014).
84. Gardai, S. J. *et al.* Cell-surface calreticulin initiates clearance of viable or apoptotic cells through *trans*-activation of LRP on the phagocyte. *Cell* **123**, 321–334 (2005).
**This article identifies LRP1 as the main receptor that drives the uptake of dying cells exposing CALR on their membrane by myeloid cells.**
85. Basu, S., Binder, R. J., Ramalingam, T. & Srivastava, P. K. CD91 is a common receptor for heat shock proteins gp96, hsp90, hsp70, and calreticulin. *Immunity* **14**, 303–313 (2001).
86. Lillis, A. P. *et al.* Murine low-density lipoprotein receptor-related protein 1 (LRP) is required for phagocytosis of targets bearing LRP ligands but is not required for C1q-triggered enhancement of phagocytosis. *J. Immunol.* **181**, 364–373 (2008).
87. Cirone, M. *et al.* Primary effusion lymphoma cell death induced by bortezomib and AG 490 activates dendritic cells through CD91. *PLoS ONE* **7**, e31732 (2012).
88. Gilardini Montani, M. S. *et al.* Capsaicin-mediated apoptosis of human bladder cancer cells activates dendritic cells via CD91. *Nutrition* **31**, 578–581 (2015).
89. Garg, A. D. *et al.* Resistance to anticancer vaccination effect is controlled by a cancer cell-autonomous phenotype that disrupts immunogenic phagocytic removal. *Oncotarget* **6**, 26841–26860 (2015).
90. Suzuki, S. *et al.* CD47 expression regulated by the miR-133a tumor suppressor is a novel prognostic marker in esophageal squamous cell carcinoma. *Oncol. Rep.* **28**, 465–472 (2012).
91. Majeti, R. *et al.* CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. *Cell* **138**, 286–299 (2009).
92. Wang, H. *et al.* Expression and significance of CD44, CD47 and c-met in ovarian clear cell carcinoma. *Int. J. Mol. Sci.* **16**, 3391–3404 (2015).
93. Fucikova, J. *et al.* Calreticulin expression in human non-small cell lung cancers correlates with increased accumulation of antitumor immune cells and favorable prognosis. *Cancer Res.* **76**, 1746–1756 (2016).
94. Stebbing, J. *et al.* The common heat shock protein receptor CD91 is up-regulated on monocytes of advanced melanoma slow progressors. *Clin. Exp. Immunol.* **138**, 312–316 (2004).

95. Ghiringhelli, F. *et al.* Activation of the NLRP3 inflammasome in dendritic cells induces IL-1β-dependent adaptive immunity against tumors. *Nat. Med.* **15**, 1170–1178 (2009).
96. Ma, Y. *et al.* Anticancer chemotherapy-induced intratumoral recruitment and differentiation of antigen-presenting cells. *Immunity* **38**, 729–741 (2013).
97. Ma, Y. *et al.* Contribution of IL-17-producing γδ T cells to the efficacy of anticancer chemotherapy. *J. Exp. Med.* **208**, 491–503 (2011).
98. Zitvogel, L., Galluzzi, L., Kepp, O., Smyth, M. J. & Kroemer, G. Type I interferons in anticancer immunity. *Nat. Rev. Immunol.* **15**, 405–414 (2015).
99. Sims, G. P., Rowe, D. C., Rietdijk, S. T., Herbst, R. & Coyle, A. J. HMGB1 and RAGE in inflammation and cancer. *Annu. Rev. Immunol.* **28**, 367–388 (2010).
100. Bergmann, C. *et al.* Toll-like receptor 4 single-nucleotide polymorphisms Asp299Gly and Thr399Ile in head and neck squamous cell carcinomas. *J. Transl Med.* **9**, 139 (2011).
101. Tittarelli, A. *et al.* Toll-like receptor 4 gene polymorphism influences dendritic cell *in vitro* function and clinical outcomes in vaccinated melanoma patients. *Cancer Immunol. Immunother.* **61**, 2067–2077 (2012).
102. Gast, A. *et al.* Association of inherited variation in Toll-like receptor genes with malignant melanoma susceptibility and survival. *PLoS ONE* **6**, e24370 (2011).
103. Ladoire, S. *et al.* Combined evaluation of LC3B puncta and HMGB1 expression predicts residual risk of relapse after adjuvant chemotherapy in breast cancer. *Autophagy* **11**, 1878–1890 (2015).
104. Yamazaki, T. *et al.* Defective immunogenic cell death of HMGB1-deficient tumors: compensatory therapy with TLR4 agonists. *Cell Death Differ.* **21**, 69–78 (2014).
105. Chiba, S. *et al.* Tumor-infiltrating DCs suppress nucleic acid-mediated innate immune responses through interactions between the receptor TIM-3 and the alarmin HMGB1. *Nat. Immunol.* **13**, 832–842 (2012).
106. Goritzka, M. *et al.* α/β interferon receptor signaling amplifies early proinflammatory cytokine production in the lung during respiratory syncytial virus infection. *J. Virol.* **88**, 6128–6136 (2014).
107. Lazear, H. M. *et al.* A mouse model of Zika virus pathogenesis. *Cell Host Microbe* **19**, 720–730 (2016).
108. Robinson, N. *et al.* Type I interferon induces necroptosis in macrophages during infection with *Salmonella enterica* serovar Typhimurium. *Nat. Immunol.* **13**, 954–962 (2012).
109. Cervantes-Barragan, L. *et al.* Type I IFN-mediated protection of macrophages and dendritic cells secures control of murine coronavirus infection. *J. Immunol.* **182**, 1099–1106 (2009).
110. Bidwell, B. N. *et al.* Silencing of Irf7 pathways in breast cancer cells promotes bone metastasis through immune escape. *Nat. Med.* **18**, 1224–1231 (2012).
111. Chan, S. R. *et al.* STAT1-deficient mice spontaneously develop estrogen receptor α-positive luminal mammary carcinomas. *Breast Cancer Res.* **14**, R16 (2012).
112. Salaun, B. *et al.* TLR3 as a biomarker for the therapeutic efficacy of double-stranded RNA in breast cancer. *Cancer Res.* **71**, 1607–1614 (2011).
113. Perretti, M. & D'Acquisto, F. Annexin A1 and glucocorticoids as effectors of the resolution of inflammation. *Nat. Rev. Immunol.* **9**, 62–70 (2009).
114. Leib, D. A., Alexander, D. E., Cox, D., Yin, J. & Ferguson, T. A. Interaction of ICP34.5 with beclin 1 modulates herpes simplex virus type 1 pathogenesis through control of $CD4^+$ T-cell responses. *J. Virol.* **83**, 12164–12171 (2009).
115. Galluzzi, L. *et al.* Viral strategies for the evasion of immunogenic cell death. *J. Intern. Med.* **267**, 526–542 (2010).
116. Kepp, O. *et al.* Viral subversion of immunogenic cell death. *Cell Cycle* **8**, 860–869 (2009).
117. Taxman, D. J., Huang, M. T. & Ting, J. P. Inflammasome inhibition as a pathogenic stealth mechanism. *Cell Host Microbe* **8**, 7–11 (2010).
118. Galluzzi, L., Kepp, O., Chan, F. K. & Kroemer, G. Necroptosis: mechanisms and relevance to disease. *Annu. Rev. Pathol.* (in the press).
119. Cordano, P. *et al.* The E6E7 oncoproteins of cutaneous human papillomavirus type 38 interfere with the interferon pathway. *Virology* **377**, 408–418 (2008).
120. Mittal, D., Gubin, M. M., Schreiber, R. D. & Smyth, M. J. New insights into cancer immunoediting and its three component phases — elimination, equilibrium and escape. *Curr. Opin. Immunol.* **27**, 16–25 (2014).
121. Gabrilovich, D. I., Ostrand-Rosenberg, S. & Bronte, V. Coordinated regulation of myeloid cells by tumours. *Nat. Rev. Immunol.* **12**, 253–268 (2012).
122. Li, M. O. & Rudensky, A. Y. T cell receptor signalling in the control of regulatory T cell differentiation and function. *Nat. Rev. Immunol.* **16**, 220–233 (2016).
123. Fucikova, J. *et al.* Prognostic and predictive value of DAMPs and DAMP-associated processes in cancer. *Front. Immunol.* **6**, 402 (2015).
124. Galluzzi, L., Buque, A., Kepp, O., Zitvogel, L. & Kroemer, G. Immunological effects of conventional chemotherapy and targeted anticancer agents. *Cancer Cell* **28**, 690–714 (2015).
125. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674 (2011).
126. Ni, M. *et al.* Serum levels of calreticulin in correlation with disease activity in patients with rheumatoid arthritis. *J. Clin. Immunol.* **33**, 947–953 (2013).
127. Sanchez, D. *et al.* Occurrence of IgA and IgG autoantibodies to calreticulin in coeliac disease and various autoimmune diseases. *J. Autoimmun.* **15**, 441–449 (2000).
128. Zhou, Z. *et al.* Immunoproteomic to identify antigens in the intestinal mucosa of Crohn's disease patients. *PLoS ONE* **8**, e81662 (2013).
129. Menger, L. *et al.* Cardiac glycosides exert anticancer effects by inducing immunogenic cell death. *Sci. Transl Med.* **4**, 143ra99 (2012).
130. van der Burg, S. H., Arens, R., Ossendorp, F., van Hall, T. & Melief, C. J. Vaccines for established cancer: overcoming the challenges posed by immune evasion. *Nat. Rev. Cancer* **16**, 219–233 (2016).



**Acknowledgements**

The authors are supported by the French Ligue contre le Cancer (équipe labellisée); Agence National de la Recherche (ANR) – Projets blancs; ANR under the frame of E-Rare-2, the ERA-Net for Research on Rare Diseases; Association pour la recherche sur le cancer (ARC); Cancéropôle Ile-de-France; Institut National du Cancer (INCa); Institut Universitaire de France; Fondation pour la Recherche Médicale (FRM); the European Commission (ArtForce); the European Research Council (ERC); the LeDucq Foundation; the LabEx Immuno-Oncology; the SIRIC Stratified Oncology Cell DNA Repair and Tumour Immune Elimination (SOCRATE); the SIRIC Cancer Research and Personalized Medicine (CARPEM); and the Paris Alliance of Cancer Research Institutes (PACRI).


**Competing interests statement**

The authors declare no competing interests.