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Review

Immunogenic *versus* tolerogenic phagocytosis during anticancer therapy: mechanisms and clinical translation

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Phagocytosis of dying cells is a major homeostatic process that represents the final stage of cell death in a tissue context. Under basal conditions, in a diseased tissue (such as cancer) or after treatment with cytotoxic therapies (such as anticancer therapies), phagocytosis has a major role in avoiding toxic accumulation of cellular corpses. Recognition and phagocytosis of dying cancer cells dictate the eventual immunological consequences (i.e., tolerogenic, inflammatory or immunogenic) depending on a series of factors, including the type of 'eat me' signals. Homeostatic clearance of dying cancer cells (i.e., tolerogenic phagocytosis) tends to facilitate pro-tumorigenic processes and actively suppress antitumour immunity. Conversely, cancer cells killed by immunogenic anticancer therapies may stimulate non-homeostatic clearance by antigen-presenting cells and drive cancer antigen-directed immunity. On the other hand, (a general) inflammatory clearance of dying cancer cells could have pro-tumorigenic or antitumorigenic consequences depending on the context. Interestingly, the immunosuppressive consequences that accompany tolerogenic phagocytosis can be reversed through immune-checkpoint therapies. In the present review, we discuss the pivotal role of phagocytosis in regulating responses to anticancer therapy. We give particular attention to the role of phagocytosis following treatment with immunogenic or immune-checkpoint therapies, the clinical prognostic and predictive significance of phagocytic signals for cancer patients and the therapeutic strategies that can be employed for direct targeting of phagocytic determinants.
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Facts

- Recognition and clearance of dying cells is affected by the molecular nature, spatiotemporal frame and overall balance of 'eat me' and 'don't eat me' signals exposed on the surface of dying cells.
- During carcinogenesis, both cell death and phagocytic clearance mechanisms tend to become inefficient and cooperate to expand premalignant clones that resist antitumour immunity.
- The mechanisms of cancer cell death elicited by anticancer therapy and the type of phagocytes (e.g., tumour-resident *versus* therapy-recruited) interacting with dying cells are decisive factors in making a difference between anti-inflammatory or pro-inflammatory responses.
- At the two extremes of a spectrum, tolerogenic phagocytosis represents a tolerogenic 'eat me' signal-dependent engulfment of dying cancer cells that leads to active immunosuppression. On the other hand, immunogenic phagocytosis is an immunogenic 'eat me' signal-dependent engulfment of dying cancer cells that facilitates immunostimulatory clearance of cancer cell corpses.

Open Questions

- It is unknown to what extent the mechanisms and/or consequences of phagocytic removal tend to be cell death pathway specific.
- It is unknown if specific 'eat me' signals govern the intracellular processing route of the engulfed cargo and thereby regulate the presentation of cancer antigens.
- The mechanisms and immunological consequences of immune cell-mediated endocytosis of cellular fragments, microparticles and/or exosomes released from dying cells need urgent characterization in near future.
- It remains enigmatic whether immune cells showing preimmunosuppressed state can mature or turn immunostimulatory upon immunogenic phagocytosis.
- For a large majority of FDA-approved anticancer therapies, there is no clarity on specific 'eat me' signals or immunological consequences of phagocytosis – this needs further characterization.
- In the future, it would be crucial to characterize whether immune-checkpoint therapies stimulate antibody-

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Abbreviations: ADCP, antibody-dependent cellular phagocytosis; APC, antigen-presenting cell; CD, cluster of differentiation; CRT, calreticulin; CTL, cytotoxic $CD8^+$ T lymphocyte; DAMP, damage-associated molecular pattern; DC, dendritic cell; DD1α, death domain 1α; Ecto, surface exposure; ER, endoplasmic reticulum; HSP, heat shock protein; ICD, immunogenic cell death; ICT, immune-checkpoint therapy; IFN, interferon; IL, interleukin; MΦ, macrophages; PDT, photodynamic therapy; PtdSer, phosphatidylserine; ROS, reactive oxygen species; SIRPα, signal regulatory proteins α; TAA, tumour-associated antigen; TCD, tolerogenic cell death; TLR, Toll-like receptor; Treg, T regulatory cell
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Box 1 Major cell death pathways and their immunobiological profiles

Apoptosis:[2] Is a physiological cell death pathway that is executed in a programmed or regulated manner by caspases and involves the degradation of DNA, cellular shrinkage and membrane blebbing. *In vivo*, apoptosis tends to avoid leakage of cellular contents until the phagocytes can arrive. Physiological apoptosis tends to facilitate immunologically 'silent' phagocytic clearance resulting in induction of tolerogenicity or even active immunosuppression.[9] This is the reason behind physiological apoptosis being also termed as 'tolerogenic cell death (TCD)' to emphasize its immunobiological profile.[9]

Secondary necrosis:[2,9] Is a terminal process experienced by late-apoptotic cells, if they fail to be cleared by phagocytes, and is characterized by general cellular-content spill over.

Autophagic cell death:[2,157] Is a form of regulated cell death driven by autophagic proteins. It is often, but not uniquely, induced by overactivation of autophagy, which results in irreversible and lethal cellular self-digestion.

Necrosis:[2] Is a form of cell death that occurs in an accidental (primary necrosis) or regulated (e.g., necroptosis, ferroptosis and parthanatos) manner and is characterized by cellular swelling and subsequent breakdown of the plasma membrane. Normally, necrosis is accompanied by inflammatory consequences; however, in certain contexts it may also exhibit a TCD-like low or null immunogenic profile.

Regulated necrosis:[2] Is a form of programmed cell death, controlled by a signalling cascade and terminally resulting in necrotic cell demise. Depending on the signalling cascade leading to regulated or programmed necrosis, it can be further defined as necroptosis, ferroptosis or parthanatos. Necroptosis[31] is executed by the interplay of proteins such as receptor interacting protein kinase-1/-3 (RIPK1/3), mixed lineage kinase like (MLKL), caspase-8 and FADD (among others), often collectively constituting a 'necrosome'. Parthanatos is regulated by the hyper-activation of poly(ADP-ribose) (PAR) polymerase 1 (PARP1) that leads to cellular depletion of NAD^+ and consequent ATP and nuclear translocation of AIF. Ferroptosis is mediated by iron-dependent production of reactive oxygen species (ROS), glutathione depletion and inactivation of GPx4, which is elicited by pharmacological inhibition of the Na^+ independent antiporter system (x^{c-}) exchanging extracellular cysteine for intracellular glutamate.

Immunogenic cell death (ICD):[1,19] Is induced by an assorted set of therapies capable of activating danger signalling pathways within the cancer cells leading to spatiotemporally defined emission of damage-associated molecular patterns (DAMPs).[1,21] DAMPs are normal endogenous molecules that are 'hidden' by the cancer cells under normal conditions but tend to get exposed or secreted/released in certain stressed or cell death conditions and bind their cognate receptors on the immune cells. The ability of ICD to expose certain DAMPs, such as surface-calreticulin (CRT), secreted-ATP and released-high mobility group box 1 (HMGB1) that act as danger signals, mediates its immunogenic potential.[1,55] Beyond danger signals, especially in the context of anthracycline-induced ICD, immunogenic potential can also be mediated by secretion of type I interferon (IFN) response-related cytokines (e.g., IFN-α/β)[158] and release of Annexin A1, which can help in recognition of dead/dying cells through immune cell-associated formyl peptide receptor-1 (FPR1).[47]

dependent cellular phagocytosis with immunogenic consequences.
- An important challenge is to develop methodologies to detect active phagocytosis in clinical tumour samples and ascertain its prognostic or predictive impact.

Clearance mechanisms of dying cells. Homeostatic tissue turnover is facilitated by regulated cell death, mainly in the form of apoptosis (a physiological form of cell death; Box 1) that avoids leaking contents and stimulates rapid, immunologically 'silent' phagocytic clearance.[1–3] Failure to clear apoptotic corpses causes release of their intracellular components possibly evoking undesired inflammatory responses (e.g., autoimmunity).[3,4] Clearance of dying cells is carried out by both professional phagocytes of the innate immune system (i.e., macrophages (MΦ), immature dendritic cells (DCs), neutrophils) and non-professional phagocytes (e.g., epithelial cells in the skin or intestine). However, the professional phagocytes are better adapted at antigen cross-presentation (especially DCs, which are the principle antigen-presenting cells (APCs)).[5–9]

To ensure their efficient removal, physiologically dying cells emit 'find-me signals' (e.g., fractalkine (CX3CL1)) to recruit anti-inflammatory phagocytes,[10] or release 'keep-out' signals (e.g., lactoferrin), to avoid inflammatory cells.[7,11,12] Along with these soluble signals, clearance of dying cells is regulated by a constellation of 'eat me' signals, a collective term for surface-tethered proteins, phospholipids or protein complexes facilitating cellular engulfment by binding to phagocytic receptors on immune cells (Figure 1).[5,7,11,12] Viable cells avoid phagocytic clearance through retained presentation of surface-associated 'don't eat me' signals.[6,13] On the other hand, recognition and clearance of dying cells is affected by the molecular nature, spatiotemporal frame and overall balance of pro-phagocytic and antiphagocytic determinants,[14] for example, dying cells tend to reduce 'don't eat me' signals while increasing the 'eat

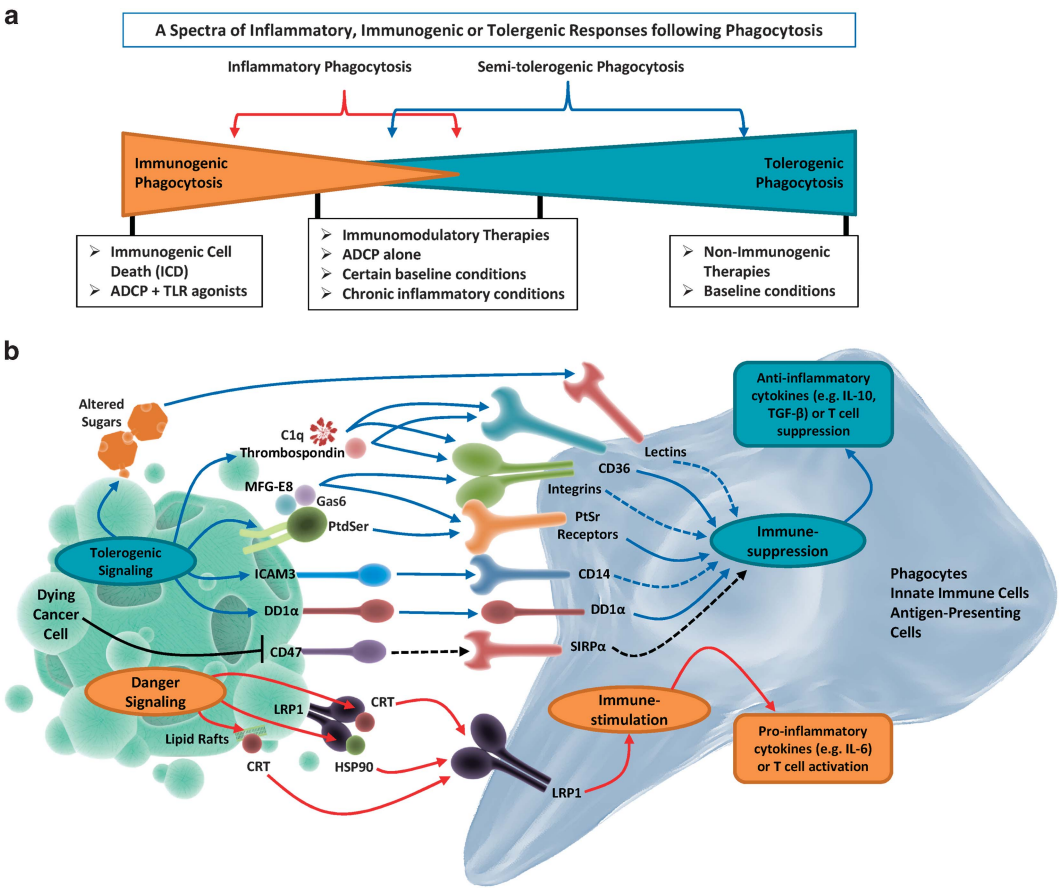

Figure 1 A schematic representation of major 'eat me' and 'don't eat me' signals regulating phagocytosis of dying cancer cells and the spectrum of subsequent immunological responses. (**a**) The immunological consequences of phagocytosis of dying cancer cells can be viewed as a spectrum of responses ranging from immunogenic and inflammatory to semi-tolerogenic and tolerogenic. Here immunogenic phagocytosis (induced by ICD inducers or the presence of ADCP plus TLR/TLR agonists) and tolerogenic phagocytosis (induced by non-immunogenic therapies or in basal conditions) occupy the two diametrically opposite poles of this spectrum, and consist of the most resolved immunological responses. On the other hand, inflammatory or semi-tolerogenic phagocytosis may result in context-specific immunological responses that are less resolved and thus more complex to decipher or exploit. (**b**) Cancer cells dying under basal conditions or following treatment with non-immunogenic therapies undergo tolerogenic phagocytosis mediated by interaction between tolerogenic 'eat me' signals (on dying cancer cells) and their respective cognate receptors (on phagocytes). This facilitates immunosuppression driven by anti-inflammatory cytokines. On the other hand, cancer cells dying following treatment with immunomodulatory therapies or inducers of ICD undergo immunogenic phagocytosis mediated by interaction between immunogenic 'eat me' signals and their respective cognate receptors. This facilitates immunostimulation driven by pro-inflammatory cytokines. Cancer cell death is also usually accompanied by downregulation of 'don't eat me' signals, such as CD47. CD, cluster of differentiation; Gas6, growth arrest-specific 6; ICAM3, intercellular adhesion molecule 3; LRP1, low-density lipoprotein receptor-related protein 1; TGF, transforming growth factor

me' signals on the surface.[5,7,11,12] Excessive cell death events can overwhelm the clearance capacity of phagocytes thereby causing a persistence of late apoptotic or secondary necrotic cells capable of disturbing tissue homeostasis.[14] Such corpses are cleared through mechanisms that have been partially deciphered[15] and involve a complex repertoire of receptors, opsonins and cell-associated ligands.[14] Finally, the relevance and contribution of non-apoptotic cell death mechanisms, including various forms of regulated necrosis such as necroptosis (Box 1),[16] in tissue homeostasis remains unclear.

Besides physiological events, phagocytosis also has a vital role in the control of injured, infected or diseased cells, such that inefficient phagocytosis may exacerbate disease.[15] For example, during carcinogenesis the inefficient phagocytosis of dying cancer cells, resulting from the overwhelming of the phagocytic system, may cause the persistence of necrotic cells in tumours (a prominent negative prognostic factor).[3,5,17,18] In a therapy-context, cancer cell death can occur through different mechanisms (as discussed later), which in turn can decisively affect the mechanisms of phagocytosis and its immunological consequences.[5] However, both cell death and phagocytic clearance mechanisms become inefficient in a tumour-context, thereby cooperating to expand premalignant clones resisting antitumour immunity. In this scenario, the main purpose of anticancer treatments should not only be limited to inducing cancer cell death but should also involve facilitating efficient phagocytosis-based transfer of crucial tumour-associated antigens (TAAs; that include both classical and neo-antigens).[3,5,17,18] This would allow processing and presentation of TAAs on the level of effector innate immune cells such as DCs with proper co-stimulation.[3,19,20] Such activated immune cells can further activate the effector adaptive immune cells (e.g., $CD4^+$ T cells polarized for type-I antitumour immune reactions, that is,

interferon (IFN)-γ-producing $CD4^+$ T cells or Th1 cells, and cytotoxic $CD8^+$ T lymphocytes (CTLs)).[1,20] Properly activated T cells are capable of targeting and eliminating the (residual) malignant cells based on TAAs presented to them.[3,19,20]

Thus the nature, intensity and context of phagocytosis in a tumour are pivotally positioned at the interface between cancer cell death and the immune system.[3] In the present review, we discuss this pivotal role of phagocytosis in regulating responses to anticancer therapy, in particular, immunogenic and immune-checkpoint therapies. We also discuss the prognostic and predictive significance of 'eat me'/ 'don't eat me' signals for cancer patients and the clinical translation of therapies targeting these signals.

Impact of therapy-induced cancer cell death on phagocytosis. The mechanisms of cancer cell death elicited by anticancer therapy and the type of phagocytes (e.g., tumour-resident *versus* therapy-recruited) involved in their clearance, are decisive factors between inducing anti-inflammatory responses or TAA-directed immunity.[21]

In the past decades, compelling evidence has challenged the original simplistic dichotomy that classified apoptosis as a tolerogenic cell death (TCD) and necrosis as a pathological cell death inherently pro-inflammatory/immunogenic (Box 1). Indeed, certain forms of cancer cell apoptosis (termed immunogenic cell death (ICD), Box 1)[19] can be perceived as 'non physiological' by the immune system, which reacts by engaging an efficient host immune defense.[1] ICD triggered by certain anticancer modalities inducing the combined occurrence of reactive oxygen species (ROS) and endoplasmic reticulum (ER) stress[19] is highly immunogenic owing to emission of danger signals or damage-associated molecular patterns (DAMPs) and other immunostimulatory molecules (Box 1 lists the known DAMPs/immunomodulatory molecules associated with ICD)[21] and is able to elicit T-cell mediated antitumour immunity.[1] Based on the main immunological profiles of cancer cell death (i.e., TCD and ICD), the subsequent phagocytic contexts can also be mainly associated with tolerogenic and immunogenic responses (Figure 1). Here tolerogenic phagocytosis can be defined as homeostatic engulfment of dying cancer cells that leads to induction of tolerogenicity (also owing to anti-inflammatory factors released by dying cells, Box 1) (Figure 1). Conversely, immunogenic phagocytosis can be defined as a non-homeostatic engulfment of dying cancer cells[19] that causes increased production of pro-inflammatory cytokines/chemokines (also owing to further co-stimulation provided by danger signals[21] and/or Toll-like receptor (TLR) agonists released by dying cells, Box 1), resulting in immunostimulatory clearance of cancer cell corpses (Figure 1).[1] It is also possible (albeit still poorly characterized) that the immunological consequences of phagocytosis are differentially modulated by the type of phagocytes that are recruited by TCD (anti-inflammatory MΦ or neutrophils) or ICD (inflammatory monocytes or specific DCs, for example, $CD11c^+CD11b^+Ly6C^{hi}$DCs[22] or $CD8\alpha^+$ DCs)[1] and the (inflammatory) microenvironment where clearance takes place.

However, it should be noted that tolerogenic phagocytosis and immunogenic phagocytosis represent two extreme polar-ends of the clearance mechanism. In reality, phagocytosis of dying cancer cells can give rise to a spectrum of inflammatory responses, which may be associated with ambiguous immunological reactions[23,24] that can facilitate pro- or antitumorigenic responses in a context-dependent manner (Figure 1).[25,26] Such responses tend to be quite distinct from pure tolerogenic or immunogenic responses on the levels of cytokines, chemokines, DAMPs and balance or misbalance between 'eat me' or 'don't eat me' signals[23–26] (as detailed more exhaustively elsewhere[27–29]). For the sake of clarity and focussed discussion, in this review we will only elaborate upon the two extreme polar-ends of this continuum (i.e., tolerogenic and immunogenic phagocytosis; Figure 1).

Anticancer therapies evoke various cancer cell death mechanisms, which may even coexist. The biological or therapeutic contexts where apoptosis or necrosis can evoke tolerogenicity or immunogenicity have been described.[30] However, similar knowledge is seldom available for other cell death pathways[2] such as necroptosis, autophagic cell death, mitotic catastrophe, parthanatos and ferroptosis (Box 1) – a gap in knowledge that requires urgent attention. For instance, necroptosis[31] and autophagic cell death[32] offer a therapeutic alternative to kill apoptosis-resistant cancer cells,[33] but recognition and clearance of necroptotic/autophagic cells by phagocytes is not completely understood. It is presumable that the uptake of necroptotic cells involves similar inflammatory mechanisms as applicable to necrosis owing to a resemblance in their terminal morphologies.[14,16,34] However, it is also possible that kinase-driven signalling events during necroptosis modify cellular components, generating a different immunobiology, for example, immunosuppression.[35] However, recent evidence suggests that heightened autophagy in cancer cells can suppress the emergence of immunogenic 'eat me' signals, such as surface-calreticulin (ecto-CRT).[36–38] In another context, autophagic dying cells have been found to undergo (phosphatidylserine (PtdSer)-based)[8] phagocytosis associated with inflammatory response.[39] Thus in the future it would be crucial to identify the molecular determinants driving the recognition and phagocytic removal of cancer cells dying through these non-apoptotic pathways.

Tolerogenic 'eat me' signals: from PtdSer to DD1*α*. Tolerogenic 'eat me' signals are predominantly exposed not only by cells dying through TCD or physiological apoptosis but also sometimes by necrotic cells[40] (Box 1, Figure 1).[29] Differential phagocytosis of disintegrated cells is still a matter of debate although recently F-actin was documented as an engulfment signal for (primary or secondary) necrotic cells, binding Clec9a on $CD8\alpha$+ DCs.[41]

The best known of the tolerogenic 'eat me' signals is externalized PtdSer (Figure 1).[8,15,40] PtdSer is a phospholipid that normally faces the inner lumen of the bilayered plasma membrane in living cells. However, during the early apoptotic phases it becomes externalized on the outer leaflet of the plasma membrane owing to the coordinated activity of caspases and scramblases (and inactivation of flippases).[29,42,43] PtdSer binds a large number of immune receptors in a phagocyte-type- and context-specific manner (please refer to other reviews for further insight[29]). The pro-phagocytic task of PtdSer is further assisted by the presence of phagocytosis-augmenting bridging molecules, for example,

Milk-fat globule-EGF factor VIII (MFG-E8) and Gas6 (Figure 1). Such bridging molecules can also support pro-tumorigenic immune reactions. For example, MFG-E8 promotes tumour progression/invasion by favouring tolerogenic phagocytosis-mediated recruitment of immunosuppressive T regulatory cells (Tregs), which are major inhibitors of antitumour immunity.[44]

Beyond PtdSer, some other surface membrane moieties act as tolerogenic 'eat me' signals in a context-dependent manner (although the exact compositional balance of these with PtdSer is still debatable). These include externalized cardiolipin,[45] oxidized low-density lipoproteins, annexin-A1, thrombospondin, complement C1q and changes in membrane glycosylation status or charges (Figure 1).[6,15,46] Interestingly, in the context of chemotherapy-induced ICD, secretion of annexin-A1 by dying cancer cells followed by its binding to the formyl peptide receptor 1 on DCs, was found to facilitate recruitment of tumour-infiltrating DCs in close vicinity to the dying cancer cells and the formation of dead corpse/DC conjugates, resulting in immunogenic phagocytosis.[47] This finding further reinforces the concept that the inflammatory context and array of spatiotemporally exposed/secreted factors by the dying cancer cells govern the ultimate immunological responses.[48]

The most recent molecule to join the 'club' of tolerogenic 'eat me' signals is a p53 target, namely, immunoglobulin superfamily receptor death domain 1*α* (DD1*α*) (Figure 1).[49] Of all the known 'eat me' signals, DD1*α* exhibits the most unique and complex immunoregulatory mechanism. On one hand, the (homophilic) DD1*α*–DD1*α* interactions between apoptotic cells and phagocytes help in the uptake of the apoptotic cells. On the other hand, these interactions also inhibit the proliferation of $CD4^+$/$CD8^+$ T cells.[49] Moreover, the p53-induced expression of DD1*α* facilitates apoptotic (cancer) cells' phagocytosis in a PtdSer-independent manner.[49] This establishes DD1*α* as a major immune checkpoint.

Immunogenic 'eat me' signals: the role of surface-exposed CRT and heat shock protein 90 (HSP90). Immunogenic phagocytosis is mediated by a limited number of known 'eat me' signals, namely ecto-CRT[50] and surface-HSP90 or ecto-HSP90 (Figure 1), which facilitate antitumour immunity.[20,51,52] The co-existence of an array of such surface-exposed signals is predominantly elicited by cells dying through ICD (Box 1).[3,53–55] However, in some contexts, specific chemotherapeutics (e.g., melphalan)[56] or targeted therapies (e.g., $BRAF^{V600E}$ inhibitor, vemurafenib)[57] that are not *bona fide* ICD inducers can evoke a partial and specific subset of these ICD-associated 'eat me' signals/DAMPs and thereby mediate phagocytic clearance with partial immunogenic properties. A very complex interplay between ER stress (centred on the ER stress sensor, protein kinase RNA-like ER kinase (PERK))[58] and ROS helps in trafficking of ecto-CRT through the conventional secretory pathway.[1,59,60] This core trafficking mechanism displays some degree of plasticity[3] and has been found to be also regulated by some pro-apoptotic proteins (BAX/BAK/caspase-8), cytosolic Ca^{2+} or the unfolded protein response signalling proteins (ERp57/eIF2*α*), depending on the ICD inducer utilized.[1,59–61] For more on danger signalling pathways, please refer to other recent reviews.[1,3,19,54,62] On the surface of cancer cells, ecto-CRT tends to dock on either lipid rafts and/or LRP1,[50,59] whereas ecto-HSP90 binds prevalently to LRP1 (Figure 1).[63] Interaction of these 'eat me' signals with some phagocytic receptors on immune cells (e.g., LRP1) aids in removal of cancer cells undergoing ICD (Figure 1, Box 1).[38,52,64] Ecto-CRT elicits the production of pro-inflammatory cytokines, such as interleukin (IL)-6 and tumour necrosis factor-*α* (TNF-*α*) from DCs, thereby facilitating Th1 and/or Th17 polarization.[65,36] Moreover, overall expression of CRT mRNA (*CALR*) in tumour tissue samples (derived from ovarian or lung cancer patients treated with paclitaxel or radiotherapy, respectively) linearly correlates with the levels of genes coding for phagocytosis-related proteins (involved in phagosome maturation or degradation).[52] In fact, dying cancer cells naturally incapable of presenting ecto-CRT (owing to an intrinsic resistance mechanism) fail to mediate an anticancer vaccination effect.[52] Similarly, HSP90–CD91 binding on immune cells facilitates DC maturation and Th1/17 priming.[65] In some contexts, ecto-HSP90 and ecto-CRT are interchangeable in mediating immunogenicity;[66] while in other cases, ecto-CRT is the superior immunogenic signal.[56] In fact, an *in silico* analysis suggests that CRT (but not HSP90) possesses close homologues of crucial phagocytosis-assisting motifs.[61] Also, ecto-CRT may correlate better with a phagocytosis increase than ecto-HSP90 in the context of anthracycline-induced ICD.[67]

Surface CD47: a ubiquitous 'don't eat me' signal? A number of 'don't eat me' signals have been characterized that act in a context-dependent manner (with the context being type of tissue, type of cells or type of phagocytes).[13,29] However, evidence over time has characterized CD47 as a rather ubiquitous 'don't eat me' signal (Figure 1). The binding of CD47 to the immune-receptor signal regulatory proteins *α* (SIRP*α*) on phagocytes, inhibits the phagocytosis of CD47-proficient cells.[13] Thus, not surprisingly, CD47-deficient cells are critically sensitive to phagocytic clearance.[68–71] Concerning cell death, there are two prevailing models that explain CD47's antiphagocytic functions. The most widely accepted model entails downregulation of CD47 paralleled by upregulation of 'eat me' signals (Figure 1).[50] The second model entails spatial repositioning of CD47 away from 'eat me' signals.[50,71] CD47 is abundantly overexpressed on cancer cells (especially on cancer stem cells) belonging to various cancer types,[72] representing a potent strategy for immune evasion. Moreover, CD47–SIRP*α* interaction and subsequent SIRP*α* signalling restricts the efficacy of cancer therapeutic antibodies.[73] Beyond SIRP*α*, CD47 can also interact with some integrins or thrombospondins to modulate IgG antibody-mediated phagocytosis and other inflammatory responses.[74–76] On the level of cancer cells, a HIF1*α* target protein BNIP3 has been found to regulate CD47 expression levels;[77] however, further clarity on CD47-regulating signalling pathway is urgently needed, as it is not entirely known how cancer cell death links with CD47 downregulation or relocalization. Nevertheless, CD47 forms a formidable barrier against cancer cell clearance and thus represents an interesting therapeutic target.

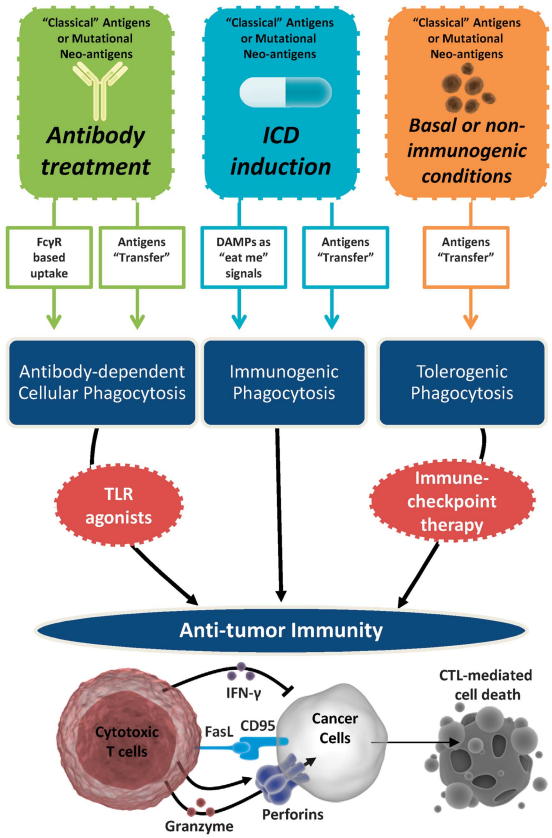

Figure 2 Therapeutic exploitation of phagocytosis of dying cancer cells for T-cell-mediated cancer cell elimination. Treatment with antibody-based anticancer therapies induces FcγR-mediated, ADCP of cancer cells that facilitates antitumour immunity in the presence of co-stimulatory signals such as TLR agonists. Similarly, treatment with ICD-inducing anticancer therapies induces immunogenic phagocytosis driven by immunogenic 'eat me' signals or immunogenic DAMPs, components that eventually facilitate antitumour immunity. On the other hand, under basal conditions or after treatment with non-immunogenic anticancer therapies, dying cancer cells undergo tolerogenic phagocytosis that tends to inhibit antitumour immunity by facilitating immunosuppression. Despite these distinct mechanisms and immunological consequences, all three scenarios result in the transfer of cancer antigens ('classical' or mutational neo-antigens) from dying cancer cells to the phagocytes. The immunosuppression propagated by tolerogenic phagocytosis can be reversed by treatment with ICT. Antitumour immunity resulting from these treatment scenarios is expected to culminate into cancer cell-eliminating activity exerted by CTLs through IFN-γ (exerts cytostatic effects and polarizes immune cells towards type I-immune reactions), FasL–CD95 interactions (exerts extrinsic apoptosis) or granzyme-perforins secretion (exerts direct cytotoxicity through perforin-driven membrane-pore formation followed by granzyme-induced cell death)

Antibody-dependent cellular phagocytosis: bypassing the 'eat me'/'don't eat me' signals' interplay? Phagocytes possess Fcγ receptors (FcγRs) through which they interact with the Fc regions of antibodies to further exert antigen-specific effector functions.[78] Interestingly, FcγRs can also mediate antibody-dependent cellular phagocytosis (ADCP) that bypasses the need for canonical phagocytic determinants. More specifically, predominantly type I FcγRs on macrophages or DCs can help in phagocytosis of targets bound to antibodies or antibody complexes (mainly IgG antibodies).[78] Such IgG-bound target cells can be efficiently processed and the resulting TAAs can be used for cross-presentation by APCs, thereby enhancing cancer antigen-directed CD4$^+$/CD8$^+$ T-cell responses.[78] Importantly, while ADCP proceeds through interactions with type I FcγRs alone, the subsequent immunogenic consequences of such uptake are more tightly governed. In particular (especially in DCs), the activating effects of type I FcγRs are balanced by the inhibitory FcγRIIb receptors,[78] which are overcome only if phagocytosis of target cells happens in the presence of additional co-stimulatory signals (e.g., TLR ligands).[78] This latter point shows that ADCP might have immunogenic consequences only if the cancer cells die through ICD or necrosis, cell death routines known to release danger signals, including TLR agonists.

Tolerogenic and immunogenic consequences of phagocytic clearance. Besides the nature or balance of the 'eat me' signals, the differentiation state of phagocytes can also be a critical factor in defining immunological consequences. In general, tolerogenic 'eat me' signals interacting with immature APCs and/or APCs exhibiting immunosuppressive phenotypes (e.g., M2 MΦ, N2 neutrophils, myeloid-derived suppressor cells or MDSCs)[79] might favour tolerogenic phagocytosis (Figure 1).[20,25] Instead, immunogenic 'eat me' signals interacting with immature APCs might favour immunogenic phagocytosis (Figure 1).[20,25] It is unclear whether APCs showing a preimmunosuppressed state (e.g., M2 MΦ, MDSCs) can mature upon immunogenic phagocytosis; however, based on available literature this is plausible.[80] Of note, 'eat me' signals alone are not exclusive immunological determinants as their exposure is invariably accompanied by the emission of other signals (e.g., DAMPs or immunosuppressive cytokines/chemokines).[3,19,53] Thus, APC's commitment to tolerogenicity or immunogenicity is regulated by a complex program integrating a variety of signals (Figure 1).

APCs performing phagocytosis (and ADCP) eventually prime the T cells for respective TAAs[81] (owing to innate programming of APCs, which constantly process and present any captured antigens to the T cells).[82,83] However, APCs that carry out tolerogenic phagocytosis fail to reach functional maturation and thus present TAAs to CD4$^+$ T cells in the absence of proper co-stimulatory signals (e.g., surface CD80/CD86/CD40/CD83) but possibly in the presence of immunosuppressive cytokines (IL-10/TGF-β), ultimately facilitating the formation of immunosuppressive Tregs (overexpressing immune-inhibitory CTLA-4/PD-1).[9,25,84–86] Treg cells not only fail to attack the cancer cells, as their immunosuppressive phenotype categorizes them as 'safe'/'self',[9,86,87] but also actively secrete pro-tumorigenic cytokines (e.g., IL-6/TNF) and directly eliminate CTLs (through FasL or TRAIL expression).[9,85,86] Also, APCs that have carried out tolerogenic phagocytosis facilitate cancer progression by disrupting the cross-talk between CD4$^+$ T cells and CTLs. More specifically, tolerogenic APCs present TAAs only to CTLs but not to CD4$^+$ T cells, thereby causing sub-optimal CTL activation.[9] Eventually, if re-exposed to TAAs, such CTLs may orchestrate a deranged cytotoxic response that also targets the CD4$^+$ T cells (through TRAIL), thereby facilitating tolerance in the long run.[9] Beyond TAA presentation, tolerogenic phagocytosis actively suppresses the secretion of pro-inflammatory

Table 1 Summarization of 'eat me' signals, antibody-dependent cell phagocytosis (ADCP) and inhibitory effect on phagocytic activity associated with major anticancer therapies

Cancer therapy	ICD or TCD	Specific 'eat me' signals exposed on cancer cells (beyond PS)	Effect on phagocytosis confirmed by blocking 'eat me' signal?	Direct inhibition of phagocytic activity?	Comments	Refs.
Anthracyclines (Doxorubicin, Epirubicin, Daunorubicin, Idarubicin, Mitoxantrone) Oxaliplatin Bortezomib	ICD	Surface-CRT Surface-HSP90	Yes	—	Surface-CRT has been found to be the predominant 'eat me' signal and mediator of immunogenicity through *in vivo* analysis; resistance against immunogenic effects of mitoxantrone can be mounted by defects in surface-CRT	19,52,53,118
Hypericin-based photodynamic therapy (Hyp-PDT)	ICD	Surface-CRT Surface-HSP90	Yes	—	Surface-CRT has been found to be the predominant 'eat me' signal and mediator of immunogenicity through *in vivo* analysis; resistance against immunogenic effects of Hyp-PDT can be mounted by defects in surface-CRT	52,53,118,119
Certain forms of radiotherapy	ICD	Surface-CRT Surface-HSP90 Surface-DD1α	Yes	—	In radiotherapy-treated lung cancer patients, overall *CALR* levels correlate with levels of phagocytosis-associated genes	19,49,52,53,118
Certain oncolytic viruses	ICD	Surface-CRT	No	—	—	53,91,118
Cyclophosphamide, Bleomycin, Vorinostat	ICD	Surface-CRT	No	—	—	19,53,118
High-hydrostatic pressure	ICD	Surface-CRT	No	—	—	53,118
Paclitaxel	ICD	Surface-CRT	No	?	In paclitaxel-treated ovarian cancer patients, overall *CALR* levels correlate with levels of phagocytosis-associated genes	52
Photofrin-based PDT	ICD	Surface-CRT Surface-HSP90	No	—	—	120,121
5-Fluorouracil	—	Surface-CRT?	—	—	—	122
Cytarabine	—	—	—	—	Combination of Cytarabine with anti-CD47 antibody was found to improve *in vivo* efficacy against AML	123
Rose Bengal acetate-based PDT	—	Surface-CRT Surface-HSP90	No	—	—	124
Prednisone	—	—	—	—	Treatment with prednisone assists in phagocytosis of cells through interaction between PS and the extracellular opsonin MFG-E8	42
Trastuzumab	—	—	—	No	Mediates increased antibody-dependent cellular phagocytosis (ADCP) by binding to FcγRIV receptor on phagocytes	125
Cetuximab, Ofatumumab, Obinutuzumab, Rituximab	—	—	—	No	Mediates antibody-dependent cellular phagocytosis (ADCP)	126–128
Arsenic trioxide	TCD	—	—	No	Increases phagocytic capacity of immune cells by activating Syk kinase signalling within the immune cells	129
BRAFi drugs (Dabrafenib, Vemurafenib)	TCD?	Surface-CRT Surface-HSP90	No	—	—	57
Cisplatin	TCD	—	—	—	Induces 'eat me' signal KIM-1 in kidney injury context	130
Docetaxel, Mitomycin C	TCD	Surface-CRT	No	—	—	122
Topotecan/Camptothecin, Etoposide	TCD	Surface-DD1α	—	—	In topotecan-treated ovarian cancer patients, overall *CALR* levels either do not or even negatively correlate with levels of phagocytosis-associated genes	49,52

Table 1 *Continued*

Cancer therapy	ICD or TCD	Specific 'eat me' signals exposed on cancer cells (beyond PS)	Effect on phagocytosis confirmed by blocking 'eat me' signal?	Direct inhibition of phagocytic activity?	Comments	Refs.
Zoledronic acid	TCD	—	—	Yes	Increases surface-CRT, only in combination with Doxorubicin in MDR-positive cells	122,131
Methotrexate, Ibrutinib, Aflibercept, Bevacizumab, Toremifene, Vinblastine, Vincristine, Idelalisib, Mercaptopurine, Thalidomide	—	—	—	Yes	—	132–141
Sorafenib	—	—	—	Yes	Sorafenib-treated human macrophages exhibit low phagocytosis and CD80 expression	142
Tamoxifen	—	Surface-CRT	—	Yes	Phagocytosis of tamoxifen-treated cells is mediated in a phagocyte-dependent manner, either by PtdSer or surface-CRT	8, 143

Major FDA-approved anticancer therapies for which either specific 'eat me' signals are not clear or direct effects on immune cell phagocytic activity are unavailable: Abiraterone acetate, Afatinib, Aldesleukin, Aldesleukin, Alemtuzumab, Anastrozole, Axitinib, Belinostat, Bendamustine, Bicalutamide, Blinatumomab, Bosutinib, Brentuximab, Busulfan, Cabazitaxel, Capecitabine, Carboplatin, Carfilzomib, Carmustine, Ceritinib, Clofarabine, Crizotinib, Dacarbazine, Dactinomycin, Dasatinib, Degarelix, Denileukin, Denosumab, Enzalutamide, Eribulin, Erlotinib, Everolimus, Exemestane, Exemestane, Fludarabine, Fulvestrant, Gefitinib, Goserelin, Ibritumomab, Imatinib, Ipilimumab, Irinotecan, Ixabepilone, Lapatinib, Lenalidomide, Letrozole, Leucovorin, Leuprolide, Lomustine, Mechlorethamine, Megestrol, Nelarabine, Nilotinib, Nivolumab, Olaparib, Omacetaxine, Palbociclib, Pamidronate, Panitumumab, Panobinostat, Pazopanib, Pegaspargase, Pembrolizumab, Pemetrexed Disodium, Pertuzumab, Pierixafor, Pomalidomide, Ponatinib, Pralatrexate, Procarbazine, Radium 223, Ramucirumab, Regorafenib, rIFNa-2b, Romidepsin, Sunitinib, Temozolomide, Temsirolimus, Thiotepa, Tositumomab, Trametinib, and Vinorelbine

cytokines and causes an exaggerated polarization of MΦ into a pro-tumorigenic phenotype (owing to the production of pro-tumorigenic cytokines TGF-β and IL-10)[88], while promoting the production of anti-inflammatory factors.[5,7,11,12,88]

Instead, APCs that carry out immunogenic phagocytosis present TAAs to $CD4^+$ T cells in the presence of heightened levels of co-stimulatory molecules and increased levels of pro-inflammatory cytokines (e.g., IL-6/IL-12/IL-1β) (Figure 1).[25,52,55] This, in concert, facilitates the differentiation of Th1 cells that orchestrate a type-I immunity-based anticancer programme (consisting of IFN-γ-driven cancer-directed cytostatic effects and suppression of Treg differentiation).[19,36,89,90] Simultaneously, these immunogenic APCs allow a productive cross-talk between Th1 cells and CTLs and thereby facilitating CTL-elicited malignant cell elimination (mediated through IFN-γ, FasL–CD95 interaction and perforin-granzyme action) (Figure 2).[19,90]

Phagocytic clearance by anticancer therapies. Clinical anticancer therapies that either augment immunogenic potential of cancer cells (e.g., through ICD) or facilitate ADCP in the presence of co-stimulatory ligands are most likely to encourage immunogenic phagocytosis and thus warrant urgent identification (Figure 2). However, clinical therapies that encourage TCD, although less preferable, also need to be identified in order to design smart combinatorial strategies (Figure 2).

To gain a wider view on this important point, we carried out a survey of PubMed publications to ascertain what was known about the immunological consequences of phagocytosis associated with various anticancer therapies (including several FDA-approved ones) (Table 1). To our dismay, only a handful of FDA-approved drugs had some clarity on specific 'eat me' signals or immunological consequences of phagocytosis (Table 1). Among these, some agents, such as tamoxifen, sorafenib, bevacizumab, vinblastine and vincristine, exhibited the unfavourable activity of directly inhibiting phagocytic activity of APCs, while others tended to divide between three phagocytic profiles, that is, tolerogenic or immunogenic phagocytosis and ADCP (Table 1).

Harnessing immunogenic phagocytosis via immunogenic anticancer therapies: A number of major anticancer therapies can induce ICD associated with immunogenic phagocytosis-driven anticancer immunity.[1] Known ICD inducers, as Table 1 details, include some chemotherapeutics, photodynamic therapy (PDT), radiotherapy, some oncolytic viruses and some physical therapies (Figure 2).[1] The immunogenic 'eat me' signal mostly characterized for these ICD inducers and confirmed through blockade or intervention strategies (at least for anthracyclines, oxaliplatin, bortezomib, hypericin-PDT, radiotherapy; Table 1) is ecto-CRT. Of note, while many of these anticancer therapies induce apoptotic ICD, it has also emerged that ICD can be necroptotic if induced by the oncolytic Newcastle disease virus.[91] It will be important to discover more ICD inducers capable of eliciting a high diversity of immunogenic 'eat me' signals.

Encouraging immunogenic phagocytosis via antibody-based immunotherapies. Various anticancer therapeutic antibodies induce ADCP (Table 1), such as rituximab (anti-CD20 antibody), trastuzumab (anti-HER2 antibody) and cetuximab (anti-EGFR antibody). Rituximab and trastuzumab

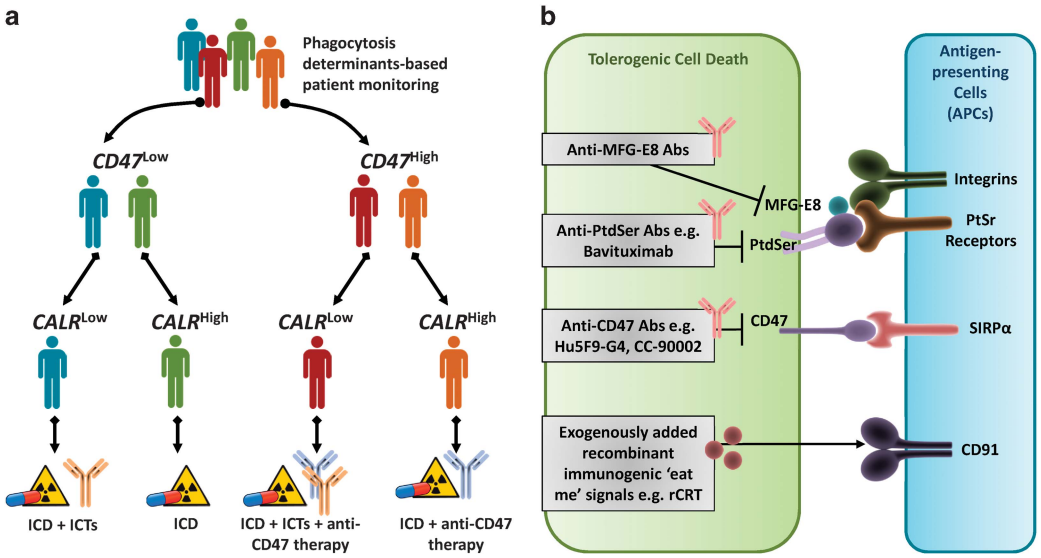

Figure 3 Clinical exploitation of phagocytic determinants for therapeutic targeting or biomarker-driven patient treatment/management. (**a**) Phagocytosis determinants (i.e., 'eat me' signal such as calreticulin (*CALR*) or 'don't eat me' signal such as *CD47*) can be used as prognostic or predictive biomarkers for stratification or segregation of cancer patients into different risk groups allowing further decision-making regarding specific treatment options. For instance, overall tumour-associated expression levels of CD47 and CALR can be used in synchrony to segregate patients in different treatment groups who would eventually receive different treatments or combinations thereof involving ICD inducers, ICTs and/or anti-CD47 therapy. (**b**) Phagocytic determinants can also be more directly exploited to therapeutic ends, for example, by targeting through antibodies (against CD47, MFG-E8 or PtdSer) and/or by exogenously providing recombinant versions (e.g., recombinant calreticulin or rCRT), as applicable. CD, cluster of differentiation

can induce FcγR-mediated anticancer immunity possibly through immunogenic phagocytosis.[92] Some clinical observations suggest a correlation between FcγRIIIa (CD16) or FcγRIIa (CD32) polymorphisms and a response to rituximab, trastuzumab and cetuximab.[93] Moreover, few of these therapies can liberate co-stimulatory signals from cancer cells (required to make ADCP immunogenic), for example, rituximab causing release of the TLR agonist, HMGB1.[94,95] Overall, these results show that besides their targeted activities, ADCP can help antibody-based therapies to achieve the desirable 'off-target' induction of antitumour immunity (Figure 2).[93] This raises similar precedence for other immunotherapies targeted towards the cancer cells, that is, anti-PD-L1 antibodies. This is further supported by recent observations of FcγRs modulating the activity of the PD-1/PD-L1 axis.[96] In the future, it would be crucial to characterize better the ADCP-associated immunogenic consequences of these antibody-based therapies.

Tolerogenic phagocytosis: foundation for eventual responses to immune-checkpoint therapy (ICT)? As TAAs are ultimately transferred from cancer cells to the APCs during tolerogenic phagocytosis, does this TAA transfer matter? If so, can the immunosuppressive consequences be reversed? Recent evidence emerging from the ICTs[51] suggests that such immunosuppressive consequences can still be reversed (Figure 2). ICTs are therapeutic agents that target regulatory pathways in T cells (e.g., CTLA-4 or PD-1) to enhance antitumour immunity.[97–99] ICTs, however, do not induce a *de novo* immune reaction,[97–99] since they simply block the immunosuppressive molecules on preexisting T cells already primed for TAAs.[97–99] It is presumable that most of this initial TAA priming of T cells occurred through APCs performing tolerogenic phagocytosis in the tumour microenvironment (Figure 2).[97–99] In the future, it would be crucial to find new targets for ICTs that are exploited by tolerogenic phagocytosis.[98,99] Moreover, considering that currently many clinically applied anticancer therapies tend to induce TCD, and thereby facilitate tolerogenic phagocytosis (Table 1),[30] it would be crucial to combine these with ICTs (Figure 2).[100]

Clinical applications of pro-phagocytic and antiphagocytic determinants. Beyond therapeutic induction of immunogenic phagocytosis/ADCP or reversing the consequences of tolerogenic phagocytosis through ICTs, phagocytic clearance of cancer cells can be more directly exploited for clinical benefits (Figure 3). In the following subsections, these direct applications of pro-phagocytic and antiphagocytic determinants are discussed in further details (Figure 3).

'Eat me' or 'don't eat me' signals as prognostic or predictive biomarkers in cancer. Prognostic and predictive biomarkers, especially those that can be detected in human tumour samples, are valuable for patient management and clinical decision-making (Figure 3a).[101–104] It is technically challenging to detect surface localization of phagocytic determinants in tumour tissue samples[90] – a hurdle that has hampered research in this direction. This is particularly important as elevated surface presence of immunogenic 'eat-me' signals, such as CRT, should be distinguished from their overall intracellular expression, which is often elevated as a result of stress adaptation and leads to increased cancer cell resistance.[105] However, in the right context, the overall expression of the respective molecules can be utilized for patient prognostic analysis or to predict therapy response.[90]

Table 2 Summarization of prognostic or predictive effects of 'eat me' or 'don't eat me' signals in human cancer patients

Parameter	Cancer	Treatment	No. of patients	Prognostic or predictive impact	Ref.
Phosphatidylserine (PtdSer)	Ovarian carcinoma	—	76	Increased PtdSer expression is associated with higher tumour grade and poor overall survival	144
Calreticulin (CRT or *CALR*)	Acute myeloid leukemia	Anthracyclines	20	Ecto-CRT on blasts correlated with improved relapse-free survival	145
	Bladder carcinoma	Surgery	195	High *CALR* correlated with poor disease outcome	13
	Breast carcinoma	Surgery	23	High *CALR* correlated with poor metastasis-free survival	146
	Breast carcinoma	Surgery alone or combined with chemotherapy	1115	High *CALR* correlated with marginally improved overall survival	90
	Colorectal carcinoma	Surgery+chemotherapy	68	High *CALR* correlated with improved five-year survival	147
	Gastric carcinoma	Gastrectomy and lymphadenectomy	79	High *CALR* correlated with poor disease outcome	148
	Lung carcinoma	Radiotherapy	23	High *CALR* correlated with prolonged overall survival	52
	Lung carcinoma	—	58	High *CALR* correlated with tumour grade and malignancy	149
	Lung carcinoma	Surgery alone or combined with chemotherapy/chemo-radiotherapy	1432	High *CALR* correlated with poor overall survival	90
	Mantle cell lymphoma	Surgery	163	High *CALR* correlated with poor disease outcome	13
	Neuroblastoma	Surgery alone or combined with chemotherapy	729	High *CALR* correlated with poor disease outcome	13
	Neuroblastoma	Surgery alone or combined with chemotherapy	68	High *CALR* correlated with good disease outcome	150
	Non-Hodgkin's lymphoma	Autologous cancer cell-based vaccine	18	Ecto-CRT associated with positive clinical responses	66
	Ovarian carcinoma	Paclitaxel	220	High *CALR* correlated with prolonged disease-free survival and overall survival	52
	Ovarian carcinoma	Surgery alone or combined with chemotherapy	1436	High *CALR* correlated with improved overall survival	90
CD47	Acute myeloid leukemia	—	137	High *CD47* correlated with poor overall survival	72
	Breast carcinoma	Surgery alone or combined with chemotherapy	255	High *CD47* correlated with poor overall survival	151
	Breast carcinoma	—	738	High *CD47* in bone marrow or peripheral blood associated with poor disease-free survival	152
	Esophageal carcinoma	Surgery	102	High *CD47* correlated with poor overall survival	153
	Gastric cancer	Surgery	115	High *CD47* was an adverse prognostic factor	154
	Ovarian carcinoma	Surgery	86	Low *CD47* correlated with good disease outcome	155
HSP90 (or *HSP90AA1*)	Breast carcinoma	Surgery alone or combined with chemotherapy	1115	High *HSP90AA1* correlated with poor overall survival	90
	Colorectal carcinoma	—	182	High serum levels of HSP90 correlated with oncogenesis	156
	Lung carcinoma	Surgery alone or combined with chemotherapy	1432	High *HSP90AA1* correlated with improved overall survival	90
	Non-Hodgkin's lymphoma	Autologous cancer cell-based vaccine	18	Ecto-HSP90 associated with positive clinical responses	66

Abbreviations: CD, cluster of differentiation; Ecto-, surface exposed; HSP, heat shock protein.

Table 2 summarizes the prognostic or predictive impact of major phagocytic determinants, among which CRT/*CALR* and *CD47* are the most studied (Table 2). High expression of *CD47* is a definitive negative prognostic factor across various cancer types (Table 2),[106] whereas the overall picture is much more complex for ecto-CRT/*CALR*.[106] Increased ecto-CRT or high *CALR* levels predict positive responses to immunogenic anticancer therapies, such as anthracyclines, radiotherapy, paclitaxel and DC vaccines (Table 2).[106] However, as a prognostic factor the utility of *CALR* is limited to only a few cancer types.[90] This discrepancy could be because of differences in phagocytic context. In a prognostic biomarker set-up, no differentiation is made between treated or untreated patients, thereby meaning that tumour tissues with both tolerogenic and immunogenic phagocytosis might be tested for *CALR* levels, thereby confounding the ultimate prognostic impact. On the other hand, in a predictive biomarker set-up, a clear distinction is made between treated and untreated patients.[106] This would explain why in contexts of immunogenic anticancer therapies (where immunogenic phagocytosis is likely) high *CALR*/ecto-CRT levels are positive predictive factors.[52] Indeed, in this context, *CALR* levels tend to positively correlate with levels of phagocytosis-related genes.[52] We propose that *CD47* and

CALR could be used in synchrony for efficient stratification of high- or low-risk cancer patients and for further decision-making regarding the choice of anticancer therapy to be given, as depicted in Figure 3a.[106]

It is clear that very few phagocytic determinants have been tested so far as prognostic or predictive biomarkers and more studies are needed to reach further clarity. Another challenge would be to detect active phagocytosis in human tumour tissue and directly detect its prognostic or predictive impact.

Combinatorial therapy with recombinant immunogenic 'eat me' signals in cancer. Chemotherapeutic ICD inducers cannot be integrated in clinical cell-based vaccination protocols owing to their residual amounts being capable of exerting side effects or toxicity.[107] This is one of the primary reasons why clinical anticancer vaccines (whole-tumour-cell or DC vaccines) utilize physicochemical cancer cell death inducers.[108] However, while some physicochemical strategies can induce ICD (e.g., radiotherapy, PDT) yet certain others cannot (e.g., freeze/thawing-based necrosis).[1,52,59,109] In the latter cases, exogenous addition of recombinant immunogenic 'eat me' signals (such as recombinant CRT)[52,56,64,110,111] can complement immunogenic phagocytosis (Figure 3b). In line with this, several studies have used recombinant CRT to augment the immunogenicity of otherwise low immunogenic cancer vaccines (Figure 3b),[52,56,64,110,111] or to promote the immunogenic potential of cancer cells treated with loco-regionally applied chemotherapeutics, such as melphalan.[56] Melphalan is a genotoxic drug used often for treatment of limb-confined melanoma through an isolated-limb perfusion/infusion (ILP/ILI) procedure, which involves shunting the limb circulation in order to allow high-concentration melphalan treatment for a limited time followed by its withdrawal from circulation.[112,113] This raises a further prospect of administering the cancer patients with melphalan plus recombinant CRT in an ILP/ILI set-up. This is an exciting prospect that could be safer than systemic treatment (which has autoimmunity-related concerns).[7]

Combinatorial therapy involving blockade of 'don't eat me' or tolerogenic signals in cancer. Direct blockade of a 'don't eat me' signal (CD47) or a tolerogenic 'eat-me' signal (PtdSer)[114] are interesting strategies to combine with anticancer therapies/ICTs (Figure 3b). Anti-CD47 antibodies can achieve durable tumour regression in preclinical settings.[13,46,72] Interestingly, anti-CD47 blockers can synergize with rituximab/trastuzumab in increasing cancer cell clearance and preclinical tumour regression.[46] Moreover, while initially anti-CD47 therapy was presumed to mainly involve macrophages, recently it was reported to also activate DC-based priming of antitumour T cells.[115] All these promising preclinical results have paved the way for multiple clinical trials with anti-CD47 monoclonal antibodies (Figure 3b), whose results are eagerly awaited (NCT02216409, NCT02367196, NCT02447354, NCT02488811).[116] PtdSer can also be a negative prognostic factor (Table 2) and thus an attractive therapy target (Figure 3b).[43] In fact, an anti-PtdSer therapeutic antibody, that is, bavituximab has yielded positive results (improved progression-free and overall survival) in a Phase II trial involving lung carcinoma/NSCLC patients.[98] Based on these encouraging results and FDA approval, a Phase III trial of bavituximab as a second-line therapy is currently underway for lung carcinoma.[98] Preclinical studies have recently also shown that anti-PtdSer can synergize with ICTs to exert antitumour effects.[98] Last but not least, pro-phagocytic-bridging molecules can also be therapeutically targeted (Figure 3b). For instance, systemic MFG-E8 blockade increases the effectiveness of conventional chemo-radiotherapy and anticancer vaccines by augmenting apoptosis and potentiating DC-driven immunity.[117]

Conclusions. The process of phagocytosis was discovered more than a century ago, much before the finer details of cell death regulation and mechanisms came to be described. Despite this, phagocytosis and cell death research have not progressed in synchrony and it is only in the past decade that finer details of cell death pathway-specific phagocytic mechanisms have emerged. Most researchers recognize that the currently known 'eat me' and 'don't eat me' signals are only a fraction of what might exist; however, discovery of new phagocytic determinants has been slow. This has also further affected research on immunological consequences of phagocytosis. Although the differentiation between tolerogenic and immunogenic phagocytosis is now starting to emerge, much remains to be resolved. Very few immunogenic 'eat me' signals have been discovered. During ICD, while both immunogenic and tolerogenic 'eat me' signals co-exist, it still remains unclear how the immunogenic ones ultimately supersede the effects of the tolerogenic ones. Last but not least, not enough FDA-approved therapies have been associated with relevant 'eat me' signals, thereby hampering knowledge on the immunogenic or tolerogenic consequences of such therapies. With the clinical success of cancer immunotherapy, it is imperative that more research is carried out on dying cancer cells' phagocytosis as this is the major route for ordered acquisition of cancer antigens.

Conflict of Interest

The authors declare no conflict of interest.

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