Drugging the efferocytosis process: concepts and opportunities

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Abstract | The daily removal of billions of apoptotic cells in the human body via the process of efferocytosis is essential for homeostasis. To allow for this continuous efferocytosis, rapid phenotypic changes occur in the phagocytes enabling them to engulf and digest the apoptotic cargo. In addition, efferocytosis is actively anti-inflammatory and promotes resolution. Owing to its ubiquitous nature and the sheer volume of cell turnover, efferocytosis is a point of vulnerability. Aberrations in efferocytosis are associated with numerous inflammatory pathologies, including atherosclerosis, cancer and infections. The recent exciting discoveries defining the molecular machinery involved in efferocytosis have opened many avenues for therapeutic intervention, with several agents now in clinical trials.

Cell death and cellular turnover are fundamentally critical to our healthy living. Roughly 200–300 billion cells are turned over in the human body daily, primarily via the death process of caspase-dependent apoptosis. This apoptotic process has been established to be critical for routine tissue homeostasis, embryonic development, wound healing and inflammation resolution in various tissues1,2. For example, neutrophils, the major innate immune cells that are the first line of defence against invading pathogens, have a short lifespan (~24 h), and >100 billion neutrophils are turned over daily. In the thymus or bone marrow, millions of immature T lymphocytes or B lymphocytes are generated, of which only a small fraction matures with the rest being eliminated by apoptosis. During organ development and embryogenesis, a rapid turnover of cells via apoptosis supports tissue restructuring. In addition, ‘used’ cells such as aged red blood cells are eliminated in large numbers on a daily basis as part of our bodily ‘house cleaning’3. Furthermore, during inflammation resolution, infiltrating neutrophils, monocytes and lymphocytes undergo apoptosis after their job is ‘done’. There are also other types of cell death such as necroptosis, pyroptosis and ferroptosis that arise from the death cue and tissue context4,4. But the questions are, what happens to all of these dying cells and what comprises the clearance crew?

Despite the large numbers of apoptotic cells being generated routinely, they are hardly observable in tissues in vivo. This is because cell death is remarkably well coupled to the highly effective phagocytic removal of apoptotic cells, a process termed ‘efferocytosis’4. In most tissues, efferocytosis is performed by either tissue-resident professional phagocytes with high phagocytic capacity — such as macrophages and dendritic cells — or non-professional phagocytes with lower phagocytic capacity that can be neighbouring cells — such as epithelial cells and fibroblasts. In certain tissues there are also ‘specialized’ phagocytes, such as retinal pigmented epithelial cells (eye) or Sertoli cells (testes), that perform phagocytic clearance in addition to their primary role as nurse/support cells. Efferocytosis has two benefits4,4: the quick removal of the apoptotic cells prevents them from becoming secondarily necrotic, where they might release their potentially toxic intracellular contents that may induce inflammation; and, equally important, engulfing phagocytes switch to a more ‘pro-resolution’ phenotype by increasing the secretion of anti-inflammatory mediators and upregulating pro-repair transcriptional programmes. This allows billions of cells dying in the body to be removed in a manner that avoids inflammation and is non-immunogenic.

In contrast to efferocytosis, other types of phagocytosis such as Fc receptor-mediated phagocytosis are geared towards initiating an immune response. Failed or defective clearance of apoptotic cells can be causative in numerous pathological conditions such as atherosclerosis, ageing-associated inflammation, cancer and infections1. The relevance of efferocytosis to diverse physiological and pathological outcomes opens the possibility to therapeutically target the various steps in the process and to harness the beneficial anti-inflammatory aspects of efferocytosis. In contrast, anti-inflammatory features of efferocytosis tumour-associated macrophages may be detrimental to an antitumour response, and in this context, converting efferocytosis to a more inflammatory nature could potentially be beneficial.

Numerous strategies to therapeutically harness efferocytosis are emerging. For example, small molecules targeting receptors for phosphatidyserine (PS; an ‘eat-me’ signal exposed on the plasma membrane
Pyroptosis
Inflammatory caspase-driven lytic cell death, usually triggered by pathogenic insults. This involves inflammasome assembly, which via caspase 1 induces Gasdermin D activation. Gasdermin D proteins form pores in the plasma membrane, leading to membrane rupture and lytic cell death accompanied by the release of IL-1β and IL-18.

Ferroptosis
A form of caspase-independent lytic cell death induced by excess free intracellular iron inducing lipid peroxidation, causing plasma membrane pore formation and osmotic cell rupture.

Fc receptor-mediated phagocytosis
The process in which a cell-surface immune cell receptor recognizes and binds to the Fc fragment of antibodies, allowing for phagocytosis of the antibody-coated particles.

Solute carrier (SLC) family
A superfamily of membrane transporters that carry a wide number of substrates across the plasma membrane, and membranes of intracellular organelles.

Chemokines
Secreted proteins that act as chemoattractants and induce immune cell migration.

Pannexin channels
Heptameric plasma membrane channels that are activated in apoptotic cells by caspase 3 cleavage (or other modes in live cells) allowing the release of metabolites, including ATP.

Purinergic receptor P2Y
A G-protein-coupled receptor that is activated by extracellular adenosine and uridine nucleotides, and UDP-glucose. The P2Y family consists of eight receptors that can engage in intracellular signalling via adenylate cyclase or phospholipase C.

of apoptotic cells) on phagocytes are under clinical trials for numerous cancers. The application of autologous apoptotic cells for transplant tolerance is already under use. Further, the metabolites released from apoptotic cells or the molecular secretions from phagocytes engulfing apoptotic cells are actively anti-inflammatory with a beneficial role in inflammatory diseases. In addition, recent research suggests that anti-inflammatory and pro-resolution effects of efferocytosis are influenced by the solute carrier (SLC) family of transporters, which are highly druggable. In this Review, we focus on the distinct steps of efferocytosis, their linkage to human disease and the therapeutic interventions targeting this exciting physiological process, with implications for many human pathologies and aging.

The mechanics of efferocytosis
Although there are many forms of cell death, apoptosis is the primary cell death modality under homeostatic conditions. Efficient uptake of apoptotic cargo by phagocytes requires that the dynamic efferocytosis machinery operates optimally. The factors governing optimal functionality include the ratio of phagocytes to apoptotic cells, the nature of phagocytes (professional or non-professional), the size of the dying cell, the rate of death in a given spatial location, as well as the presence of stimulatory and signalling molecules facilitating the process. All of these factors can impact efferocytosis, which is a multistep process. First is the ‘smell phase’, during which phagocytes ‘sense’ the presence of apoptotic cells and locate them. Second is the ‘eating phase’, when ligands on apoptotic cells are engaged by phagocytic receptors on phagocytes to specifically recognize and ingest the dying cell. Third is the ‘digestion phase’, when the ingested corpse and its contents are processed. This digestion phase is critical, resulting in a multifold increase in the amounts of proteins, nucleotides, carbohydrates and lipids that the phagocyte must manage while maintaining its own metabolic homeostasis. Thus, enhancing any of these steps could improve efferocytosis, and, in turn, provide an opportunity to dampen tissue inflammation. The subsections below will discuss the key molecular details and strategies to therapeutically target each of the phases of efferocytosis.

The smell phase
The very first step towards successful efferocytosis is the ‘smell phase’. Apoptotic cells release soluble mediators which include multiple ‘find-me’ signals, such as the nucleotides ATP and UTP, membrane lipids such as sphingosine-1-phosphate and lysophosphatidylcholine (LysoPC), and chemokines such as fractalkine (CX3CL1). These soluble factors play dual roles: first, acting as attraction signals for the phagocytes to migrate towards the apoptotic cells; and, second, preparing the phagocytes for engulfment by modulating their cytoskeleton, enhancing the expression of engulfment receptors and the digestion machinery. The same signals also attribute anti-apoptotic, regenerative and anti-inflammatory properties to the infiltrating and resident phagocytes. The relative importance of each of these signals is dependent on the physiological context, cell type and stage of apoptosis. The variation in the find-me signals might be a mechanism to ensure apoptotic cell clearance in every tissue context; for example, whereas some find-me signals may help to attract motile phagocytes such as macrophages to carry out clearance, other find-me signals may improve the efferocytosis machinery in the non-professional phagocyte. With the rapid technological advancements in mass-spectrometric imaging, it is now feasible to decipher the relative importance and abundance of each of these find-me signals in different tissue contexts. Also, determining the local concentration and strength of the chemical gradient in vivo will help understand how these may be ‘drugged’ in the future.

Nucleotides.
The regulated release of small amounts of the intracellular nucleotides (<1%) from apoptotic cells can act as a potent inducer of monocyte/macrophage migration towards the apoptotic cells and also promote an anti-inflammatory tone in the tissue neighbourhood (see below). This nucleotide release is mediated by pannexin channels on the plasma membrane that become activated by caspase 3/7-mediated cleavage during apoptosis. The released ATP can induce migration of phagocytes via the cognate purinergic receptor P2Y, and clearance of apoptotic cells is impaired in P2Y<sup>-/-</sup> mice. Further, UDP released by damaged microglia can induce the upregulation of P6Y in the hippocampus and aid in clearance of damaged cells. Nucleotides also ‘prepare’ the phagocytes for engulfment by inducing the expression of binding and engulfment receptors such as CD11b and α5β3 integrin. In addition, nucleotides can exert immunomodulatory effects on macrophages and are, hence, called ‘calm-down’ signals. For example, the ATP or AMP released by apoptotic cells can be converted to adenosine via the sequential actions of membrane nucleotidases such as CD39 and CD73; adenosine, in turn, can dampen inflammation through adenosine receptors. This also leads to the expression of anti-inflammatory and pro-resolution molecules, such as Nr4a1, Nr4a2 and thrombospondin. It is important to note that the regulated nucleotide release in small amounts (0.1%) from early-stage apoptotic cells (when the cells are still ‘whole’) is different from complete cell lysis and the release of the intracellular ATP at high concentrations, as the latter is well known to be pro-inflammatory. Recently, it has been shown that pannexin channel-mediated ATP and AMP release from viable T cells, and their subsequent conversion to adenosine, is critical for dampening airway inflammation. It is important to note that the pannexin channels in viable T cells in this context are not activated by the irreversible caspase-mediated cleavage but, rather, by other ‘reversible’ mechanisms.

Metabolites as preparatory signals.
In addition to attracting the neighbouring phagocytes, apoptotic cells also actively release >100 metabolites during early stages of apoptosis. These metabolites, in turn, induce transcriptional changes in phagocytes and neighbouring cells. The ‘metabolite secretome’ of apoptotic cells has revealed numerous signalling metabolites such as polyamines and nucleotides released in a regulated fashion
from dying cells after induction of apoptosis. A good fraction of these metabolites (>20%) are released via the large-pore pannexin 1 channels (which can conduct molecules up to 1 kDa). Three aspects of metabolite release from apoptotic cells are particularly interesting from a therapeutic standpoint. First, apoptotic cells are not simply emptying their contents but, rather, release select metabolites among the thousands normally present in a cell; even more intriguing, whereas many pathways are shut down during apoptosis, the polyamine pathway continues to remain active, and the polyamine spermidine (but not all metabolites in the polyamine pathway) is selectively released. Second, groups of metabolites are much more active than individual metabolites in inducing responses in neighbouring cells, suggesting cooperativity among metabolites in tissue communication. Last, metabolites released from apoptotic cells are not unique to a particular type of apoptotic trigger or cell type. A few ‘core’ metabolites released during apoptosis were shared among different cell types and different apoptosis triggers. The metabolites were identified to induce gene expression linked to inflammation resolution and tissue repair. Further, administering a metabolite cocktail reduced symptoms in models of inflammatory arthritis, and minor histocompatibility mismatch in lung transplantation.

**Approaches to target the smell phase**

The immunomodulation induced by secreted soluble messengers released by apoptotic cells has brought forth an interesting therapeutic opportunity. A small subset of these conserved, often actively synthesized metabolites could be harnessed as ‘drugs’ to induce pro-repair and anti-inflammatory gene transcription programmes. For many decades now, the approach of ‘extracorporeal photopheresis’ (ECP) has been widely used to dampen inflammation in systemic lupus erythematosus (SLE) and arthritis, and facilitate organ transplantation. In ECP, the leukocytes from a patient are removed, treated with UVA light along with 8-methoxysulfoxin and, then, reintroduced into the patient, which has been highly beneficial in dampening overall inflammation in many organs. Further, the benefits of apoptotic cell infusion for transplantations have been widely accepted, and experimental transplant models ranging from murine organs to the heart, pancreatic islets and aortic transplants have shown much improved graft acceptance when the recipient was exposed to donor apoptotic cells. However, how the donor cells given during ECP function has been a mystery. Perhaps, the anti-inflammatory gene signature induced by metabolites released from apoptotic cells may be part of the answer. Thus, boosting the regulated release of AMP, other nucleotides or metabolites via pannexin channels is a potential drug targeting strategy. Recently, direct activation of pannexin 1 via deacetylation has been identified in vitro, suggesting drug-based channel activation is likely feasible. In addition, the membranes of apoptotic cells contain PS, and their contact with neighbouring cells may also provide an anti-inflammatory tone to the tissue. ECP is a highly onerous procedure, has to be done repeatedly every few months and is expensive. The ability to modulate inflammatory diseases with soluble mediators released by apoptotic cells or apoptotic cell therapy could revolutionize the concept of personalized medicine for inflammatory diseases.

**The eating phase**

The ability to phagocytes to specifically recognize apoptotic cells is based on the exposure of ‘eat-me’ signals on the surface of apoptotic cells, in contrast to ‘do not eat-me’ signals that are exposed on the surface of live cells. The ‘eat-me’ signals are recognized by a cohort of phagocytic receptors as detailed below. The most potent, evolutionarily conserved and well-studied ‘eat-me’ signal is PS exposed on the outer leaflet of the plasma membrane of apoptotic cells. Under normal conditions, PS is actively kept on the inner leaflet of the plasma membrane in a regulated and energy-dependent fashion by specific enzymes called flippases and scramblases that help maintain this PS asymmetry. Upon induction of apoptosis, caspase 3 causes the cleavage and inactivation of flippases while simultaneously activating scramblases. Other ‘eat-me’ signals exposed on apoptotic cells include calreticulin, annexin 1, oxidized low-density lipoprotein, thrombospondin 1 binding sites and complement proteins C1q or C3b binding sites. Although PS exposure is clearly central, the collection of other ‘eat-me’ signals dictates the optimal recognition and eating by phagocytes. It is important to note that not all apoptotic cells expose the same set of ‘eat-me’ signals, and a subset of them together with PS seem to be sufficient for recognition by specific receptors on phagocytes.

**Phagocytic receptors.** PS exposed on the surface of apoptotic cells is recognized via receptors that can bind PS either directly or indirectly. Receptors such as the brain-specific angiogenesis inhibitor 1 (BAI1), T cell immunoglobulin mucin receptors TIM1–TIM4 (TIM family) and Stabilin 2 bind PS directly, whereas receptors of the Tyro3/Axl/Mer (TAM) family of tyrosine kinases engage PS via soluble bridging molecules such as Gas6 and Protein S. Dendritic cells, macrophages and endothelial cells are also capable of recognizing and binding to apoptotic cells via the scavenger receptor class F member 1 (SCARF1). SCARF1 binds to the complement component C1q that is bound to the PS on apoptotic cells. This complement-mediated uptake plays an important role in the aversion of autoimmunity (as will be discussed later in the Review). Among these PS recognition receptors, there are considerable differences in how they trigger intracellular signalling. BAI1, upon binding to PS via its thrombospondin repeats (TSR), initiates intracellular signalling via the ELMO1–DOCK complex to induce Rac1-mediated actin cytoskeletal rearrangements to facilitate efferocytosis. BAI1 and downstream signalling via the ELMO1 pathway have also been linked to suppression of inflammation in models of gut inflammation. TIM4, on the other hand, can interact with PS but is incapable of independently transducing cytoplasmic signalling, and signals via other integrins, BAI1 or other receptors. Numerous reports in the past years have shed light on the fact that the different
Augmenting PS receptor expression for inflammation resolution
- Gene targeting — AAV/Crispr
- Exploring tissue specificity of PS receptor for targeted expression

Enhancing panxenin 1 expression or activity
- Apoptotic/necrotic corpse administration
  - Apoptotic cell administration for localized EMMi release
  - Necrotic cell administration for cancer immunotherapy
  - Apoptotic corpse therapy in transplant acceptance

Dietary interventions — Metabolic enzyme corpse administration
- Enhancing pannexin 1 activity
- Activity
- Role of SLC programme

Cell types express different combinations of phagocytic receptors, and this is influenced by the stage of efferocytosis, tissue context and developmental and immune states. Thus, the existence of a wide variety of engulfment receptors appears prudent to allow tissue-specific engulfment of apoptotic cargo. The binding of apoptotic cells to receptors on phagocytes triggers a complex array of cytoskeletal rearrangements, facilitating cargo engulfment. The signalling mechanism, the proteins involved and the kinetics of the process are highly variable and depend on the nature of the phagocytes and the inflammatory context.

The fusion of the apoptotic cell-containing phagosome with lysosomes, where many pH-sensitive degradative
enzymes reside, aids in the breakdown of the biomolecular content of the apoptotic cargo. How the phagosomes traffic inside phagocytes has been reviewed extensively elsewhere.

Interestingly, the TAM family of receptors are implicated in multiple physiological processes, distinct from, but closely related to, their primary role in effecrocytosis. MerTK and AXL have direct roles in suppressing inflammatory responses and building tolerance towards ‘self’ antigens. During effecrocytosis, MerTK blocks NF-kB signalling in macrophages to suppress inflammatory cytokine production and macrophage M1-like polarization. Similar observations were made in monocyte-derived dendritic cells. In dendritic cells, a strong feedback loop is activated upon TLR stimulation that causes the expression of AXL. Also, AXL was found to interact with the type I interferon receptor to trigger the expression of suppressor of cytokine signalling 1 (SOCS1) and SOCS3, which directly acts in inhibiting inflammatory cytokine production and inflammation. Further, MerTK expression and activation are associated with suppression of dendritic cell maturation and effector T cell expansion, promoting T cell tolerance. Besides suppressing an M1-like phenotype, MerTK also helps polarize macrophages to the alternative or reparative M2-like phenotype.

**Approaches to target the eating phase**

Owing to the diversity of PS receptors, their ubiquitous nature and their involvement in multiple pathologies (FIG. 2), PS receptors may serve as attractive therapeutic targets for multifactorial benefits. There are multiple methods by which the activity/effectiveness of PS receptors can be altered, and many therapeutics are already in clinical trials (TABLE 1). First, simply increasing the numbers of individual phagocytic receptors can enhance clearance. For example, whereas the levels of Bai1 mRNA progressively decreased in the gut epithelial cells and colonic tissue after DSS-induced colitis in wild-type mice, transgenic overexpression of Bai1 could attenuate the pathology of DSS-induced colitis in vivo. Consistent with this beneficial effect, Bai1 transgenic mice showed fewer apoptotic cells in the colonic epithelium and reduced colonic inflammation compared with wild-type mice. Along similar lines, Stab2 expression was found to be critical for myoblast fusion during the process of muscle differentiation and regeneration, and overexpression of Stab2 was found to enhance myoblast fusion and myotube formation, both in vitro and in vivo. Reduction in the expression of ‘anti-inflammatory’ phagocytic receptors may also be useful in cancer therapies. Targeting the downregulation or blocking the activity of MerTK has shown benefit in non-small lung cell carcinoma, gastric cancer cells, head and neck cancers, and glioblastoma. Thus, modifying the expression levels/activity of PS receptors to optimal levels, in the context of individual pathologies, could be therapeutically beneficial. Second, the fact that there are so many PS recognition receptors and that they are not all equally expressed in all phagocytes strongly suggests specificity, both in signalling and in outcome, after corpse ingestion. Therefore, defining context-specific PS receptors would need to be a priority for drug targeting of the eating phase. A third method could be to engineer a ‘universal’ booster of effecrocytosis that can increase the expression of multiple PS recognition receptors in an inflammatory tissue context. An alternative possibility might be to engineer effecrocytic receptors to increase their efficiency, that can then be expressed in a tissue context during ongoing inflammation to improve apoptotic cell clearance and, in turn, dampen the source of inflammation (FIG. 1).

**Anti-phagocytic receptors and ‘do not eat-me’ signals.**

The exposure of ‘do not eat-me’ signals on live cells helps avoid their effecrocytosis by phagocytes. Numerous such ‘do not eat-me’ molecules have now been discovered, including CD47 and CD24. These ‘do not eat-me’ signals, in turn, enable ‘anti-phagocytic receptors’ on phagocytes, and thereby help evade effecrocytosis. For example, CD47 on live cells binds to SIRPs on macrophages, and this interaction causes tyrosine phosphorylation of the cytoplasmic domain of SIRPs, leading to the recruitment and activation of the phosphatases SHP1/2. SHP1/2 then inhibit effecrocytosis by inhibiting non-muscle myosin IIA. This signal, therefore, suppresses macrophage interaction with tumour cells, and this interaction causes tyrosine phosphorylation of the cytoplasmic domain of SIRPs, leading to the recruitment and activation of the phosphatases SHP1/2. SHP1/2 then inhibit effecrocytosis by inhibiting non-muscle myosin IIA. Thus, targeting the ‘do not eat-me’ signals to promote active engulfment of ‘live’ tumour cells is currently being pursued as part of anticancer immunotherapies (discussed in greater detail in the following sections). Another ‘do not eat-me’ signal, CD24 was recently reported to be highly expressed on tumour cells and the interaction of CD24 with sialic acid binding immunoglobulin-like lection 10 (Siglec10) on tumour-associated macrophages prevented the effecrocytosis of cancer cells. Blocking the CD24–Siglec10 interaction via monoclonal antibodies allowed for improved clearance of tumour cells. CD31, expressed on viable cells, via homotypic interactions, is thought to mediate the detachment of live cells from the surface of phagocytes. Although targeting anti-phagocytic receptors to promote effecrocytosis of apoptotic cells is less studied, this could also be a powerful approach in the context of ongoing inflammation.

**The digestion phase**

Very often, the number of apoptotic cells far exceeds that of phagocytes, causing each phagocyte to successively ingest multiple apoptotic corpses, some of which can be very large in size. The processing and degradation of the apoptotic cargo is carried out either by the canonical degradation programme or in other ways that may work in parallel. The canonical phagocytosis pathway involves the maturation of the early phagosome to late phagosome with eventual fusion with the lysosomal compartment. The additional pathway entails microtubule-associated protein 1A/1B light chain 3 (LC3)-associated phagocytosis and has been covered in other reviews. Phagocytosis of apoptotic corpses brings with it a multilayered complexity: upon ingesting multiple corpses, the phagocyte needs to manage its volume and surface area; the ingested cargo is a metabolic burden, bearing membranes, cholesterol, proteins, nucleic acids and so on, which need to be metabolized and either rerouted into the metabolic flux cycles of the...
phagocyte or excreted; and the ingested cargo might bring with it some harmful content, and managing digestion and degradation of the apoptotic cargo while shifting gears towards an anti-inflammatory, pro-repair phenotype after efferocytosis is a unique challenge for the phagocytes.

**Lipids and associated receptors.** Degradation of apoptotic cargo causes phagocyte cholesterol levels to increase, a phenomenon exacerbated in atherosclerosis when apoptotic ‘foamy’ macrophages (foam cells) undergo efferocytosis and have to be taken up by other macrophages. A complex cascade of signalling events aid

| Causes of defective efferocytosis | Potential therapeutic opportunities |
|----------------------------------|-----------------------------------|
| • Reduced PS exposure | • Antibodies against soluble PS receptor cleavage products |
| • Cleavage of PS receptors by ADAM17/10 | • PPARγδ agonists |
| • C1q deficiency | • Recombinant Fc multimers |
| • Loss of DNase1/L1 | • DNAse1, DNAse1L3 and DNAse2 treatment |
| • Reduced LXR/PPARγ signalling | • PPARγδ agonists |
| • SCARF1 autoantibodies | • Overexpression of miR-126 via non-particle delivery |

| SLE/EAE | Diabetes/obesity | Atherosclerosis |
|---------|-----------------|-----------------|
| Reduced Xkr1 | mER/Tyro | High circulating oxidized lipids/inflammatory cytokines |
| Reduced PS | Pro-resolution signalling | Degradation |
| Therapeutic anti-sAxl/merTK/Tyro antibodies | sMer | Circulating TNF |
| SCARF1 | sMer | TNF |
| ADAM17/10 | sMer | Foam cell |
| sAxl | Anti-sAxl | Anti-CD47 |
| sMer | EPO | ABCA1 transporter |
| sTyro | SCARF1 | LRP1 |
| PPAR agonists | S1P receptor | CD47 |
| HIF1α/β | PS receptor expression | Cholesterol |
| ADAM9 | Pro-resolution cytokines | Degradation |
| miR-126 | PS receptor | | |
| Pro-resolution signalling | Enhanced efferocytosis | |

**Fig. 2 | Opportunities to target efferocytosis steps in specific pathologies.** Systemic lupus erythematous (SLE): defects in multiple steps of efferocytosis have been recognized in SLE. High levels of autoantibodies against scavenger receptor class F member 1 (SCARF1) (a phosphatidylserine (PS) receptor acting in conjunction with C1q) are found in patients with SLE, and correlated with accumulation of apoptotic cells. Enhanced cleavage of PS receptors MerTK, Axl, Tyro and Tim by ADAM17/10 proteases, and reduced activity and bioavailability of DNase1L3, further suppress efferocytosis, exacerbating inflammation. Reduction in scramblase Xkr1, responsible for PS exposure, is also seen in mouse models of SLE. Plausible therapeutic opportunities in SLE could include: targeting PS receptors to improve efferocytosis; targeting PPAR/ligand-X receptor (LXR) to enhance efferocytosis and LC3-associated phagocytosis (US Patent US20190145961); and extending administration of nanosphere-coupled DNase1 to SLE. Diabetes and obesity: in diabetes, defective efferocytosis and reduced expression of ABCA1 further suppress efferocytosis, exacerbating inflammation. Reduction in scramblase Xkr1, responsible for PS exposure, is also seen in mouse models of SLE. Plausible therapeutic opportunities in SLE could include: targeting PS receptors to improve efferocytosis; targeting PPAR/ligand-X receptor (LXR) to enhance efferocytosis and LC3-associated phagocytosis (US Patent US20190145961); and extending administration of nanosphere-coupled DNase1 to SLE.

| Potential therapeutic opportunities | Molecules (Mfge8, MerTK, CD-36, Gas6) is defective in obese mice and a potential point of intervention in diabetes. Further, administration of endogenous metabolic modulators (EMMIs) may also be effective strategies for enhancing efferocytosis in diabetes and obesity. Atherosclerosis: defective efferocytosis contributes to accumulation of lipid-laden apoptotic cells, which eventually add to necrotic lesions. Causes for poor efferocytosis include ADAM17/10-dependent cleavage of MerTK and decrease in ABCA1 upregulation. Reduced cholesterol export from phagocytes further diminishes engulfment capacity of cells. Enhanced expression of CD47 on apoptotic cells further suppresses apoptotic cell engulfment in atherosclerotic plaques. Numerous clinical trials are underway, targeting CD47–SIRP1α interaction in cancers, and repurposing these for atherosclerosis can be of value. Turning specialized pro-resolving mediator (SPM) ratios in favour of resolution and efferocytosis may help delay atherogenesis. Induction of foam cell apoptosis and localized administration of EMMIs in plaques may be a supplementary therapeutic strategy. EAE, experimental autoimmune encephalomyelitis; LRP1, low-density lipoprotein receptor-related protein 1; TIM, T cell immunoglobulin mucin receptor. |
| Target | Drug/company (trial details) | Pathologies | Trial phase | Clinical trial ID/refs |
|--------|-----------------------------|-------------|-------------|------------------------|
| Axl    | Bemcentinib; also known as BGB324 and R428/trial by BerGenBio ASA | COVID-19 | II | NCT04890509 (REFS\(^{206,207}\)) |
|        | Bemcentinib/Rigel Pharmaceutical/BerGenBio (alone or in combination with other chemotherapeutics) | Recurrent glioblastoma, advanced solid tumours, metastatic breast cancer, AML, non-small cell lung cancer, malignant mesothelioma | II | NCT03824080 |
|        | | | II | NCT03184571 |
|        | | | II | NCT03654833 (REFS\(^{218,219}\)) |
|        | | | I | NCT03649321 |
|        | | | I | NCT03965494 |
|        | Bemcentinib/trial by BerGenBio | Non-small cell lung cancer | I | NCT02488408 |
|        | Amuvatinib/trial by Astex Pharmaceuticals (in combination with platinum–etoposide) | Small cell lung cancer | II | NCT01357395 (REFS\(^{212–214}\)) |
|        | Cabozantini/trial by Memorial Sloan Kettering Cancer Centre and Exelixis | Non-small cell lung cancer | II | NCT01639508 |
|        | TP-0903/trial by Sumitomo Dainippon Pharma Oncology | Advanced solid tumours | I | NCT02729298 (REFS\(^{215–218}\)) |
| MerTK  | ONO-7475/Ono Pharmaceuticals (alone or in combination with venetoclax) | Cancers\(^{279}\), acute leukaemia and myelodysplastic syndromes | I-II | NCT03176277 |
|        | MRX-2843/trials by Meryx, Inc. (NCT04872478 and NCT03510104), Emory University (NCT04762199) and Betta Pharmaceuticals Co., Ltd (NCT04946890) (alone or in combination with osimertinib) | Solid tumours, non-small cell lung cancer, refractory AML | I-II | NCT04946890 |
|        | | | I | NCT03510104 |
|        | | | I | NCT04762199 |
|        | | | I | NCT04872478 |
|        | PF-07265807/trial by Pfizer | Metastatic solid tumours | I | NCT04562529 (REF\(^{119}\)) |
|        | Gene therapy (AAV-mediated delivery) | Retinitis pigmentosa | I | NCT01482195 (REFS\(^{220–222}\)) |
| CD47   | Magrolimab/trials by Gilead Sciences (alone or in combination with pembrolizumab/docetaxel/azacitidine/neopacitaxel/venetoclax/mitoxantrone/etoposide/cytarabine/CC-486/cetuximab) | AML | III | NCT04313881 |
|        | | | III | NCT04778397 |
|        | | | III | NCT05079230 |
|        | | | II | NCT04788043 |
|        | | | II | NCT04827576 |
|        | | | II | NCT04958785 |
|        | | | II | NCT04892446 |
|        | | | II | NCT04854499 |
|        | | | II | NCT04778410 |
|        | | | I-II | NCT04541017 |
|        | | | I-II | NCT04435691 |
|        | | | I-II | NCT02953782 |
|        | | | I-II | NCT02953509 (REF\(^{223}\)) |
|        | | | I | NCT05169944 |
|        | | | I | NCT04751383 |
|        | ALX148/most trials by ALX Oncology (alone or in combination with venetoclax/azacitidine/rituximab/lenalidomide/zahtidamab) | B cell non-Hodgkin lymphoma, myelodysplastic syndromes, AML, advanced head and neck squamous carcinoma, advanced solid tumours, Her2+ cancers, microsatellite stable metastatic colorectal cancer | II-III | NCT05001217 (REF\(^{222}\)) |
|        | | | II | NCT04675294 |
|        | | | II | NCT04675333 |
|        | | | II | NCT05167409 |
|        | | | I-II | NCT04755244 |
|        | | | I-II | NCT04417517 |
|        | | | I-II | NCT05025800 |
|        | | | I-II | NCT05027139 |
|        | | | I | NCT03013218 |
|        | AO-176/trial by Arch Oncology and Merck Sharp & Dohme Corp. | Malignancies/multiple myeloma | I-II | NCT04886271 |
|        | | | I-II | NCT04445701 |
### Transcription Factors in Efferocytosis

Transcription factors play a critical role in regulating the expression of genes involved in efferocytosis. The liver X receptors (LXRs) α/β are transcription factors belonging to the nuclear receptor family, and they are activated by the presence of oxysterols. This activation leads to the expression of ABCA1, which facilitates cholesterol efflux from macrophages, thereby reducing the burden of cholesterol-rich cargo. The receptors can also suppress the release of inflammatory cytokines by downregulating the expression of pro-inflammatory genes, such as TNF, IL-1β, IL-6, COX2, and iNOS. This suppressive effect is mediated through the repression of the NF-κB pathway, which is involved in the pro-inflammatory response. Additionally, the activation of LXRα/β can suppress the secretion of pro-inflammatory cytokines such as IL-10 and TGFβ, which helps to maintain a tolerogenic environment.

#### Liver-X Receptors (LXRs) α/β

- **Transcription factors** belonging to the nuclear receptor family, involved in regulating cellular metabolism and immune cell behavior, and anti-inflammatory responses.
- **PPARγ**: A transcription factor involved in the regulation of cholesterol metabolism and the induction of anti-inflammatory responses.

### Efferocytosis and Metabolism

Efferocytosis is an essential process for the resolution of inflammation and the re-establishment of metabolic homeostasis. The engulfment of apoptotic cells by phagocytes is mediated by the recognition of specific surface markers, such as CD47, which binds to the receptor Tim4 on phagocytes. This interaction triggers the engulfment of the apoptotic cell.

#### Phagocytes in Handling Excess Lipid

Phagocytes play a crucial role in handling the excessive lipid overload that occurs during cell death. The uptake of cholesterol-rich cargo, such as damaged lipids, can be regulated by the nuclear receptor family, including the vitamin D receptor (VDR) and liver X receptors (LXRs). Upon binding to their ligands, these receptors activate a series of signaling cascades that modulate the expression of genes involved in lipid metabolism and inflammation.

#### Role of PPARγ in Efferocytosis

PPARγ is a transcription factor that plays a significant role in the resolution of inflammation and the maintenance of metabolic homeostasis. It is activated by the presence of oxysterols, which are products of cholesterol degradation. PPARγ activation leads to the repression of pro-inflammatory genes and the induction of anti-inflammatory genes, including those involved in the suppression of NF-κB activity. This results in the suppression of inflammatory cytokines and the induction of tolerogenic molecules, such as IL-10 and TGFβ.

### Clinical Trials for Efferocytosis

**Table 1 (cont.): Ongoing or completed clinical trials for therapeutics targeting efferocytosis**

| Target | Drug/company (trial details) | Pathologies | Trial phase | Clinical trial ID/refs |
|--------|-----------------------------|-------------|-------------|------------------------|
| CD47 (cont.) | AK117/Akesobio Pharmaceuticals | AML/myelodysplastic syndrome, malignant neoplasm | I-II | NCT04900350 |
| | | | I-II | NCT04980885 |
| | | | I | NCT04349969 |
| | | | I | NCT04728334 |
| AO-176 | trial by Arch Oncology (alone or in combination with paclitaxel/pembrolizumab/magrolimab or with bortezomib/dexamethasone) | Advanced solid tumours, relapsed/refractory multiple myeloma | I-II | NCT03834948 |
| | | | I-II | NCT0445701 (REF.14) |
| HX009 | trial by Waterstone Han X Bio Pty Ltd | Advanced solid tumours | I | NCT04097769 |
| Tim4/3 | Sabatolimab; also known as MBG453 trials by Novartis (alone or in combination with PDR001/azacitidine/decitabine/venetoclax) | Cancers: advanced malignancies/lower risk myelodysplastic syndrome/AML/myelodysplastic syndromes | III | NCT04266301 |
| | | | II | NCT04823624 |
| | | | I | NCT04150029 |
| | | | II | NCT04878432 |
| | | | I | NCT03946670 |
| | | | I | NCT04812548 |
| | | | I | NCT04623216 |
| | | | I | NCT02608268 |
| TSR-022 | trials by Tersaro, Inc. (alone or in combination with TSR-042) | Advanced solid tumours/liver cancer/melanoma | II | NCT03680508 |
| | | | I | NCT0281763 |
| | | | I | NCT03307785 |
| | | | I | NCT03099109 (REF.12) |
| LY3321367/ | Eli Lilly and Company (alone or in combination with LY3300054) | Advanced/refractory solid tumours | | |

**A|ML, acute myeloid leukaemia; TIM, T cell immunoglobulin mucin receptor.**

**Sugars, nucleotides and transporters.** In the quest to uncover the utilization of apoptotic cell-derived metabolic load, multiple interesting facts about metabolic flux occurring in phagocytes have emerged. Continuous efferocytosis is an essential mechanism in resolution and repair. Macrophages have been shown to utilize the arginine and ornithine derived from the first round of engulfed apoptotic cells to facilitate subsequent rounds of efferocytosis. Arginine and ornithine were shown to be converted to putrescine via the action of macrophage-expressed ornithine decarboxylase and arginase. Interestingly, putrescine could increase the activity of the small GTPase Rac1, which then facilitates actin rearrangement and subsequent rounds of efferocytosis. The additional observations from this study gain importance in tissues with high rates of cell turnover, where multiple cargoes need to be engulfed rather rapidly (FIGS 1 and 3). An interesting recent finding also highlights how efferocytosis of apoptotic cargo can enable proliferation of the pro-resolution macrophages in vitro and in vivo. The nucleotides released by the hydrolysis of apoptotic
cell-derived DNA could enhance Myc activity via the mTPRC2/Rictor pathway, leading to the proliferative phenotype and inflammation resolution\textsuperscript{66}. The subcellular compartmentalization of metabolites has emerged as an important aspect governing cellular metabolic fluxes. Metabolite transport is mediated via a specialized family of transmembrane proteins called the SLC family. SLC family members (numbering >400) are localized in the plasma membrane as well as in intracellular membranes. The different phases of efferocytosis rewire the SLC expression network in both macrophages and fibroblasts\textsuperscript{61}. The mere presence of the ‘secretome’ of apoptotic cells was shown to induce the expression of the mRNA for the enzyme Sgk1, which phosphorylates SLC2a1 (a glucose transporter) and increases SLC2a1 abundance in the plasma membrane\textsuperscript{62}. Further, the engagement of PS with cognate phagocytic receptors was shown to increase the expression of SLC2a1 itself\textsuperscript{63}. The enhancement of glucose uptake and glycolysis, owing to the increased activity and expression of SLC2a1, was found to be essential for initial and continuous efferocytosis both in vitro and in vivo. Later stages of efferocytosis, possibly tending towards ‘digestion’ of the apoptotic cargo, were found to increase gene expression of the lactate transporter SLC16a1. The lactate released via SLC16a1 was biologically active — extracellular lactate derived via the upregulation of SLC16a1 was capable of triggering the expression of pro-resolution genes such as \textit{il10} (REF \textsuperscript{66}). Recent studies also suggest that mitochondrial dynamics within the phagocytes may play a significant role in regulating their homeostasis, and successive corpse uptake\textsuperscript{64}. The fusion of the phagosome containing the apoptotic cargo with the lysosomes leads to the release of a large number of lipid metabolites within the macrophage. The lipid substrate abundance and oxidative phosphorylation were linked to an increase in the NAD\textsuperscript{+}/NADH ratio, in turn triggering a member of the sirtuin family of proteins, SIRT1, ultimately inducing the release of IL-10 via Pbx1 (FIG. 3). Further, efferocytosis by mouse bone marrow derived macrophages (BMDMs) led to increased cytosolic calcium and mitochondrial fission mediated by Drp1, and mitochondrial morphology could influence high-burden efferocytosis\textsuperscript{65}.

\section*{Approaches to target the digestion phase}

The intricate protein interaction networks that operationally link immunological behaviour to metabolic fluxes have recently become high-priority drug targets\textsuperscript{6}. Efferocytosis provides a unique phenomenon in this regard, wherein the ingestion of an apoptotic cargo triggers metabolic reprogramming that aids the phagocyte to suppress inflammatory signalling (FIGS 1 and 3). Aberrations in this signalling network contribute to multiple pathologies, as detailed below. Pioglitazone, a drug approved for type 2 diabetes, is a PPAR\gamma agonist. In recent years, PPAR\gamma has been demonstrated to boost efferocytosis (via increasing PS receptor expression) and induce anti-inflammatory gene expression signatures when tested on monocytes derived from patients with human chronic granulomatous disease\textsuperscript{67}. Repurposing of PPAR\gamma/LXRa agonists to boost efferocytosis in the context of inflammatory diseases and infections is a
Foam cells
Macrophages carrying high lipid and cholesterol burden seen in atherosclerotic plaques, due to enhanced lipoprotein uptake.

ADAM family proteases
A disintegrin and metalloprotease (ADAM) family of proteins. A family of transmembrane proteins with strong proteolytic activity. The substrates for ADAM proteases are very often the extracellular domains of transmembrane proteins.

Specialized pro-resolvin mediators (SPMs). A class of immunologically active lipid derivatives that limit acute inflammatory responses and aid in inflammation resolution. The family includes lipoxins, E-series and D-series resolvins, protectins and maresins.

pro- resolution phenotype. However, in advanced atherosclerotic plaques, the ratio of Resolvin D1 to the pro-inflammatory lipid mediator, leukotriene B4, was inversely correlated with the efferocytosis capacity of macrophages as these levels of 12(S)-HETE) were inversely correlated with the SLC family of proteins by efferocytosis implies that metabolite localization, subcellular compartmentalization and temporal fluctuations during the process of cargo engulfment have a major immunomodulatory role. Boosting efferocytosis by altering the activity or expression of selected solute carrier proteins such as SLC2a1, while simultaneously modulating the activity of ABCA1 and SLC16a1, could prove powerful in pathologies where poor efferocytosis is observed and is pro-inflammatory (for example, atherosclerosis). Interestingly, β-hydroxybutyrate increases SLC2a1 expression levels (epigenetically), β-Hydroxybutyrate, as a dietary supplement, is already in clinical trials for type1 diabetes (NCT03999853). In conditions where successive efferocytosis by phagocytes is hampered (for example, during inflammation), the ability to enhance the activity of unique SLC family members, or the lysosomal arginine transporter Pqcl2, which can then drive continued efferocytosis in vitro and in vivo, poses a novel window of therapeutic opportunity. The research on how phagocytes utilize apoptotic cargo to drive their immunological programmes is bound to provide more leads into novel therapeutic avenues. A large ongoing body of research on SLCs, which represent the second largest family of proteins in the human genome (after GPCRs), has identified numerous small-molecule inhibitors/activators, and targeting specific SLCs relevant during efferocytosis could be an approach to improve outcomes in inflammatory diseases (Fig. 3).

Efferocytosis in pathologies and therapeutic opportunities
A large number of human disorders, including atherosclerosis, cancer, SLE, diabetes, obesity, rheumatoid arthritis and ageing, are now linked to defects or alterations in the efferocytosis machinery. Efferocytic machinery is highly sophisticated, involving a network of receptors, cytosolic signalling molecules, rapid cytoskeletal rearrangements, cargo digestion and subsequent immunomodulatory signalling. Owing to these distinguishable and well-defined steps during efferocytosis and the accessible molecular pathways, the steps in efferocytosis are emerging as highly attractive for therapeutic intervention in a broad range of diseases, some of which will be discussed below.

Atherosclerosis
Atherosclerosis is a condition that develops due to the accumulation of oxidized lipids and lipoproteins in the sub-endothelial layer of arteries in the heart. The heart vasculature contains phagocytes such as dendritic cells, macrophages and monocytes, and the further accumulation of lipids and lipoproteins during atherogenesis also causes infiltration of leukocytes into the vasculature. Initially, the resident and infiltrating macrophages ingest these sub-endothelial lipid deposits, but when these macrophages are overwhelmed with intracellular lipid and cholesterol (‘foamy macrophages’), this triggers their apoptosis. Defective efferocytosis and accumulation of apoptotic foam cells have been demonstrated in the necrotic core of atherosclerotic plaques and large necrotic cores are correlated with higher risk of myocardial infarction. Multiple causes for reduced efferocytosis in lesions have emerged including defective ABCA1 expression, as well as increased degradation of efferocytic receptors such as MerTK and LR1. MerTK is liable to cleavage by a member of the ADAM family proteases, ADAM17, and a cleavage-resistant mutant of MerTK accelerated resolution in the model of zymosan-stimulated peritonitis. MerTK cleavage might be a phenomenon in atherosclerosis, as macrophages in the necrotic cores of atherosclerotic plaques exhibit decreased expression of MerTK on the cell surface and have heightened expression of ADAM17.

A well-understood phenomenon associated with macrophage-mediated efferocytosis is the generation of a class of lipids called specialized pro-resolvin mediators (SPMs), which include Resolvens D1, D2 and E4, lipoxin A4 (LXA4) and Maresin. The precursors of SPMs are often carried by apoptotic cargo and efferocytosis leads to the activation of a critical enzyme, 12/15-lipoxygenase, needed for SPM biosynthesis from these precursors. Molecules such as LXA4 and Resolvin D1 have been demonstrated to boost efferocytosis in macrophages and promote an overall anti-inflammatory, pro-resolution phenotype. However, in advanced atherosclerotic mice with Resolvin D1 to the pro-inflammatory lipid mediator, leukotriene B4, was skewed towards inflammation. Treatment of atherosclerotic mice with Resolvin D1 suppressed reactive oxygen species (ROS) levels and necrosis in the lesions, while simultaneously inducing efferocytosis. Further, the levels of 12(S)-hydroxyeicosatetraenoic acid (12(S)-HETE) are increased in patients with acute coronary syndrome. The levels of 12(S)-HETE were inversely correlated with the efferocytosis capacity of macrophages as these compounds activate RhoA, which inhibits apoptotic cargo internalization.

Lastly, the expression of CD47 as a ‘do not eat-me’ signal prevents efferocytosis within the plaque. The inflammatory milieu of atherosclerotic lesions is linked to the aberrant expression of CD47 on apoptotic cells, causing them to accumulate and, in turn, become secondarily necrotic, further exacerbating lesional inflammation. Inflammation-induced TLR signalling can further suppress the expression of ligands such as MFGE8. Thus, owing to an increasingly inflammatory environment and greatly dysregulated metabolic machinery, efferocytosis in atherosclerotic plaques is suppressed, furthering disease pathology.

Opportunities for targeting the efferocytic machinery in atherosclerosis. The most common therapeutic intervention for atherosclerosis and coronary heart diseases is statin administration. Along with the direct metabolic consequences, statin treatment also aids in enhancing efferocytosis by inhibiting RhoA isoprenylation, as RhoA is a negative regulator of efferocytosis. Inhibiting ROCK1, the downstream target of RhoA, also improves efferocytosis, and has shown promise in atherosclerosis, reducing the lesion size and inflammatory macrophage accumulation in the plaques.
ROCK activity has been implicated in numerous inflammatory diseases and ROCK1 inhibition (along with statin treatment) is used in multiple models of inflammatory diseases including inflammatory arthritis, acute lung injury, systemic sclerosis\(^{106}\), LPS-induced lung injury and Crohn’s disease\(^{107,108}\). Although most of the beneficial effects of ROCK inhibition (for example, fasudil treatment) are attributed to the anti-inflammatory phenotype that emerges owing to the suppression of NF-κB activation\(^{109}\), fasudil treatment also increased neutrophil apoptosis and efferocytosis in a model of intraperitoneal LPS challenge\(^{110}\). CD47 blocking antibodies have also shown promise by enhancing efferocytosis in atherosclerotic lesions in numerous mouse models\(^{111}\). The expression of CD47 and SIRP1α was increased in human atherosclerotic arteries and the loss of SIRP1α signalling protected mice from atherosclerosis\(^{112}\). In addition, enhanced CD47−SIRP1α signalling in atherosclerotic lesions has been observed to lead to a high burden of apoptotic and secondary necrotic cells. Delivery to atherosclerotic plaques of a small-molecule inhibitor of SHP1, a downstream effector of SIRPα receptor on macrophages that recognizes CD47, increased lesional phagocytosis, reduced the inflammatory gene expression and allowed plaque regression in apoE\(^{-/-}\) mice\(^{113}\). An added complication in atherosclerosis was identified to be the accumulation and reduced efferocytosis of necroptotic cells along with an associated reduction in SPM secretion\(^{114}\). The administration of RevD1 or CD47 blocking antibodies aided in enhancing necroptotic cell efferocytosis\(^{115}\).

As ABCA1 and MerTK expression are influenced by LXR and PPAR, agonists of these nuclear receptors seem to have beneficial effects on efferocytosis and anti-inflammatory signalling during efferocytosis in vivo\(^{116}\). Similarly, LXR agonists also suppressed inflammation in vivo\(^{117}\). Although targeting these multifunctional nuclear receptors is complicated, a selected and localized delivery approach may hold promise in promoting efferocytosis to remove the lesional apoptotic burden. Further, efferocytosis-associated factors seem to improve plaque stability, and boosting efferocytosis may again provide additional benefits. Overall, great progress has been made in unravelling the mechanistic underpinnings of efferocytosis in atherosclerosis\(^{118}\). With the advent of nanomaterials for localized and targeted delivery of pro-efferocytic compounds and Resolvin-like lipid mediators, their combination with classical drugs such as statins may provide new therapeutic strategies (FIG. 2). In another approach, mesenchymal stem cells induced M2-like behaviour in macrophages in vitro\(^{119,120}\), and administering mesenchymal stem cells in mouse models of cardiac inflammation led to enhanced M2 macrophage infiltration and efferocytosis, aiding clearance of apoptotic neutrophils and improving cardiac function\(^{121}\).

### Aging

Ageing has been associated with sterile, chronic low-grade inflammation, called ‘inflamming’\(^{100}\). In recent years, numerous studies have noted the increasing presence of cellular debris with age contributing to this inflamming. The presence of cellular debris and mismatched ‘self-antigens’ such as cellular and mitochondrial DNA increase in circulation in aged human subjects, and this has been shown to be positively correlated with inflammatory mediators such as TNF and IL-6 (REF.\(^{101}\)). Aged mice show impaired capacity to clear either apoptotic cells injected into the peritoneum or apoptotic keratinocytes generated by UV irradiation of the skin\(^{102}\). Similarly, in a self-resolving model of peritonitis, aged mice have dramatically reduced SPMs, reduced capacity of inflammation resolution and heightened circulating inflammatory cytokines\(^{103}\). Further, ex vivo differentiated BMDMs from older mice have reduced efferocytic capacity that could be enhanced by SPMs such as Resolvin D3 (REF.\(^{103}\)).

An age-related decline in inflammation resolution is well documented\(^{103,104}\). During acute inflammation in a dermal model of sterile inflammation, efferocytic clearance of the infiltrating polymorphonuclear neutrophils was reduced in older human subjects compared with younger subjects\(^{105}\). This phenotype was, in part, mediated by p38MAPK-mediated inhibition of p300, which caused a reduction in the expression of Tim4, and correlated with reduced expression of pro-resolution signalling and TGFβ release. Blocking p38MAPK with the inhibitor losmapimod enhanced Tim4 expression and efferocytosis in mice. These findings indicate that the resolution phase of inflammation is impacted with age and can be therapeutically targeted.

The low-grade chronic inflammation associated with ageing is accompanied by the presence of senescent cells. Senescence is defined as an irreversible and long-term arrest of the cell cycle, and is caused by shortened telomeres, DNA damage\(^{106}\), defects in mitochondrial function and alteration of the epigenomic status\(^{107,108}\). Accumulation of senescent cells is seen at the sites of age-related pathologies such as atherosclerosis, Alzheimer disease, osteoarthritis, chronic obstructive pulmonary disease and Parkinson disease\(^{109–111}\). Bioactive molecules (SASP) secreted by senescent cells such as IL-1, IL-6, IL-18, TNF, IL-8, GM-CSF and MCP1–MCP4 induce paracrine signalling in the neighbouring cells to activate senescence\(^{112}\). It is plausible that the presence of senescent cells and associated SASP could feed into the general ‘polarization’ of macrophages and other phagocytes towards a non-efferocytic phenotype with age. Conditioned media from senescent cells induced MerTK cleavage and decreased macrophage efferocytosis in vitro, whereas cleavage-resistant mutants of MerTK were non-responsive to the presence of the conditioned medium from senescent cells\(^{113}\). This decreased efferocytosis could be countered by the administration of Resolvin D1 (REF.\(^{114}\)). Thus, a decrease in efferocytosis of apoptotic cargo, and accumulation of apoptosis-resistant senescent cells along with the influence of SASP on inflammation resolution and efferocytosis, could be major players in inflamming.

**Opportunities for targeting the efferocytic machinery in ageing**. Current ‘senotherapies’ are directed towards induction of apoptosis in senescent cells and suppression of the damaging effects of SASP. Following the

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**Senotherapies**

Therapies aimed at killing senescent cells and diminishing the effects of the senescence-associated secretory phenotype that is a key driver of sterile inflammation during ageing.

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elegant work by Baker et al.,

efforts are underway to develop pharmacological agents that could potentially trigger cell death in senescent cells via senolytic agents. Owing to a decline in efferocytosis and an increase in the accumulation of apoptotic and senescent cells with age, combinatorial therapies increasing efferocytosis and inducing apoptosis in senescent cells could be a powerful intervention in increasing healthspan. In this context, compounds such as resolvins could be administered in combination with senolytic compounds. The co-treatment with potentially localized delivery of senolytic compounds could be useful for conditions such as osteoarthritis. Localized delivery could be further enhanced by pro-resolving approaches, as demonstrated in the zymosan model of inflammation. Spermidine is a bioactive polyamine, which has been proven beyond doubt to have beneficial effects in ageing and increasing lifespan in human subjects. Apoptotic cells synthesize and secrete spermidine, and administration of spermidine in combination with purine nucleotides (GMP and IMP) was demonstrated to improve the pathology in an arthritis model as well as in a lung transplant rejection model. This reveals an exciting possibility of combining apoptotic metabolite-based or oral therapies with senolytic compounds and SPMs to affect suppression of inflammation to improve efferocytosis.

Cancer
Cancer is often referred to as a disease with inflammation as one of its hallmarks, and prolonged, unresolved inflammation as a contributing factor to oncogenesis. On the contrary, the tumour microenvironment is increasingly recognized as immunosuppressive, allowing escape of tumour cells from immunosurveillance. Macrophages exist in large numbers in the tumour microenvironment (referred to as ‘tumour-associated macrophages’) and play significant immunomodulatory roles at progressive stages of cancer from initiation to progression, epithelial–mesenchymal transition, metastasis, angiogenesis, tumour cell motility and matrix degradation. Macrophages are also linked to suppression of tumour immunosurveillance, leading to poor disease prognosis. Efferocytosis machinery promotes naïve macrophages to adopt an ‘M2’-like phenotype, which is proven beyond doubt to be a major contributor to poor cancer prognosis by suppressing antitumoural activity, aiding in tissue repair-type responses and angiogenesis (via production of VEGF).

The reason for the existence of the M2 polarizing milieu in the tumour mass is an area of active research. Cell death is a common occurrence in solid tumours as they progress towards malignancy, with chemotherapies increasing the number of dying cells. It is plausible that anti-inflammatory mediators such as TGFβ and IL-10, released by tumour macrophages engulfing apoptotic cells within the tumour mass, could be the drivers of the observed alternative M2 polarization. Further, as noted in the previous sections, PS receptor activation actively interferes with NF-κB signalling and type I interferon production, in turn, limiting antitumour immune responses. Thus, a combination of pro-survival function of TAM receptors on tumour cells and their ability to suppress inflammation in the tumour microenvironment (via the macrophages) may promote immunosuppression and tumour progression. The TIM family of PS receptors are also implicated in poor prognosis of malignant tumours including colorectal cancer, non-small cell lung cancer and gastric cancer. A potential deleterious effect of anti-inflammatory signalling-associated efferocytosis within the tumours is the link to myeloid-derived suppressor cells (MDSCs) within the tumour microenvironment. MDSCs can protect the tumour from assaults on the patient’s immune system via regulatory T cells, promoting angiogenesis (by secreting VEGF) and production of type I interferons. Cytokines released during efferocytosis such as IL-10, PGE2 and VEGF promote MDSC development and function. MDSCs in tumour-bearing mice have dramatically upregulated TAM receptors and ligands, and knocking down the TAM receptors in MDSCs led to increased T cell proliferation and tumour immunosurveillance. Transplantation of MerTK−/− bone marrow in a mouse model of metastatic breast cancer led to a higher number of cytotoxic CD8+ T cells and increasing antitumour immune responses. The roles of TAM receptor activation in tumours have been reviewed extensively elsewhere.

Opportunities for targeting the efferocytosis machinery in cancer. Owing to their role in aiding tumour growth, angiogenesis and metastasis, the TIM and TAM families of PS receptors are hot topics of research for antitumour therapy, along with antibodies aimed at PS blockade. R428 (bemcentinib) is a selective Axl inhibitor with a half-maximal inhibitory concentration of 14 nM, which could suppress breast cancer metastasis, angiogenesis and inflammatory cytokine production by inhibiting Axl and downstream Akt phosphorylation. Axl inhibition by bemcentinib could also sensitize cells that have undergone epithelial–mesenchymal transition to a large number of anti-mitotic drugs — particularly docetaxel in vivo. Bemcentinib is currently in phase II clinical trials (NCT03824080) for acute myeloid leukaemia (AML) and low/high-risk myelodysplastic syndrome. Cabozantinib, a broad receptor tyrosine kinase inhibitor also suppressing Axl activity, is under phase II clinical trials (NCT01639508). Multiple other compounds targeting Axl are under clinical trials for different types of cancers. TP-0903, a novel oral Axl inhibitor, and amuvatinib are under phase I and phase II clinical trials respectively, for small cell lung cancer.

Apart from Axl, small molecules targeting MerTK and TIM4/3 are in advanced stages of clinical trials. ONO-7475 is a dual Axl–MerTK inhibitor tested in acute leukaemia and myelodysplastic syndromes, either alone or in combination with the chemotherapeutic venetoclax (NCT03176277; TABLE 1). Cabozantinib, a broad receptor tyrosine kinase inhibitor also suppressing Axl activity, is under phase II clinical trials (NCT01639508) (TABLE 1). Multiple other compounds targeting Axl are under clinical trials for different types of cancers. TP-0903, a novel oral Axl inhibitor, and amuvatinib are under phase I and phase II clinical trials respectively, for small cell lung cancer.

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activity when combined with anti-PD1 therapy\textsuperscript{134}, is also undergoing phase I clinical trials (NCT04458259; \textbf{TABLE 1}). The TIM3 inhibitor sabotilomab (MBG453) was recently granted fast-track designation by the US Food and Drug Administration (FDA) for myelodysplastic syndrome and is being tested in numerous clinical studies for myelodysplastic syndromes, either alone or in combination with other chemotherapeutics\textsuperscript{135–137} (\textbf{TABLE 1}). In addition, the novel anti-TIM3 antibody TSR-02 is currently undergoing phase I clinical trials against solid tumours (NCT02817633; \textbf{TABLE 1}) and is being tested in combination with anti-PD1 antibody for liver cancer (in phase II clinical trials, NCT03680508; \textbf{TABLE 1}). Finally, LY3321367, an anti-TIM3 monoclonal antibody, is undergoing phase I clinical trials alone or in combination with anti-PD1 antibody for the treatment of solid tumours (NCT03099109; \textbf{TABLE 1}). Thus far, no dose-limiting toxicity has been observed in either the monotherapy or combinatorial therapy\textsuperscript{138}.

A surprising phenomenon in patients with myeloproliferative disease is the ability of tumour cells to upregulate the expression of the ‘do not eat-me’ signal molecule CD47, to limit engulfment by phagocytes. CD47 classically interacts with the macrophage receptor SIRPα, suppressing efferocytosis of live cells. Increased CD47 expression has been observed in patients with ovarian cancer, glioma, myeloid leukaemia, non-Hodgkin lymphoma and glioblastoma, which correlated with poor prognosis and tumour growth with successive stages of malignancy\textsuperscript{139,140}. Anti–CD47 monoclonal antibody-mediated engulfment of tumour cells by macrophages facilitated the priming of CD8\textsuperscript{+} T cells to mount a cytotoxic response\textsuperscript{141}. Although anti–CD47 therapies have shown promise in animal models, due to CD47 expression also on red blood cells, specific targeting of tumours without interfering with other ‘normal’ roles of CD47 is an ongoing challenge\textsuperscript{142–144}. So far, the observations point towards a beneficial role for CD47 blockade in cancer. It is worth noting that the phagocytosis mediated under conditions of CD47 antibody blockade is not typical efferocytosis, and is more akin to FcR-mediated phagocytosis, and may induce a more pro-inflammatory response within the tumours\textsuperscript{145}. A large number of clinical trials are underway, testing the efficacy of anti–CD47 therapy in combination with pre-existing chemotherapeutics in various cancer types. AO-176, a humanized IgG2 antibody that specifically binds and blocks CD47, is being tested in multiple myeloma (NCT04445701; \textbf{TABLE 1}). Ao-176 has been shown to promote efferocytosis along with inducing antitumour T cell responses, as well as T cell-mediated killing of CD47-expressing tumour cells\textsuperscript{146}. HX009 is a humanized bispecific antibody targeting CD47 and PD1, which is undergoing clinical trials for patients with advanced tumours and malignancies (NCT04886271, NCT03834948)\textsuperscript{147}. In addition, AK117, a humanized IgG4 monoclonal antibody against CD47, has shown promise in phase Ia trials (NCT04980885, NCT04900350), being tolerated up to 20 mg kg\textsuperscript{-1} and achieving sustained peripheral receptor occupancy at 10 mg kg\textsuperscript{-1} and higher\textsuperscript{148}. Notably, ALX14 is a high affinity anti–CD47 antibody with lower molecular weight, allowing for greater dosage\textsuperscript{149}, and is under clinical trials for a large number of cancers (\textbf{TABLE 1}). Magrolimb is an anti–CD47 monoclonal antibody that was recently given FDA breakthrough therapy designation for interventions in myelodysplastic syndrome due to promising clinical trial data\textsuperscript{150}. The drug, in combination with either azacitidine or venetoclax + azacitidine, is in phase III trials for TP53-mutated AML (NCT04778397). About 20 clinical trials are currently underway using magrolimb in combination with other available chemotherapeutic drugs (\textbf{TABLE 1}).

Recently, a few other inhibitory receptors and cognate molecules have been identified on tumour-associated macrophages and/or the tumour cells, such as the LILRB1/MHC class I and PD1/PDL1, and these appear to impact clearance of tumour cells and, in turn, disease prognosis\textsuperscript{151}.

Another approach to promote the development of antitumour immunity involves induction of non-apoptotic and immunogenic forms of cell death, such as necroptosis. Immunogenic cell death is characterized by chronic exposure of intracellular damage-associated molecular patterns — particularly calreticulin, HMGB1, HSP70/90 and ATP — to the tumour microenvironment, stimulating the otherwise suppressed antitumour immune niche\textsuperscript{152}. A cohort of drugs repurposed for inducing immunogenic cell death are in various stages of clinical trials. These include anthracyclines, glycosides, oxaliplatin, bleomycin and cyclophosphamide, among others, and have been extensively reviewed\textsuperscript{153,154}.

Although efferocytosis is generally anti-inflammatory, this can be modified. For example, whether macrophages ‘interpret’ a dying cell as anti-inflammatory versus pro-inflammatory depends on the expression of SLC12a2 and the associated chloride efflux during efferocytosis\textsuperscript{155}. Blocking SLC12a2 promoted enhanced apoptotic corpse uptake but the response of the efferocytic macrophages was pro-inflammatory. As SLC12a2 can be targeted via small molecules, targeting SLC12a2 within the tumour microenvironment may help antitumour responses; an approach which remains to be tested. In summary, efferocytosis, effecrocytic receptors and subsequent responses of efferocytic phagocytes could play important roles in suppressing antitumour immunity. Finding the correct balance between dead cell clearance and development of antitumour immunity is a challenging aspect of drug development, and combinatorial therapies coupled to localized delivery of these drugs might be a way forward.

\textbf{Infectious diseases}

Host cell death is a well-defined characteristic of pathogenic insults. The modality of host cell death, such as necroptosis, pyroptosis or apoptosis, is largely dependent on the strain of the infecting pathogen. Whether host cell death triggered by infection benefits or harms the host is governed by numerous factors such as the nature of cell death, kinetics of death post infection, degree of death and extent of the removal of dead cell debris. Efferocytosis of dying infected cargo plays a crucial role in clearing infection, or, as it happens in some cases, provides a new host niche for the pathogen upon engulfment.
**Beneficial role of efferocytosis.** *Mycobacterium tuberculosis* uses macrophages as its primary host. Virulent strains of the bacteria are known to suppress apoptosis of infected macrophages while pushing necrotic death. Upon efferocytosis of infected apoptotic macrophages by healthy macrophages, the bacterial load has been demonstrated to be significantly reduced both in vitro and in the lungs of infected mice\(^\text{156}\). Further, blocking TIM4 in resident peritoneal macrophages significantly increased the incidence of secondarily infected cells, and this was attributed to secondary necrosis of the primary infected cells\(^\text{156}\). Along with aiding bacterial clearance, efferocytosis promotes antigen presentation. Dendritic cells taking up *M. tuberculosis*-infected apoptotic vesicles was shown to increase antigen presentation to CD8\(^+\) T cells\(^\text{157}\). Pulmonary tuberculosis is often defined by the presence of caseous granulomas with a lipid-rich necrotic core, formed as a result of rapid infection-induced death of lipid-laden macrophages. It is possible that those treatments activating efferocytosis in the early stages of granuloma development, before fibrotic cuff formation, might aid in reducing the bacterial burden. Other bacterial infections also show efferocytosis-mediated clearance of pathogens, albeit by different processes. Pyroptotic cell death, triggered by *Klebsiella pneumoniae* infection of macrophages, aided efficient clearance of the pathogen via efferocytosis of the pyroptotic debris\(^\text{158}\). *Salmonella typhimurium* infection could trigger pyroptotic cell death in host macrophages leading to the formation of pore-induced intracellular traps. Pore-induced intracellular traps expose numerous ‘find-me’ and ‘eat-me’ signals, and these structures could aid in efferocytosis and clearance of bacilli.

Efferocytosis also plays a critical role in viral infections. Influenza virus H1N1-infected mouse lungs show a rapid influx of macrophages and neutrophils. These phagocytes then engulf influenza virus-infected apoptotic cells\(^\text{159},\text{160}\). Severe cases of influenza often lead to ARDS (acute respiratory distress syndrome) and pneumonia-like symptoms owing to virus-induced alveolar epithelium damage and the ensuing immune cell infiltration and cytokine storm. How efferocytosis of the dying neutrophils aids in infection clearance was recently demonstrated\(^\text{159},\text{160}\). Influenza strain H3N2 infected in mouse airway elicited a massive, yet temporary, infiltration of neutrophils and monocytic cells into the trachea. The infiltrating neutrophils were engulfed by tissue-resident phagocytes and the infiltrating monocytes. Monocyte depletion in chemokine receptor 2 (CCR2) knockout mice led to a significant accumulation of apoptotic neutrophils in the lungs. Apoptotic neutrophil-educated Ly6C\(^+\)/CD64\(^+\) inflammatory monocytes were critical antigen-presenting cells, responsible for CD8\(^+\) T cell activation. Further, EGF secreted from apoptotic neutrophils promoted the differentiation of infiltrating monocytes into antigen-presenting cells, and also facilitated CD8\(^+\) T cell effector roles\(^\text{160}\). Efferocytosis may also play a role in human immunodeficiency virus (HIV-1), which primarily infects CD4\(^+\) T cells, causing a steady decline in the numbers of these cells\(^\text{157}\). Similar to other viral infections, HIV-1 infection induces PS exposure on infected cells by triggering either apoptotic or pyroptotic cell death\(^\text{157},\text{162},\text{163}\). The efferocytosis of HIV-infected T cells was facilitated by MerTK-mediated uptake by macrophages and this was found to be aided by Gas6 and Protein S\(^\text{164}\). Efferocytosis was shown to play a positive role in HIV infections, as the engulfment of apoptotic HIV-1-infected cells by dendritic cells aided the expansion of viral antigen-specific CD4\(^+\) and CD8\(^+\) T cells\(^\text{165}\).

**Detrimental role of efferocytosis.** Although efferocytosis aids in suppressing infections in several scenarios, this is not always the case. *Listeria monocytogenes* has evolved to utilize efferocytosis machinery as a means of cell to cell spread. The bacteria were shown to induce membrane damage and localized PS exposure on the host cell membrane\(^\text{166}\). Eventually, these areas of high-pathogen density were found to form bacteria-carrying vesicles, which could infect surrounding macrophages by means of PS binding to TIM4 (REF.\(^\text{166}\)). In *M. tuberculosis* infections, the outcome of efferocytosis is often heavily dependent on the nature of the infecting strain, as the virulent strains of *M. tuberculosis* triggered necrosis of infected host neutrophils. The efferocytosis of these infected and necrotic neutrophils by macrophages, instead of infection clearance, has been shown to lead to further replication of the bacilli inside the efferocytic macrophages and the spread of infection\(^\text{167}\). Parasites such as *Leishmania major* and *Trypanosoma cruzi* have also evolved strategies to manipulate the efferocytosis machinery for their advantage. In addition to interfering with phagolysosomal fusion, efferocytosis of *L. major*-infected neutrophils by dendritic cells led to a reduction in the presentation of the parasite-derived antigens, and was dependent on MerTK signalling\(^\text{168}\). Engulfment of *L. major*-infected neutrophils by macrophages also led to parasite replication and suppression of inflammatory cytokine production by macrophages\(^\text{169}\). *T. cruzi*-infected apoptotic cells, when engulfed by macrophages, acted as Trojan horses for the parasite, allowing parasite growth in the engulfing cells. This was linked to TGF\(\beta\), which not only suppressed inflammatory cytokines but also triggered the activity of ornithine decarboxylase, leading to increases in putrescine, which aided intracellular parasite replication\(^\text{170}\).

**Opportunities for targeting the efferocytic machinery in infections.** The possibility of targeting efferocytosis for therapeutic benefit during infections is context-dependent. Some PS receptors may aid virus binding and entry into host cells. TIM family members are involved in viral infections including Marburg virus, HIV (in vitro)\(^\text{171}\), Ebola virus (in vivo)\(^\text{172}\), Japanese encephalitis virus\(^\text{173}\), Dengue virus\(^\text{174},\text{175}\), and so on. Similarly, Gas6 was found to facilitate the entry of Ebola virus by bridging the viral envelope-expressed PS to Axl on target cells\(^\text{176}\). In the context of viral infections, there are two plausible mechanisms of therapeutically exploiting efferocytosis. First, antibodies or small peptides that block different PS receptors from interacting with vital PS molecules could be administered during the replicative phases of viral infections to inhibit cellular entry;...
Severe COVID-19 is characterized by an infiltration of monocytes, macrophages and T cells into the lungs, causing the build-up of a cytokine storm. This phenomenon, if unchecked, ultimately leads to pulmonary oedema, pneumonia and ARDS (acute respiratory distress syndrome), resulting in lung and multiorgan damage. Patients with severe COVID-19 also display macrophage activation syndrome (SMA). The causes for the development of ARDS in patients with COVID-19 are decreasing as the world has seen mass vaccination programmes. A detailed single-cell transcriptomics and proteomics study highlighted a highly dysregulated myeloid cell behaviour in patients with severe COVID-19 with myelopoiesis and expansion of dysfunctional neutrophils expressing PDL1 [REF 226]. Further, an elevated neutrophil to lymphocyte ratio in the blood has been described as a potential indicator of disease severity and ARDS development [227].

Classically, patients with ARDS have been reported to show reduced neutrophil clearance of neutrophil extracellular traps (NETs), largely due to the reduced efferocytosis capacity of macrophages [228], and a study of 33 patients with COVID-19 demonstrated evidence for NET-mediated immunothrombosis [229]. It is tempting to speculate that reduced efferocytosis in the lungs of patients with COVID-19, with exacerbated NET formation and death of infiltrating cells, contributes to the development of ARDS. It has recently been demonstrated that the efferocytosis of SARS-CoV-2-infected apoptotic cells augmented the production of inflammatory cytokines and diminished successive cargo uptake by macrophages in vitro. Furthermore, patient-derived lung monocytes and macrophages showed reduced expression of efferocytic receptors [230]. Thus, it is tempting to speculate that improving efferocytosis may facilitate clearance of dying cells (in specific affected tissues), and thereby help avoid chronic inflammation associated with ‘long COVID’.

Phosphatidylycerine (PS) on membranes of viruses has long been shown to be relevant in infection. Interestingly, a recent finding highlighted PS exposure and the presence of multineucleated syncytia in the pneumocytes of patients with COVID-19 to the SARS-CoV-2 Spike protein. Through candidate drug screens, compounds inhibiting syncytia formation in vitro were identified and a prime candidate was found to be niclosamide. This compound is a strong inhibitor of the Ca²⁺-activated TMEM16 family of calcium-activated lipid scramblases and channels. Out of the ten members of this family, the expression of TMEM16F was found to be enhanced by the Spike protein. Inhibition of TMEM16F by niclosamide or benz bromarone reduced the PS exposure on cells expressing the Spike protein and, in turn, dramatically reduced syncytia [231].

Proof of principle for the concept of PS receptor blocking as a potential therapeutic approach to reducing the viral load. The role of efferocytosis in SARS-CoV-2 infections has been reviewed [232]. As proof of concept, a reagent capable of blocking PS and PE mediated viral entry into host cells was highly effective in blocking Zika virus replication in vivo [233]. Second, perhaps enhancing the expression of PS receptors during later stages of enveloped virus infection may help inhibit viral shedding. During the late stages of HIV-1 replication, viral budding and release is associated with incorporation of PS receptors on HIV-1 virions [234], and these HIV-1 particles are retained on the plasma membrane, inhibiting viral release. Targeting efferocytosis is also emerging as an interesting opportunity in COVID-19 infections (BOX 1).

In bacterial infections, the plasticity with which bacterial pathogens engage the efferocytic machinery dictates the choice of intervention. The cytoplasmic efferocytosis protein ELMO1 was found to be essential for Salmonella enterica subsp. enterica serovar Typhimurium infection of enteric macrophages, bactericidal ROS production and LC3-mediated degragation of the engulfed bacteria [235,236]. M. tuberculosis infection induces the expression of miR-143 and miR-365, which suppress ELMO1 expression, aiding intracellular bacterial replication [237]. Small molecules that enhance intracellular signalling by PS receptors, while not increasing their membrane expression, might be an extremely successful host-directed therapy. The strategy will retain the antibacterial benefits while keeping the levels of bacterial invasion low. Owing to the complexity of host–pathogen interactions, the highly variable role of efferocytosis during infections and the ever-evolving nature of such interfaces, designing therapeutic interventions for directly inhibiting pathogenic infections is likely to be challenging.

**Systemic lupus erythematosus**

SLE is an autoimmune disease in which patients classically carry antibodies against self-antigens such as DNA, phospholipids and nuclei. Out of the numerous triggers associated with SLE induction, defective efferocytosis is considered a major contributor and patients with SLE often demonstrate accumulation of apoptotic cells [238], which eventually turn secondarily necrotic, releasing damage-associated molecular patterns. Multiple molecules associated with efferocytosis have now been identified as defective or malfunctioning in patients with SLE. ADAM10 and ADAM17 metalloproteases exhibit higher cleavage activity in SLE models, leading to the cleavage of cell surface PS receptors, reducing efferocytosis efficiency [239,240]. Genetic deletion of MerTK induced lupus-like symptoms in older mice. The heightened amounts of soluble PS receptors (generated by ADAM10/17 activity) compete for PS binding, further reducing efferocytosis, a phenomenon observed in juvenile SLE onset [241]. Complement plays an important role in the clearance of apoptotic cells and immune complexes, and defects in early complement components such as C1q/s/r, C4 and C2 lead to SLE development [242,243]. Further, mice deficient in the phagocytic receptor SCARF1 develop SLE-like systems with large pools of uncleared apoptotic cells. A significant proportion of patients with SLE carry anti-SCARF1 autoantibodies [244]. Along with suppressing apoptotic cell engulfment, autoantibody accumulation in patients with SLE diminished DNAse1L3 activity, further exacerbating pathology [245]. Variations in the behaviour of apoptotic cells themselves can also contribute to the development of SLE. Further, mice lacking phospholipid scramblase Xcr1 also exhibit SLE-like symptoms [246] (FIG. 2).

**Opportunities for targeting the efferocytic machinery in SLE**

Efferocytosis of apoptotic cells, as discussed earlier, is a potent inducer of nuclear receptor signaling (PPAR/LXR), which leads to an anti-inflammatory and pro-efferocytosis gene expression programme [247]. Owing to the pro-efferocytosis and resolution properties, treatment with the PPARγ agonist pioglitazone could improve vascular defects in patients with SLE (NCT02338999) [199]. A parallel study pointed to benefits of PPARγ/LXR pathway agonists in aiding LC3-associated phagocytosis (US20190145961A1). With the accumulation of DNA released by secondary necrotic cells, another complementary approach towards SLE treatment could be the administration of DNAse1L3 via nanoparticle-mediated delivery methods (FIG. 2). As discussed above, the cleavage of PS receptors such as...
as MerTK is a contributor to poor prognosis in SLE. However, patients with SLE often develop glomerular nephritis owing to heightened proliferation signalling-mediated by Gas6–Axl. The Axl-specific inhibitor R428 suppressed disease progression in the mouse model of immune glomerulonephritis (TABLE 1). It can be appreciated that organ-specific roles of PS receptors in SLE provide a unique window of opportunity for targeted and personalized therapy. Combinatorial therapy, where classical treatment lines are augmented with agents such as Resolvins or PPARγ/LXR agonists, offer additional opportunities.

**Diabetes and obesity**

One of the major manifestations of defective efferocytosis in diabetes or obesity-induced diabetes is in the form of delayed wound healing in animal models. In multiple diabetic animal models, apoptotic cells and increased inflammatory cytokines accumulate at the wound site. Although the contributions of other efferocytic cells, such as dendritic cells/epithelial cells/fibroblasts at wound sites are still under study, the mechanisms mediating defective efferocytosis have started to emerge. Macrophages in vitro and from diabetic db/db mice exposed to high glucose had reduced efferocytosis capacity and reduced levels of miR-126 — responsible for suppressing the expression of ADAM9. Greater ADAM9-mediated MerTK cleavage was linked to a reduction in efferocytosis (FIG. 2). Aberrant signalling events are also responsible for reduced efferocytosis, and lower inflammation resolution in high-fat diet-induced obesity mouse models. Under normal conditions, SIP released by apoptotic cells engaged HIF1α in macrophages leading to expression and release of erythropoietin (EPO). EPO was a strong inducer of PPARγ expression via the Jak2/ERK pathway, leading to the expression of pro-efferocytosis and anti-inflammatory genes. Interestingly, the levels of secreted EPO, macrophage EPO receptor and p-Jak2 were significantly lower in obese mice with zymosan-induced peritonitis, compared with lean mice. The obese mice in the peritonitis model also exhibited significantly lower efferocytosis and increased accumulation of apoptotic cells (FIG. 2). Although detailed mechanistic understanding of the influence of high glucose or circulatting TAGs on the efferocytic capacity of phagocytes is still incomplete, the studies above may point to routes for targeting efferocytic steps in obesity.

**Opportunities for targeting the efferocytic machinery in diabetes and obesity.** COVID mRNA vaccines and their wide acceptance has paved the way for exploring mRNA-based therapeutics. In diabetic mice, miR-126 levels were suppressed. A plausible intervention in such mice could be the delivery of miR-126 via nanoparticles or REDV peptide-modified chitosan to sites of heavy apoptotic cargo burden such as diabetic wounds. Another interesting approach could be neutralization of the cleaved soluble PS receptors. Obese mice in the zymosan peritonitis model, when treated with recombinant EPO, were shown to exhibit significantly reduced apoptotic cell burden and improved inflammation resolution. Recombinant EPO is already used as treatment for kidney disorders and can be potentially repurposed as a treatment for obesity to decrease the apoptotic cell burden and associated inflammation. Another interesting finding in diabetes and obesity suggests that an increase in saturated fatty acids with a concomitant decrease in ω3-fatty acids leads to a dramatic reduction in macrophage efferocytic capacity. The efferocytosis capacity could be restored in obese mice by altering the diet balance to ω-fatty acids, hinting towards dietary interventions to improve efferocytic capacity. Administration of anti-inflammatory metabolites/diet-based therapy may play out to be an effective treatment option depending on the patient's medical history, opening new avenues for personalized therapeutics.

**Challenges and future perspectives**

The multistep process of efferocytosis has come to the forefront in the context of numerous pathologies, either as an aetiopathological or a contributor to poor prognosis when this process is disturbed or impaired. Increased understanding of the roles and molecular mechanisms of efferocytosis has revealed many avenues for therapeutic intervention, but has also highlighted numerous challenges.

Efferocytosis is a ubiquitous process involving highly tissue-specific PS receptors and apoptotic cell burden. One evident challenge in targeting efferocytosis therefore lies in targeting the correct receptor type in the organ involved in disease aetiology. In addition, although the SLC family of proteins are highly druggable, and they heavily influence the immunological and efferocytic ability of phagocytes, targeting this family of proteins is challenged by the redundancy in some transporters. Multiple solutes may be transported via a single SLC, which must also be considered.

In pathologies such as cancers, mechanisms have evolved to undermine the beneficial effects of efferocytosis. Whereas inhibiting efferocytosis in cancer can help the development of inflammatory responses, it can also further reduce efferocytosis of cancerous cells. Although pathogenic infections, such as *M. tuberculosis* infections, often manifest a dependence on efferocytosis for bacterial spread, this is highly dependent on the infecting strain and prognosis. Despite these challenges, efferocytosis with its highly sophisticated multistage signalling — offers unique windows of therapeutic opportunities in both cancers and pathogenic infections. Depending on the nature and stage of cancer, combinatorial drugs targeting efferocytosis along with standard chemotherapy could prove valuable. Further, in pathogenic insults, when the infecting strain is known, personalized treatment targeting efferocytosis can be moulded to prevent bacterial spread and aid clearance of infected cells.

In recent years, PS receptors on phagocytes have been thoroughly explored in the context of cancer therapy, and drugs targeting these receptors have shown promising results (TABLE 1). Steps to redirect some of these therapeutics for atherosclerosis and diabetes are already underway and might open novel therapeutic avenues. The ability of efferocytosis to aid senescent cell...
removal and reduce chronic inflammation associated with inflamming holds great potential. Thus, novel therapeutics capable of enhancing efferocytosis (even moderately) could be useful in improving quality of life for ageing individuals. A plethora of exciting findings linking the metabolic changes in efferocytic cells to their immunological behaviour have added a new dimension to how this process can be viewed and therapeutically targeted. Oral or topical administration of endogenous metabolic mediators is already being explored as a therapeutic intervention in numerous other pathologies via mechanisms distinct from efferocytosis. Exploring metabolic combinations that can improve efferocytosis and, thus, disease prognosis can be a novel therapeutic tool (for example, administration of u-3-fatty acids). The development of methodologies for directed metabolite delivery into sites of inflammation may improve disease prognosis (for example, arthritis) and methods for safe administration of apoptotic and necroptotic cells have the potential to revolutionize transplant biology and cancer therapy, respectively.

Immune and metabolic profiles of patients with the same pathology often vary widely. Efferocytosis can engage directly with immune and metabolic pathways and provides opportunities to fine-tune immunological behaviour via metabolic interventions. The developments in the field of pharaco-metabolomics, coupled to the highly drug targetable nodes in efferocytosis, make this combination a unique avenue for personalized medicine. The future of therapeutics for lifestyle disorders and inflammatory diseases rests on multipronged treatments, at the centre of which lies the dynamic process of efferocytosis, connecting immunological outcomes to metabolic cues and cell death.

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Tumour-associated macrophages are a distinct M2 phenotype. They express high levels of CD163 and promote an anti-inflammatory cytokine profile. These macrophages are crucial for the growth and progression of many types of cancer, including breast cancer, melanoma, and glioblastoma. They contribute to the immune evasion and survival of cancer cells by producing anti-inflammatory cytokines and chemokines, inhibiting the function and proliferation of cytotoxic T cells, and facilitating the recruitment of other immune-suppressive cells such as regulatory T cells and myeloid-derived suppressor cells (MDSCs).

Macrophages play a dual role in the immune response. On one hand, they are crucial for the clearance of dead or dying cells, which can activate an anti-tumour immune response. On the other hand, they can also promote tumour growth by producing immunosuppressive factors. The balance between these two functions is critical in determining the overall outcome of the immune response in cancer.

The role of TIM-4 in cancer progression and the advantages of using inhibitors of TIM-4 as therapeutic targets is an area of active research. TIM-4 is a receptor that is expressed on the surface of immune cells and plays a role in the regulation of cell-cell interactions, including those involving phagocytosis and antigen presentation.

Inhibitors of TIM-4 have shown promise in preclinical studies and are being evaluated in clinical trials for the treatment of various cancers, including breast cancer, melanoma, and glioblastoma. These inhibitors have the potential to modulate the immune response and improve the effectiveness of antitumour therapies.

Further studies are needed to fully understand the mechanisms by which TIM-4 regulates the immune response and to identify the most effective strategies for targeting this receptor in cancer therapy.

This review highlights the importance of TIM-4 in cancer biology and the potential therapeutic implications of targeting this receptor.
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