Context-dependent roles of complement in cancer

Lubka T. Roumenina[1,3]*, *Marie V. Daugan*[1,3], *Florent Petitprez*[1,2], *Catherine Sautès-Fridman*[1] *and Wolf Herman Fridman*[1]*

Abstract | The tumour microenvironment (TME) highly influences the growth and spread of tumours, thus impacting the patient's clinical outcome. In this context, the complement system plays a major and complex role. It may either act to kill antibody-coated tumour cells, support local chronic inflammation or hamper antitumour T cell responses favouring tumour progression. Recent studies demonstrate that these opposing effects are dependent upon the sites of complement activation, the composition of the TME and the tumour cell sensitivity to complement attack. In this Review, we present the evidence that has so far accrued showing a role for complement activation and its effects on cancer control and clinical outcome under different TME contexts. We also include a new analysis of the publicly available transcriptomic data to provide an overview of the prognostic value of complement gene expression in 30 cancer types. We argue that the interplay of complement components within each cancer type is unique, governed by the properties of the tumour cells and the TME. This concept is of critical importance for the design of efficient therapeutic strategies aimed at targeting complement components and their signalling.

The interactions of malignant cells with supporting and reactive non-transformed host cells are orchestrated by the density, location and functional activity of the latter and by soluble mediators released into the tumour microenvironment (TME)[1]. Frequently neglected elements of the TME are the components of the complement system, produced by the tumour and infiltrating cells or originating from the circulation[2]. Complement is a key player in the innate immune defence against pathogens and in the maintenance of host homeostasis. It is composed of more than 50 plasma components produced mainly by the liver and released into the circulation as well as receptors expressed on the membranes of different cell types. The individual components interact with each other in the extracellular space[3] (FIG. 1). Recent discoveries have made clear that complement effectors can also be generated intracellularly, leading to locally occurring complement activation, and that complement proteins have non-canonical functions, which are independent of the plasmatic cascade[4,5]. Cumulative evidence over the past 10 years has proved that complement proteins are present in the TME and that malignant and infiltrating cells have the capacity to produce in situ a large spectrum of these components[6].

Complement and cancer is an emerging field and most of the phenomena have been described in a single study or for a single type of cancer. Nevertheless, a solid body of evidence has accumulated to demonstrate that the functionality and level of expression of complement proteins by malignant cells or in the TME can modulate the fate of the tumour. In cancer, the impact of complement is diverse, ranging from antitumour defence to potent tumour promotion. The data in the literature, mostly focused on animal models and in vitro studies, have yielded mixed and sometimes contradictory conclusions. Analyses of human cancers are scarce and it is still unclear whether complement is overactivated or, on the contrary, inhibited in patients with cancer. In this Review, we present the evidence showing the high diversity of actions of complement components in cancer and the heterogeneity of their production and activation pathways. Using data from human cancers and mouse models, mechanisms of tumour control and tumour promotion are discussed. We also compare the expression of complement genes and their clinical impact in different cancer types, using publicly available data sets, which highlights the context-dependent effects of complement across cancer types. Finally, we argue that the most appropriate therapeutic approaches to activate or neutralize complement will be dependent on the tumour context and, therefore, will require a personalized approach.

The complement system

Complement is a central part of immunity that serves as a first line of defence against pathogens and stressed host cells[3]. The complement system is composed of

[1]*INSERM, UMR_S 1138, Centre de Recherche des Cordeliers, Sorbonne Universités, Université de Paris, Paris, France.*

[2]*Programme Cartes d'Identité des Tumeurs, Ligue Nationale Contre le Cancer, Paris, France.*

[3]*These authors contributed equally: Lubka T. Roumenina, Marie V. Daugan.*

**e-mail: lubka.roumenina@crc.jussieu.fr; herve.fridman@crc.jussieu.fr*

https://doi.org/10.1038/s41568-019-0210-0

plasma proteins that react with one another to opsonize pathogens, inducing a series of inflammatory responses that concomitantly help immune cells to fight against infections and to maintain homeostasis[2]. The initiation of the complement cascade is dependent both on the context (for example, the nature of the trigger or the type of antigen) and on the tissue location (FIG. 1a).

Conventional complement activation pathways. Historically, complement was considered to be initiated by three distinct pathways — classical, lectin and alternative. Immune complexes and apoptotic cells activate the classical pathway, after recognition of the target molecules by C1q. The lectin pathway is triggered after recognition of sugar motifs, foreign to a healthy tissue. The alternative pathway is constitutively active at low levels, serving as a sentinel to attack any surface which is not specifically protected by complement regulators.

Each of these pathways leads, through a sequence of conformational changes and enzymatic reactions, to the cleavage of the central component C3 into bioactive fragments C3a and C3b. This is achieved by enzymatic complexes — C3 convertases of the classical, lectin and alternative pathways. The downstream component C5 is cleaved by C5 convertases, thus triggering the terminal pathway, generating C5a and the terminal complement complex C5b-9, known as the membrane attack complex (MAC)[2,7]. The anaphylatoxins C3a and C5a, the opsonizing C3 activation fragments C3b, iC3b and C3d, and the MAC are the canonical effectors of the complement system. Complement is also tightly controlled by negative regulators, such as factor H (FH), FI, CD35, CD46, CD55, CD59, C4BP and C1 inhibitor (C1inh), to avoid damage to healthy cells.

C3a and C5a bind to their respective receptors, C3a receptor (C3aR) and C5aR1 or C5aR2, and play a critical role in inducing inflammation and activation of immune cells as well as endothelial cells, epithelial cells, fibroblasts and certain malignant cells, which express the anaphylatoxin receptors[3]. Anaphylatoxins induce the oxidative burst in macrophages, eosinophils and neutrophils, which supports inflammation[3]. In a physiological context, these events contribute to acute inflammation, and eradication of pathogens. Within tissues, locally produced C3a and C5a activate C3aR and C5aR1, respectively, on antigen-presenting cells and T cells, and consequently control proliferation, differentiation, expansion and viability[8,9]. In the context of cancer, complement anaphylatoxins are continually generated and in the majority of the studied mouse models lead to tumour-promoting chronic inflammation[6].

MAC assembly creates a transmembrane pore that causes prompt osmotic lysis of certain bacteria and metabolically inert targets (such as erythrocytes and liposomes). Nucleated host cells often resist lytic killing by MAC owing to a high expression of membrane regulators. Nevertheless, when formed, the C5b-9 complex can have profound effects on cell functions, leading to activation and adaptation or cell death depending on the context[10]. To avoid inadvertent healthy host tissue damage, complement is a tightly regulated cascade, constantly kept in check. However, cancer cells acquire escape mechanisms to protect against MAC activity, such as overexpression of complement regulators[6,11]. Furthermore, one study suggests that heat shock protein 90 (HSP90) protects tumour cells from complement-dependent cytotoxicity by inhibiting, together with mortalin (also known as mitochondrial stress 70 protein or GRP75), C5b-9 assembly and/or stability at the plasma membrane[12].

Considering these effects of complement on cell activation and survival as well as on the modulation of the entire immune system, it is not surprising that tumours have evolved mechanisms to adapt to its presence and to subvert it for their benefit.

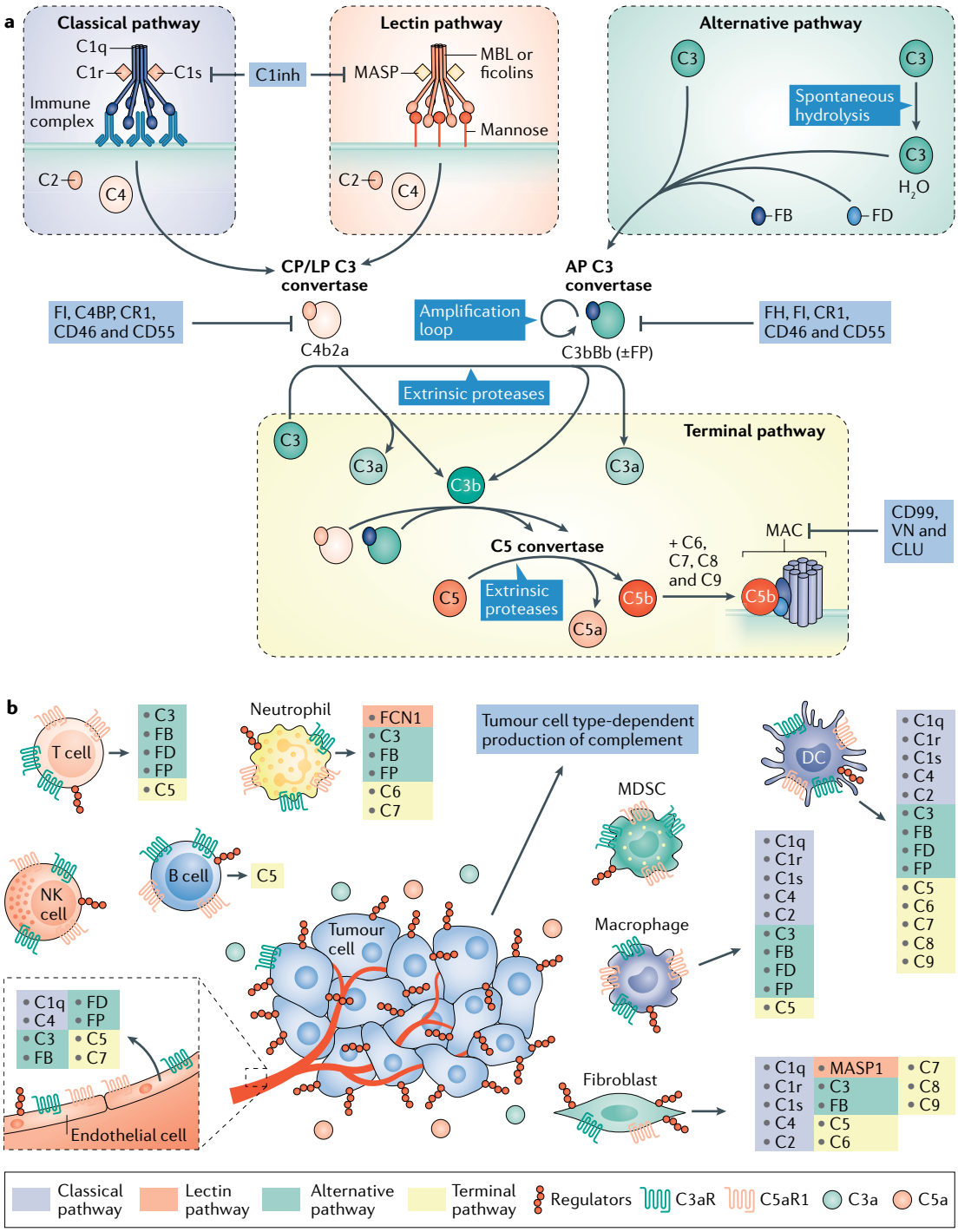
Non-canonical and intracellular complement initiation. Complement can also be activated by an unconventional, convertase-independent pathway, through other enzymes cleaving C3 and C5, such as cathepsin L, renin, thrombin and plasmin[13–17]. Although generated by non-canonical mechanisms, the C3a and C5a as well as C3b and C5b components produced are often identical in sequence to the convertase-generated anaphylatoxins. Therefore, they are canonical effectors but are generated in a non-canonical manner. This cleavage can occur in the circulation, within the tissues but also intracellularly. Interestingly, thrombin can cleave C5 at a different site from that of C5 convertases, generating an even more potent equivalent of C5b, with higher lytic activity[15]. In addition, C3a generated by mouse tumour cell lines promoted an immunosuppressive TME by acting on the tumour-associated macrophage (TAM) phenotype in such a way that could not be replicated by C3a generated exogenously[18]. The mechanism for this differential activity is still not well defined, but this study shows that local or intracellularly generated anaphylatoxins may have properties that are different from their plasmatic counterparts.

Complement activation inside cells has been described for T cells and exerts homeostatic and immunological functions[19,20]. Through their non-canonical functions, complement components modulate the fundamental processes of immune cells, including immune cell proliferation, migration, metabolism and even transcriptional activity[20–24]. The autocrine complement activation and the activity of the complement regulator CD46 are indispensable for the functioning of human $CD4^+$ and cytotoxic $CD8^+$ T cells in physiological conditions[25–27]. It should be noted that the absence of CD46 on mouse immune cells renders these phenomena human specific, emphasizing the divergent roles of innate immune sensors between mice and humans. Studies on human T cells also show unconventional intracellular C3 cleavage by cathepsin L[27]. The 'tonic' intracellular C3a that is generated is required for homeostatic T cell survival. Although it is generated intracellularly, C3a could be released locally to act in an outside-in, autocrine manner. At least in part, intracellular C3 stores derive from the internalization of the hydrolytic product of C3, $C3(H_2O)$, from the extracellular milieu[28]. It was suggested that the cleavage of C3 by cathepsin L is species specific, described only in human T cells, and does not operate in mice[27]. Nevertheless, another study described cathepsin L-mediated C3 cleavage in a mouse model of ischaemia or reperfusion[29]. These potential species differences

Anaphylatoxins
The collective name for the complement activation fragments C3a, C4a and C5a.

Oxidative burst
A rapid release of reactive oxygen species (superoxide radicals and hydrogen peroxide) from different types of cells.

Mortalin
A highly conserved heat shock protein implicated in functions ranging from the stress response to control of cell proliferation and inhibition of apoptosis.



should be taken into account if and when this mechanism is studied in animal models of cancer. In addition, in human T helper 1 (T_H1) cells, intracellular C5 activation and subsequent C5a stimulation of intracellular C5aR1 results in the assembly of the NOD-, LRR- and pyrin domain-containing 3 (NLRP3) inflammasome, needed for the optimal production of interferon-γ (IFNγ)[26]. Moreover, novel functions of intracellular C3 have emerged, such as its implication in immune cell gene transcription[23] or regulation of autophagy[30]. Soluble C1q was shown to be internalized by $CD8^+$ T cells and to modulate their metabolism in the context of autoimmunity and viral infections[22]. All of these processes have not yet been studied in the context of cancer. Nevertheless, it is tempting to speculate that the non-canonical functions of complement will shape the immune TME and play a key role in antitumour immunity.

Autophagy
A cellular stress response in which cellular proteins and organelles are digested and recycled by lysosomes in order to maintain active metabolism.

Functions of complement in cancer

Intratumoural initiation of the complement cascade. Despite strong evidence for complement activation in human tumours and mouse models (TABLES 1,2), very few studies have addressed the specific pathway by which these anaphylatoxins are generated.

◀ Fig. 1 | **The complement system in the tumour microenvironment. a** | This schematic shows the mechanisms of activation and regulation of the complement cascade. The complement system can be activated by three pathways: the classical, lectin and alternative pathways leading to the generation of C3 convertase. The classical pathway is activated after recognition by the C1 complex (composed of C1q, C1r and C1s) of immune complexes and/or apoptotic cells; the lectin pathway is initiated after fixation of the complex comprising mannose-binding lectin (MBL) or ficolins with mannose-binding lectin-associated serine proteases (MASPs) to terminal mannose residues on cell surfaces. The alternative pathway is constitutively activated at low levels by the spontaneous hydrolysis of C3 into C3(H_2O). These initiation events lead to the formation of enzymatic complexes, C3 convertases, which cleave the central component C3 into C3a and C3b. C3a is an anaphylatoxin and C3b can opsonize cells. The alternative pathway C3 convertase is composed of the activation fragments of C3 and factor B (FB) (C3bBb). The classical and lectin pathway convertase consists of the activation fragments of C2 and C4. A recent effort to harmonize the complement nomenclature postulated that it should be denoted C4b2b[141], but in the previous literature was indicated as C4b2a. C3b binds close to or on the C3 convertases, allowing formation of the C5 convertases, which cleave C5 into C5a (an anaphylatoxin) and C5bC5b that initiates the terminal pathway of the complement system, leading to the formation of the membrane attack complex (MAC). Some proteases, extrinsic to the cascade (such as plasmin), can cleave C5 in a non-canonical way, independently of convertase formation, in the circulation or within the tissues. An intracellular cleavage of C3 by cathepsin L also occurs independently of the cascade. To avoid host tissue damage, this system is tightly regulated by soluble or membranous proteins at different levels of the cascade. **b** | This schematic shows the composition of the tumour microenvironment (TME) and the complement proteins produced by different non-malignant host cell types. The tumour has a rich complement environment. All stromal and tumour cells participate in the local production of complement proteins. The immune cells, especially myeloid cells (such as macrophages, neutrophils, myeloid-derived suppressor cells (MDSCs) and dendritic cells (DCs)), can produce complement components, especially those of the classical and alternative pathways, as well as express high levels of complement receptors and multiple regulators. The endothelial cells and fibroblasts are also key players in the TME and produce complement proteins, express regulators and a lower level of complement receptors. Finally, the participation of tumour cells in the complement cascade is dependent upon the cancer type but a key feature is the high expression of complement regulators to protect against complement-dependent cytotoxicity. Of note, this is a qualitative representation. Each cell type produces different amounts of each of the complement proteins. AP, alternative pathway; C1inh, C1 inhibitor; C3aR, C3a receptor; C4BP, C4b-binding protein; C5aR1, C5a receptor 1; CLU, clusterin; CP, classical pathway; CR1, complement receptor 1; FCN1, ficolin 1; LP, lectin pathway; NK cell, natural killer cell; VN, vitronectin.

The TC-1 mouse tumour cell line used to form syngeneic tumours in mice is probably the best-characterized model for classical pathway complement activation. This cell line is derived from primary lung epithelial cells, and expresses the human papilloma virus 16 (HPV16) oncoproteins E6 and E7; it serves as a model of human tumours infected with HPV16, such as lung or cervical cancer, depending on the study. Indeed, in the seminal paper describing the pro-tumoural role of complement, the TC-1 cell line was used to demonstrate that complement activation occurred via either the C4-dependent classical or lectin pathway[31]. Recently, we established using this same model that it is in fact the classical pathway which is activated[32]. In another syngeneic lung cancer mouse model using the KRAS-mutant CMT167 lung cancer cell line, the classical pathway has also been shown to be activated, likely by intratumoural immunoglobulins[33]. Although not necessarily implicated in complement initiation, the alternative pathway can amplify the C3 activation fragment deposits, thus perpetuating intratumoural complement activation. Indeed, *C3*$^{-/-}$ mice are protected against tumour progression in nearly all of the tested mouse tumour models (TABLES 1,2). However, little evidence is available implicating the lectin pathway in tumour progression[34,35].

In cancer models, C3 and C5 can also be cleaved by complement cascade-independent proteases, thus bypassing the initial recognition events[15–17]. Early studies from the 1990s showed that (pro)cathepsin L, released by tumour cells (mouse and human cell lines), can cleave C3 (REFS[36,37]). Knock-in of cathepsin L into a mouse melanoma cell line makes it capable of cleaving exogenous C3, promoting its tumorigenic capacity and endowing it with metastatic potential. Although the cleavage pattern reported in these papers is not consistent with the recent findings for C3a generation[27], these studies suggest that the capacity of tumour cells to cleave C3 promotes tumour progression. Furthermore, in 2019, a study demonstrated acquisition of a tumour-promoting phenotype upon knock-in of C3 into various tumour cell lines with consequent intracellular activation of C3, potentially by cathepsin L[18].

C5 is cleaved by plasmin, activated by urokinase plasminogen activator (uPA)-expressing macrophages in mouse models of squamous carcinogenesis, leading to C3-independent release of C5a[16]. Using C5-producing tumour cell lines, it was shown that C5a can be generated by a, hitherto unidentified, cell membrane-bound serine protease[17]. Thrombin is produced in tumours and has potent pro-tumoural activity[38]. Therefore, it is tempting to speculate that the cleavage of C5 will be yet another mechanism contributing to its pro-tumoural activity in situ.

Certain tumour cells contain intracellular pools of C3 and C5 (REFS[32,39–41]). Although poorly studied currently, we postulate that the intracellular cleavage of C3 and C5 recently found to occur in T cells[26,27] will not be restricted to this immune cell population and it can be expected that the intracellular generation of C3a and C5a will have a major role in the function of tumour cells, as well as non-immune constituents of the TME, such as endothelial cells and fibroblasts.

Complement effectors and the immune contexture of the tumour. The immune contexture of tumours, which is the immune profile determined by the density, composition, functional state and organization of the leukocyte infiltrate, is a key determinant of tumour progression[1]. Complement receptors are expressed on the surface of immune cells. C3a and C5a, generated locally within the tumour, promote leukocyte attraction and impact their phenotype (FIG. 2). Following the discovery that C5a recruits myeloid-derived suppressor cells (MDSCs) to the TME, which in turn suppress effector T cells[31], it was found that C3a and/or C5a exerts a profound influence on the TME by inducing a series of context-dependent changes, including: the recruitment of tumour-promoting macrophages and CC-chemokine ligand 2 (CCL2) production (in a PTX3 and FH-dependent manner)[42]; a decrease in recruitment of $CD4^+$ T cells and neutrophils[43]; a decrease in recruitment of natural killer (NK) cells[44]; stimulation of a pro-tumoural phenotype for $CD4^+$ T cells[33]; inhibition of interleukin 10 (IL-10) expression by intratumoural $CD8^+$ T cells[45]; stimulation of the pro-tumorigenic properties of mast cells and macrophages, including suppression of $CD8^+$ T cell

Table 1 | **Pro-tumoural role of complement in mouse models of cancer**

Cancer mouse model	Complement component studied	Effects on tumour growth	Mechanism	Ref.
Syngeneic (using TC-1 cells)	C4, C3, C5aR1, factor B	*C4*$^{-/-}$, *C3*$^{-/-}$ and *C5ar1*$^{-/-}$ mice, but not *Cfb*$^{-/-}$ mice, have impaired tumour growth; pharmacological inhibition of C5aR1 impaired tumour growth	Modulation of migration and production of ROS and RNS by MDSCs	31
Syngeneic (using TC-1 cells)	C1q, C3, C4	*C1q*$^{-/-}$, *C4*$^{-/-}$ and *C3*$^{-/-}$ mice have impaired tumour growth	Classical complement pathway activation induced pro-tumoural effects on angiogenesis and the immune environment	32
Lung syngeneic (LLC)	C1q, C5	*C1q*$^{-/-}$ but not *C5*$^{-/-}$ mice have impaired tumour growth	C1q induced angiogenesis independently of complement activation	58
Lung syngeneic and orthotopic (CMT167-luc, EML4-ALK, LLC-luc)	C3, C3aR1, C5aR1	*C3*$^{-/-}$ mice have impaired tumour growth and a decreased number of metastases in other lobes of the lung; pharmacological blockade of C3aR or C5aR1 results in inhibition of tumour growth	C3 signalling inhibits the production of multiple cytokines from CD4^{+} T cells	33
Lung syngeneic in *Kras*$^{LSL-G12D/+}$ mice	C5aR1	Combination of C5a and C5aR1 inhibition and PD1 blockade results in decreased tumour growth and metastasis	Increased frequency of CD8^{+} T cells and decreased frequency of MDSCs within tumours	132
Experimental lung metastasis and lung xenograft (A549M1, H460M5)	C5aR1	Decreased tumour burden when mice are intravenously or intracardially injected with A549M1 cells silenced for *C5ar1*; decreased number of bone metastases	*C5ar1*-silenced tumour cells have decreased motility and a lower metalloproteolytic activity	70
Lung syngeneic (3LL)	C5aR1	Pharmacological blockade of C5aR1 results in inhibition of tumour growth	Decreased expression of bFGF and decreased numbers of MDSCs in tumours	55
Lung xenograft (A549)	Factor H	Tumour cells silenced for *Cfh* have slower tumour growth	Factor H deficiency sensitizes tumour cells to complement-mediated attack	82
Ovarian syngeneic (ID8-VEGF)	C3	Mice injected with C3 shRNA into the peritoneum have slower tumour growth	C3 enhances EMT	39
Ovarian syngeneic (ID8-VEGF) and xenograft (SKOV3ip1)	C3, C5aR1	*C5ar1*$^{-/-}$ mice have impaired tumour growth	Autocrine effect of C3a and C5a on their receptors on cancer cells and induction of proliferation through the PI3K–AKT pathway	40
Ovarian xenograft (RMA-3CF4 cells) and lymphoma syngeneic (RMA-1474)	C5aR1	Correlation between tumour burden, expression of C5a and C5aR1 signalling	Correlation between C5a and decreased number of effector T cells	47
Ovarian transgenic (TgMISIIR-TAg)	C3, C5aR1	C3 or C5aR1 deficiency attenuates tumorigenesis; pharmacological inhibition of C5aR1 impaired tumour growth	C3 induced production of pro-inflammatory cytokines; C5a induced the production of pro-angiogenic factors	56
Breast syngeneic metastatic (4T1)	C5aR1	*C5ar1*$^{-/-}$ mice or pharmacological inhibition of C5aR1 decreased metastasis	C5aR1 facilitates metastasis by suppressing effector T cells through recruitment of immature dendritic cells that produce TGFβ and IL-10	9
Breast syngeneic metastatic (4T1)	C5aR1	C5aR1 deficiency or C5aR1 blockade decreased lung metastasis	Reduced recruitment of MDSCs	8
Colon syngeneic (MC38)	C3	C3-depleted mice (using CVF) have impaired tumour growth	Increased expression of CCL5, CXCL10 and CXCL11, and migration of CD8^{+} T cells	24
Intestine transgenic (*Apc*$^{Min/+}$)	C3aR	*C3ar*$^{-/-}$ mice have impaired tumour growth	C3a induced polarization of neutrophils via C3aR towards a pro-tumorigenic phenotype	46
Colon syngeneic metastatic (SL4, CT26) and colon xenograft (HCT116, SW116)	C5aR1	*C5ar1*$^{-/-}$ mice have decreased numbers of metastases in the liver	Infiltration of inflammatory cells and production of CCL2 by macrophages via the AKT pathway	135
Experimental colon metastasis (SL4-luc)	C5aR1	*C5ar1*$^{-/-}$ mice have a decreased number of hepatic metastases	C5a–C5aR1 pathway regulates the M2 phenotype of TAMs in metastases through NF-κB signalling	145

Table 1 (cont.) | **Pro-tumoural role of complement in mouse models of cancer**

Cancer mouse model	Complement component studied	Effects on tumour growth	Mechanism	Ref.
Colitis-induced colorectal cancer (azoxymethane plus dextran sulfate sodium)	C3, C5, C5aR1	*C3*$^{-/-}$, *C5*$^{-/-}$ or *C5ar1*$^{-/-}$ mice have impaired tumour growth	C5-derived C5a promotes production of IL-1β from neutrophils via C5aR1	78
Bile duct xenograft (HuCCT1)	C5aR1	C5a-treated HuCCT1 cells overexpressing C5aR1 spread more broadly than C5a-treated HuCCT1 control cells in nude mice skin tissue	C5a enhances invasion of C5aR1-expressing cancer cells	80
Sarcoma induced by a carcinogen (3-MCA)	PTX3, factor H, C5a	*Ptx3*$^{-/-}$ mice have an increased susceptibility to carcinogenesis	Local complement activation under *Ptx3* silencing promotes M2-like macrophage infiltration; factor H is recruited by PTX3 and controls C5a levels	42
Sarcoma induced by a carcinogen (3-MCA)	C3, C3aR1	*C3*$^{-/-}$ and *C3ar1*$^{-/-}$ mice have reduced tumour growth	Deficiency of the C3–C3aR axis induces protection by reduction of macrophage recruitment and skewing of their phenotype towards M1 as well as induction of antitumour CD8^{+} T cell immune responses	146 (conference abstract)
Melanoma syngeneic (B16F10)	C1q	*C1q*$^{-/-}$ (but not *C5*$^{-/-}$) mice have impaired tumour growth and a decreased number of metastases	C1q induced angiogenesis and tumour cell adhesion and proliferation independently of complement activation	58
Melanoma syngeneic (B16F10 cells transfected with gp33)	C3	C3-depleted mice (using CVF) have impaired tumour growth	NK cell-mediated cytotoxic T cell-dependent antitumoural immune responses	44
Melanoma and colon syngeneic (B16F10, SM1WT1) and colon syngeneic (MC38)	C3aR	*C3ar*$^{-/-}$ mice have impaired tumour growth	*C3ar*$^{-/-}$ mice have increased infiltration of neutrophils and CD4^{+} T cells, and decreased numbers of macrophages	43
Melanoma and breast syngeneic (B16F10) and breast syngeneic (E0771)	C3, C3aR, C5aR1	*C3*$^{-/-}$ mice have impaired tumour growth; pharmacological blockade of C3aR or C5aR1 suppresses tumour growth	C3 inhibits IL-10 secretion by effector CD8^{+} T cells; enhanced antitumour response with combination of C3aR or C5aR1 antagonism and anti-PDL1	45
Experimental breast cancer (MDA231-LeptoM, HCC1954-LeptoM) and lung cancer (PC9-LeptoM LLC-LeptoM) leptomeningeal metastasis	C3, C3aR	MDA231-LeptoM cells silenced for *C3* injected into mice have slower tumour growth; *C3ar*$^{-/-}$ mice injected with LLC-LeptoM cells in the leptomeningeal space have impaired tumour growth; pharmacological inhibition of C3aR decreased metastasis	Leptomeningeal metastatic cells produce C3 that disrupts the choroidal blood–CSF barrier to adapt the CSF for cancer cell growth	73
Gastric syngeneic (MFC)	C5aR1	Pharmacological blockade of C5aR1 inhibits tumour growth	C5aR1 blockade inhibits phosphorylation of PI3K–AKT and increases levels of p21 and phosphorylated p21	81
Leukaemia xenograft (U937)	C3a, C5a	Tumour cells treated with C3a or C5a before subcutaneous inoculation have an increased capacity to spread to organs	C3a and C5a activates p38 MAPK, which downregulates HO1 expression and enhances motility	79
Pancreatic neuroendocrine transgenic (BT2 B6)	C5aR1	Pharmacological blockade of C5aR1 inhibits tumour growth	C5a–C5aR1 pathway increases invasiveness and macrophage recruitment	85
Cutaneous squamous cell xenograft (UT-SCC-7)	Factor I	Tumour cells silenced for *CFI* have slower tumour growth	Factor I promotes proliferation and migration	76
Squamous cell transgenic (K14-HPV16)	C5aR1	*C5ar1*$^{-/-}$ mice have impaired tumour growth; synergic effect with the combination of C5aR1 blockade and chemotherapy	C5aR1-deficient tumours have impaired angiogenesis and a decreased number of mast cells and macrophages; C5 is cleaved by plasmin	16
Squamous cell xenograft (cSCCIS)	Factor B, C3	Silencing of *CFB* or C3 in tumour cells inhibited tumour growth	Silencing of *CFB* or C3 in tumour cells decreased their migration and proliferation and resulted in inhibition of ERK1 and/or ERK2 signalling	147

bFGF, basic fibroblast growth factor; C3aR, C3a receptor; C5aR1, C5a receptor 1; CCL, CC-chemokine ligand; CF, comlement factor; CSF, cerebrospinal fluid; CVF, cobra venom factor; CXCL, CXC-chemokine ligand; EMT, epithelial-to-mesenchymal transition; HO1, haem oxygenase 1; HPV16, human papillomavirus 16; IL, interleukin; MDSC, myeloid-derived suppressor cell; NF-κB, nuclear factor-κB; NK cell, natural killer cell; PD1, programmed cell death 1; PDL1, programmed cell death 1 ligand 1; PTX3, pentraxin-related protein 3; RNS, reactive nitrogen species; ROS, reactive oxygen species; shRNA, short hairpin RNA; TAM, tumour-associated macrophage; TGFβ, transforming growth factor β; VEGF, vascular endothelial growth factor.

NETosis
A process of release of neutrophil extracellular traps (NETs) from overactivated neutrophils. NETs are defensive networks of extracellular fibres, primarily composed of DNA and histones.

cytotoxicity[16]; and promotion of pro-tumoural neutrophil extracellular trap (NET) formation (known as NETosis)[46].

The level of intratumoural C5a may be a key determinant of the composition of the immune TME. A mouse lymphoma cell line engineered to produce low levels of C5a grew more slowly in mice, resulting in increased IFNγ-producing T cells in the spleen and tumour-draining lymph nodes[47]. Conversely, mice engrafted with a high C5a-producing tumour cell line had accelerated tumour progression with more Gr-1$^+$CD11b$^+$ myeloid cells in the spleen and overall decreased numbers of T cells in the tumour, tumour-draining lymph nodes and spleen in this experimental model.

The C3 activation fragments are potent effectors, modulating the immune response[3]. In patients with ovarian cancer, mature neutrophils acquire a suppressive phenotype that is linked to complement C3 activation[48]. These immunosuppressive neutrophils completely suppressed the proliferation of naive, central memory and effector memory T cells, hampering the antitumour immune response. This process requires T cell contact and interaction between iC3b and complement receptor 3 (CR3) on neutrophils. Chemotherapeutic as well as immunotherapeutic approaches induce apoptosis in tumour cells and may induce immunogenicity[49,50]. Opsonization of apoptotic tumour cells with iC3b prevents the maturation of dendritic cells via interaction with CR3 and contributes to the induction of antigen-specific silencing and tolerance[51]. Moreover, the iC3b–CR3 interaction results in dysregulation of NK cell-dependent tumour surveillance[52].

Although the majority of the experimental models agree on the pro-tumoural role of C3a and especially C5a, the mechanisms described above are context specific. Rarely is the same mode of action of the anaphylatoxins found across different cancer models. This could reflect differences in the composition of the immune microenvironment between cancer types. Indeed, in mice, as in humans, the immune infiltration is largely controlled by the properties of the tumour cells themselves[1,53].

Neoangiogenesis and complement. Neovascularization is critical for the supply of oxygen and nutrients to the tumour. Complement contributes to this process via its canonical effectors C3a and C5a as well as by non-canonical, cascade-independent effects of the individual components (FIG. 3a).

For around 10 years, it has been known that C5a promotes migration and tube formation of endothelial cells in vitro[54,55]. In addition, *C3*$^{-/-}$ and *C5ar*$^{-/-}$ endothelial cells have impaired angiogenesis capacity[56]. Nevertheless, the impact of C3a and C5a in mouse tumours seems to be model dependent. *C3*$^{-/-}$, *C3ar*$^{-/-}$ or *C5ar*$^{-/-}$ showed either impaired tumour angiogenesis[16,56], increased blood vessel permeability without an effect on microvascular density[57] or no effect[55]. Therefore, the context-dependent impact of the anaphylatoxins on neoangiogenesis requires further investigation.

Recent evidence points towards a major role of C1q in cancer neoangiogenesis via a non-canonical, cascade-independent mechanism. A fraction of tumour vessel endothelial cells produce C1q in tumour mouse models and in human tumours[32,58]. The microvascular density was either decreased or the vascular network was disorganized in tumours growing in *C1q*$^{-/-}$ mice[32,58]. This could be explained by alteration of C1q-mediated expression of vascular endothelial growth factors (VEGFs) and VEGF receptors (VEGFRs), as shown in tumour mouse models[32] and in studies of pregnancy complications of mice[59]. The pro-angiogenic effect of C1q is again context dependent, as neoangiogenesis was enhanced in the human epidermal growth factor receptor 2 (HER2;

Table 2 | **Antitumoural role of complement in mouse models of cancer**

Cancer mouse model	Complement component studied	Effects on tumour growth	Mechanism	Ref.
Breast xenograft (MCF7)	Factor P	Factor P-overexpressing tumour cells have impaired tumour growth	Factor P induces ER stress, TES transcription and increased expression of DDIT3	84
Breast transgenic (*neuT*$^+$-*C3*$^{-/-}$)	C3	*neuT*$^+$-*C3*$^{-/-}$ mice have accelerated tumour growth and an increased number of lung metastases	Tumours in *neuT*-*C3*$^{-/-}$ mice had an increased number of T_{reg} cells and a modified vascular architecture	57
Breast transgenic (*neuT*$^+$-*C1q*$^{-/-}$)	C1q	*neuT*$^+$-*C1q*$^{-/-}$ mice have accelerated tumour growth and an increased number of lung metastases	A higher number of intratumour blood vessels and a decrease in the activation of the tumour suppressor WWOX within tumour cells; no difference in the numbers of tumour infiltrating immune cells or local complement activation	60
Melanoma syngeneic (B16F10-OVA)	C3, C3aR, C5aR1	Tumours in *C3*$^{-/-}$ mice grow slower but are resistant to radiotherapy; C3aR and C5aR1 inhibition results in resistance to radiotherapy	Local production of C3a and C5a was necessary for the tumour response to radiotherapy and for the stimulation of tumour-specific immunity	136
Colon syngeneic (CT26)	C3aR, C5aR1	*C3ar*$^{-/-}$ and *C5ar1*$^{-/-}$ results in resistance to radiotherapy	Local production of C3a and C5a was necessary for the tumour response to radiotherapy and for the stimulation of tumour-specific immunity	136
Syngeneic (using TC-1 cells) in combination with adoptive T cell therapy	C3, C5aR1	*C3*$^{-/-}$ or *C5ar1*$^{-/-}$, and pharmacological blockade of C5aR1 impaired the ability of T cells to overcome the endothelial barrier, infiltrate tumours and control tumour progression	C3 and C5aR1 expression by tumour stroma, and not leukocytes, governs T cell homing, acting on the local endothelium	137

C3aR, C3a receptor; C5aR1, C5a receptor 1; DDIT3, DNA damage-inducible transcript 3; ER, endoplasmic reticulum; TES, testin; T_{reg} cell, regulatory T cell; WWOX, WW domain-containing oxidoreductase.

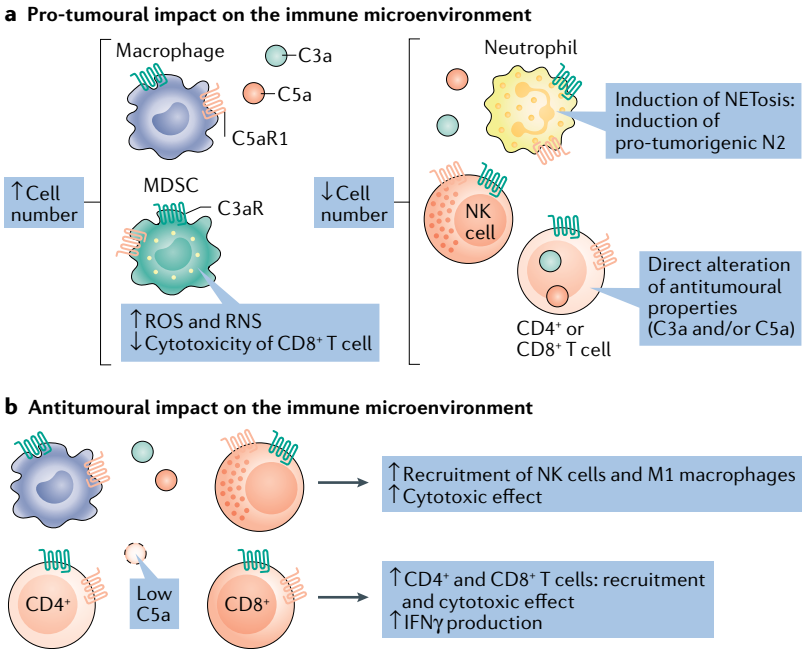


Fig. 2 | **The pro-tumoural and antitumoural impact of complement on the immune contexture.** C3a and C5a can modulate the immune microenvironment towards a pro-tumour or antitumour response depending on the tumour type and local concentrations of the anaphylatoxins. **a** | This schematic shows the pro-tumoural actions of complement effectors on immune cells. In the majority of cancer types, the anaphylatoxins can change the inflammatory milieu by increasing the recruitment of immunosuppressive cells and decreasing the number of pro-inflammatory cells through activation of their receptors. C5a induces the recruitment of myeloid-derived suppressor cells (MDSCs) and promotes their immunosuppressive functions by increasing the amount of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that in turn contribute to the suppression of antitumour T cell responses. C3a and C5a can induce CC-chemokine ligand 2 (CCL2) production, which enhances recruitment of tumour-promoting macrophages (M2-like). The C3a–C3a receptor (C3aR) axis is also involved in the recruitment of neutrophils, induction of a polymorphonuclear suppressor phenotype (known as N2) and neutrophil extracellular trap (NET) formation that promotes tumorigenesis. Indeed, all of these functions are impaired in the absence of C3aR. Complement activation blockade by cobra venom factor (CVF) resulted in an increase in the infiltration of natural killer (NK) cells in mouse models of cancer[44]. Overall, modulation of the myeloid compartment towards an immunosuppressive phenotype can lead to an ineffective T cell response. The C3a–C3aR axis is also involved in decreasing cytokine production by $CD4^+$ T cells. Moreover, this axis inhibits IL-10 production by $CD8^+$ T cells resulting in an ineffective T cell response. In addition, C3a can be generated intracellularly by cathepsin L cleavage and can interact with its receptor present at the surface of lysosomes to modulate T cell activity. **b** | This schematic shows the antitumoural actions of complement effectors on immune cells. In contrast to the pro-tumoural roles described above, in some cancer types the anaphylatoxins can play a protective role against tumour progression by promoting the recruitment of pro-inflammatory cells. In the case of an ovarian cancer mouse model, a low level of C5a is associated with recruitment of antitumoural macrophages (M1-like) and NK cells that display cytotoxic effects in contrast to high C5a levels. C5aR1, C5a receptor 1; IFNγ, interferon-γ.

also known as neu or ERBB2) transgenic *neuT*$^+$-*C1q*$^{-/-}$ breast cancer mouse model[60].

Although mouse models provide insights into the role of the complement cascade and its individual components in tumour neoangiogenesis, most of the data are generated with subcutaneously implanted tumour cell lines. Endothelial cells from different organs have unique properties, including a different spectrum of expressed complement proteins[61,62], which may differentially impact upon neoangiogenesis in each cancer type. Therefore, further studies are needed to determine the relative impact of complement on neoangiogenesis of human tumours.

Direct impact of complement effectors on tumour cells. In addition to promoting inflammation, C3a and C5a could directly affect fundamental processes of tumour cells, such as survival, proliferation, migration and stemness (FIG. 3b,c). Anaphylatoxin receptors are expressed on certain cancer cells[6]. Multiple reports show that these tumour cells also express C3 and/or C5 and generate C3a and C5a, acting in an autocrine manner. The impact of this signalling on tumour cells ranges from stimulation of proliferation[40,63] to maintenance of a multipotent state of glioblastoma stem-like cells[64], induction of the epithelial-to-mesenchymal transition (EMT)[39,65], changes in invasiveness and morphology[66], and promoting stemness[67]. Specifically, C3a enhances tumour cell proliferation, migration and stemness in mouse cutaneous squamous cell carcinoma and this activity was correlated with activation of the WNT–β-catenin pathway[67]. If the cascade proceeds to terminal MAC formation, the sublytic levels of C5b-9 mediate signalling, promoting cancer cell cycle progression[68].

Complement also promotes metastasis, as reviewed by Ajona et al.[69]. Briefly, in tumour cells, C5a triggers expression of matrix metalloproteinases (MMPs), increases tumour cell migration and invasiveness, enhances the release of pro-angiogenic factors and induces EMT. Anaphylatoxins also facilitate tumour dissemination by stimulating a hypercoagulable state (an increased predisposition to form blood clots) and NETs, and adapt specific organ environments to metastatic spread[69]. In addition, C5a induces CXC-chemokine ligand 16 (CXCL16)-mediated osteoclastogenesis and the generation of an immunosuppressive microenvironment in a mouse model of lung cancer bone metastasis[70]. Pharmacological blockade or genetic deficiency of C5aR1 was sufficient to reduce lung metastases in a breast cancer mouse model[71]. Specifically, C5aR1 signalling promoted regulatory T (T_{reg}) cell generation and suppressed T cell responses in the lungs in this context. In addition, C5aR1 expression in patients with gastric cancer is associated with cancer progression, liver metastasis and poor prognosis[72]. Cancer cell-derived C3a also promotes leptomeningeal metastasis by activation of C3aR on the choroid plexus epithelium, thus disrupting the blood–cerebrospinal fluid barrier in vivo[73].

In theory, the complement cascade may lead to tumour cell killing within primary tumours or metastases, if sufficiently strongly activated by host antitumoural immunoglobulin M (IgM) or IgG, or by therapeutic antibodies, and if abundant MACs are inserted into the cell membrane. Yet, in the context of cancer, current evidence suggests that complement can only proceed to cell-killing MAC assembly following treatment with targeted therapeutics (such as monoclonal antibodies that target tumour cells[74]). A large body of evidence has demonstrated that this escape from complement killing is in part linked to a high expression of complement regulators at the tumour cell surface[6,11,75–82].

Many complement components, such as C1q, C1s, C3, FP (also known as properdin), FH and FI, have

Leptomeningeal metastasis
A complication occurring when tumour cells spread to the leptomeninges, membranes lining the brain and spinal cord, enclosing the cerebrospinal fluid.

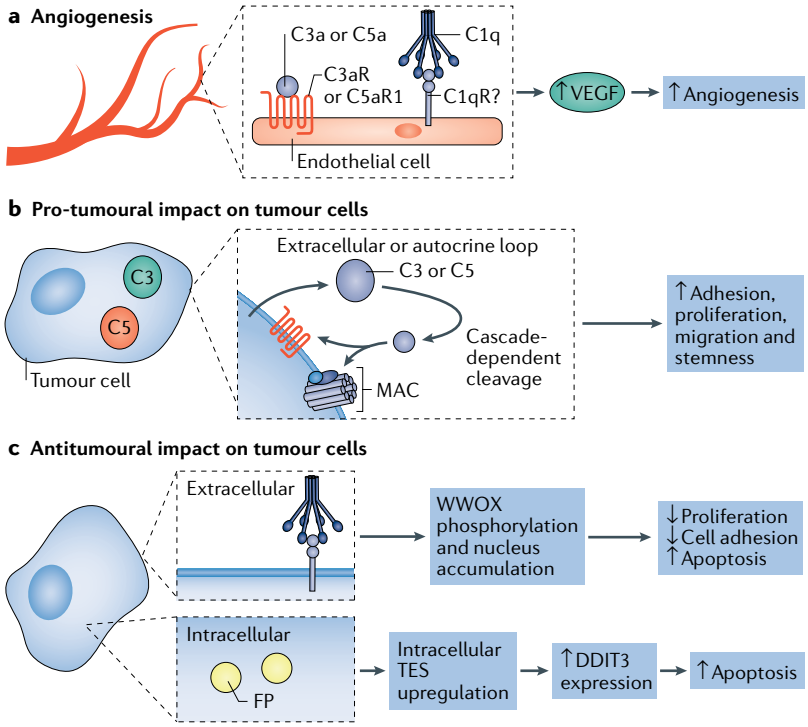


Fig. 3 | **The pro-tumoural and antitumoural impact of complement on neoangiogenesis and the biology of tumour cells. a** | This schematic shows the effects of the complement system on angiogenesis. C3a and C5a can interact with their receptors to promote angiogenesis though an upregulation of growth factor expression, such as basic fibroblast growth factor (bFGF) and vascular epithelial growth factor (VEGF), and enhancement of endothelial cell proliferation. C1q can also modulate angiogenesis in a non-canonical way through the expression of VEGFs and VEGF receptors (VEGFRs) via an unidentified C1q receptor (C1qR). **b** | This schematic shows the pro-tumoural effects of the complement system on key characteristics of tumour cells. Cancer cells can produce C3 and C5 that can be cleaved extracellularly after complement activation or potentially intracellularly through proteases. This cleavage leads to the generation of C3a and C5a that can act in an autocrine manner on tumour cells. Extracellular C3a and C5a that are generated in a canonical manner can act on their receptors at the surface of tumour cells and induce different signalling pathways such as PI3K–AKT, WNT and β-catenin, which promote tumour cell adhesion, proliferation, migration and stemness. Complement activation can also lead to sublytic membrane attack complex (MAC) formation at the surface of tumour cells. MAC formation can activate signalling pathways like ERK1 and/or ERK2, PI3K–AKT and p70 S6 kinase, which promote tumour growth. **c** | This schematic shows the antitumoural effects of the complement system on key characteristics of tumour cells. Intracellular factor P (FP; also known as properdin) induces endoplasmic reticulum stress and expression of the tumour suppressor testin (TES, a LIM domain protein), which upregulates the expression of pro-apoptotic transcription factor DNA damage-inducible transcript 3 (DDIT3) and suppresses tumour growth independently of complement activation in breast cancer cells. Extracellular C1q activates the tumour suppressor WW domain-containing oxidoreductase (WWOX), in a non-canonical manner, to induce apoptosis of prostate and breast cancer cells. C3aR, C3a receptor; C5aR1, C5a receptor 1.

non-canonical, extracellular and intracellular functions, modulating the fundamental processes of tumour cells and promoting proliferation and tumour progression in selected tumour mouse models[58,76,77,83] (TABLES 1,2). In embryonic development and in cancer models, intracellular C3 impacts EMT[39]. FP, which is the only positive regulator of the cascade, has non-canonical functions, which oppose the majority of the other tested complement proteins. For example, FP suppresses breast cancer cell growth in vitro by control of transcription[84]. Other in vitro studies show that C1q can also exert antitumoural effects by induction of apoptosis in an ovarian cancer cell line by stimulation of the tumour necrosis factor (TNF) pathway[85] or by activation of the tumour suppressor WW domain-containing oxidoreductase (WWOX) to induce apoptosis in prostate and breast cancer cell lines[60,86]. WWOX is only weakly expressed in the majority of tumour types, suggesting that this effect of C1q is context dependent, and is particularly relevant in cancers arising in hormone-regulated tissues (such as the breast, ovary and prostate). Indeed, *C1QA*, *C1QB* and *C1QC* gene expression is associated with better prognosis in basal-like breast cancer with C1q protein expression detected by immunohistochemical staining in macrophage-like cells in the stroma[87].

Complement in human tumours

The potential role of complement in the interplay between malignant cells and the TME in human cancer has begun to be uncovered. Although individual studies are quite fragmented, the accumulated literature shows that tumours develop in a complement-rich milieu. Many of the cells present in the TME produce complement components and/or bear complement receptors and complement regulators[88], suggesting a potential in situ activation of the complement pathways (FIG. 1b). In a physiological context, the complement components produced by the immune cells regulate the fundamental processes of the cells and help to fight infection. Nevertheless, in the context of the tumour, the malignant cells may produce various complement components, resulting in a disturbed complement milieu, impacting local complement activation. From the analysis of the literature, two striking observations can be made. The first observation is the presence of complement receptors, particularly C3aR and C5aR1, on most of the cell types in the TME, suggesting that the activity and functions of T and B lymphocytes, neutrophils, macrophages, MDSCs, dendritic cells, endothelial cells and fibroblasts can be modulated by the activation fragments of C3 and C5. The second observation is the presence of complement regulators at high levels, capable of inhibiting complement activation, particularly the terminal pathway, on malignant cells. This is an underappreciated mechanism of escape for tumour cells from the attack of complement, which could contribute to tumour immune escape mechanisms in general as well as to guide novel cancer immunotherapy development[89,90]. This may also explain why intratumoural C5b-9 staining has not been frequently reported[91]. Unfortunately, only a very limited number of studies address the expression and activation of complement in large cohorts of patients with different cancers. This problem can now be resolved, at least in part, owing to the data-mining and bioinformatics analyses of publicly available databases such as The Cancer Genome Atlas (TCGA) or the Pathology Atlas of the human cancer transcriptome (Human Protein Atlas)[92,93].

Expression of the complement genes in human cancers. To draw an overall picture of the impact of complement in different cancers, we compared the gene expression levels and the prognostic impact of the main complement components in 30 tumour types, using publicly available data sets[92,93]. FIGURE 4 shows an unsupervised

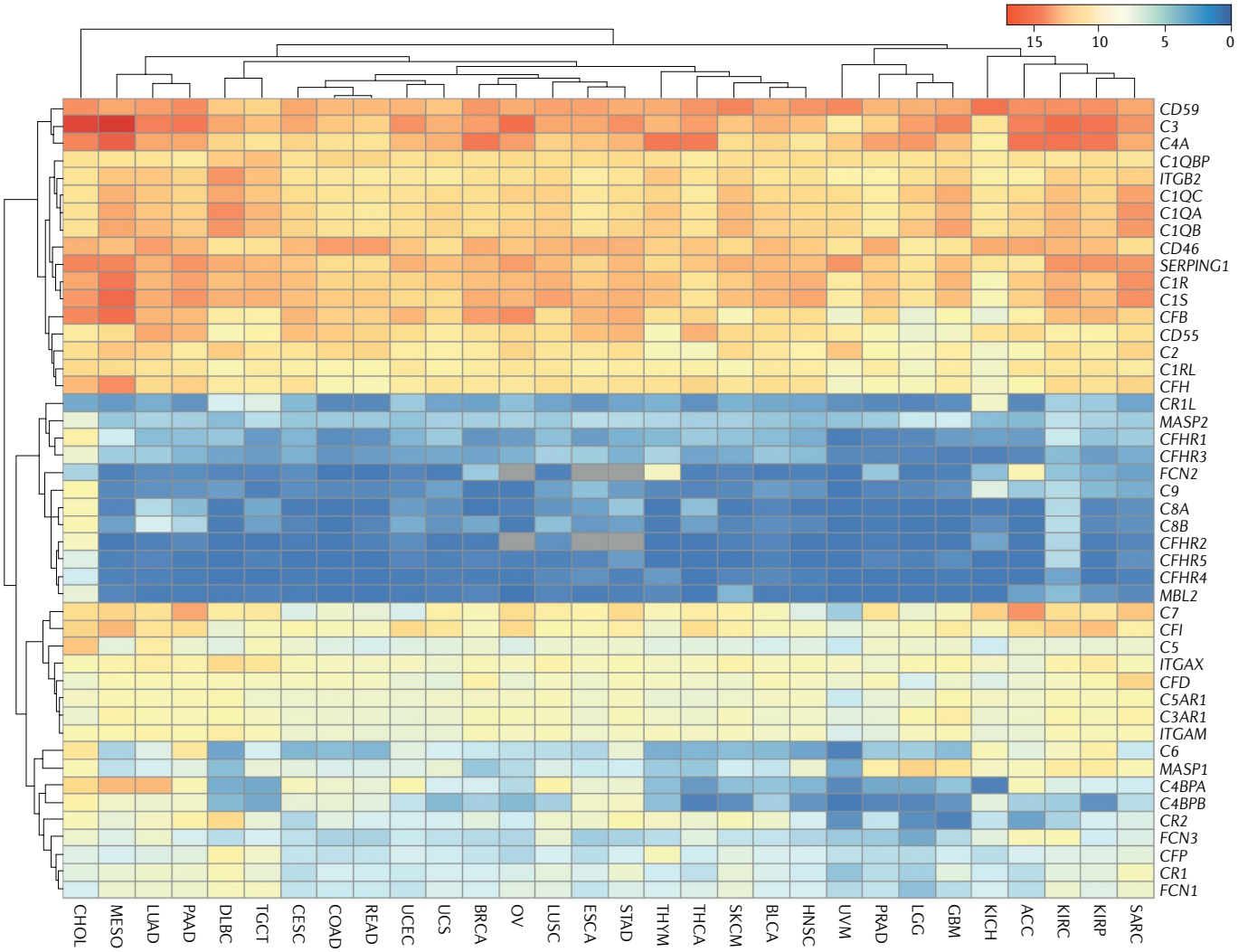


Fig. 4 | **Expression of complement genes in human cancers.** The Cancer Genome Atlas (TCGA) PanCanAtlas[142] data used in this analysis of the expression of complement genes in human cancers were downloaded through cBioPortal[143] and come from TCGA Data Coordinating Center (DCC). This heatmap shows the expression of complement genes in different cancer types. RNA sequencing (RNA-Seq) expression data, RNASeqV2, from TCGA was processed and normalized using the RSEM (RNA-Seq by Expectation Maximization) algorithm to generate transcripts per million (TPM). This method uses raw counts to quantify the abundance of transcripts and provided estimated counts for this analysis. Specifically, the data_RNA_Seq_v2_expression_median file in cBioPortal corresponds to the rsem.genes.normalized_ results file from TCGA. Thirty solid tumour types were used in this analysis. In order to avoid bias, liver hepatocellular carcinoma (LIHC) was excluded from the study because of the capacity of the liver to express very high levels of complement genes. The mean TPM of each complement gene for the patients of different TCGA cohorts was calculated and then converted into $\log_2(1 + \text{TPM})$ values. Using the R package 'pheatmap' and the clustering method 'complete', the heatmap was generated to enable visualization of the mean TPM of each complement gene for the different tumour types. Blue colours correspond to low expression, and red and orange colours to high expression. Overall, strong heterogeneity in expression among genes was revealed but not as much between cancer types. The genes of the classical pathway, *C3* and complement factor B (*CFB*), as well as genes encoding the complement regulators, were strongly expressed in the majority of the cancer types. On the contrary, the expression of the lectin and terminal pathway genes showed very low expression. ACC, adrenocortical carcinoma; BLCA, bladder carcinoma; BRCA, invasive breast carcinoma; *C1QBP*, C1q subcomponent-binding protein; *C1RL*, C1r subcomponent-like protein; *C3AR1*, C3a receptor 1; *C4BP*, C4b-binding protein; *C5AR1*, C5a receptor 1; CESC, cervical squamous carcinoma; *CFHR*, complement factor H-related protein; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; CR, complement receptor; DLBC, diffuse large B cell lymphoma; ESCA, oesophageal carcinoma; *FCN*, ficolin; GBM, glioblastoma; HNSC, head and neck squamous cell carcinoma; *ITG*, integrin; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LGG, lower grade glioma; LUAD, lung adenocarcinoma; LUSC, lung squamous carcinoma; MASP, mannose-binding lectin-associated serine protease; *MBL2*, mannose-binding lectin 2; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumours; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

hierarchical clustering of the expression of 50 complement-related genes in solid tumours, encompassing 30 cancer types. Several conclusions can be drawn from this analysis. Overall, there is strong heterogeneity in expression among genes but, surprisingly, not much heterogeneity between cancer types. The gene encoding C3, the pivotal complement component, is strongly expressed in all cancer types together with genes of the components of the classical pathway (*C1QA*, *C1QB*, *C1QC*, *C1R*, *C1S*, *C4A* and *C2*). In contrast, genes encoding components of the lectin pathway are either poorly expressed in most tumour types (mannose-binding lectin 2 (*MBL2*), mannose-binding lectin-associated serine protease 2 (*MASP2*) and ficolin 2 (*FCN2*)) or heterogeneously expressed with an overall low expression in the majority of the cancers (*MASP1*, *FCN1* and *FCN3*), arguing against a major contribution of this pathway to in situ activation of the complement cascade. As regards the alternative pathway, complement factor B (*CFB*) and *CFD* are heterogeneously expressed with a particular low expression in kidney chromophobe (KICH; also known as chromophobe renal cell cancer), uveal melanoma (UVM) and prostate adenocarcinoma (PRAD). All other tumour types exhibit higher expression levels of *CFB* and *CFD*. This, together with the high local expression of *C3*, suggests that complement could be activated via the classical or the alternative pathway.

A striking feature is the very low expression of *C8A*, *C8B* and *C9* genes in all of the tumour types (with the exception of cholangiocarcinoma (CHOL; also known as bile duct cancer)), which suggests that the terminal pathway is unlikely to be activated via components produced in situ. Moreover, the genes encoding complement regulators acting at the level of C1 (*SERPING1*, which encodes C1inh) and at the level of the C3 convertases (*CFH*, *CFI*, *CD46* and *CD55*) are highly expressed in most cancers. The gene encoding the terminal pathway negative regulator, *CD59* is among the most highly expressed of all complement genes in the analysed tumour types, suggesting efficient protection of malignant cells from complement-mediated killing. This pattern of gene expression is entirely in line with specific examples from the literature, demonstrating high expression of these regulators in different types of cancer[75,91,94–98]. Complement-mediated cytotoxicity may act as a selective pressure for tumour-intrinsic overexpression of complement regulators. Indeed, hypoxic colorectal cancer cells in vitro are resistant to complement-mediated cytotoxicity owing, in part, to hypoxia-induced expression of the complement regulator CD55 (REF.[99]). This, again, is context dependent, as the effect of hypoxia on a non-small-cell lung cancer (NSCLC) cell line in vitro was decreased expression of complement regulators and increased susceptibility of the tumour cells to complement attack[100].

The low expression levels of terminal pathway genes together with the high expression of genes encoding complement regulators reinforces the hypothesis that malignant cells evolve and adapt to avoid potential cell lytic MAC formation. Instead, intratumoural complement activation can be sustained via locally expressed classical and alternative pathway components, thus generating the largely pro-tumoural anaphylatoxins C3a and C5a.

Furthermore, we analysed gene expression in the same tumour types normalized to expression in the corresponding normal tissues (Supplementary Figure 1). The pattern which emerged is cancer dependent. Nearly half (11/26) of the analysed cancer types upregulated the majority of the complement genes relative to the corresponding normal tissue and clustered in an 'upregulated complement' group. These included kidney renal papillary cell carcinoma (KIRP), kidney renal clear cell carcinoma (KIRC), head and neck squamous cell carcinoma (HNSC), oesophageal carcinoma (ESCA), stomach adenocarcinoma (STAD), lower grade glioma (LGG), glioblastoma (GBM), pancreatic adenocarcinoma (PAAD), testicular germ cell tumours (TGCT), skin cutaneous melanoma (SKCM) and ovarian serous cystadenocarcinoma (OV). The other half (13/26) of the cancer types had 'downregulated complement', expressing less complement genes compared with the corresponding normal tissue, such as lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD), adrenocortical carcinoma (ACC), KICH, rectum adenocarcinoma (READ), colon adenocarcinoma (COAD), bladder urothelial carcinoma (BLCA), PRAD, breast invasive carcinoma (BRCA), thyroid carcinoma (THCA), uterine corpus endometrial carcinoma (UCEC), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), and uterine carcinosarcoma (UCS). Two cancers, thymoma (THYM) and diffuse large B cell lymphoma (DLBC), similarly show a mixed profile, strongly upregulating more than half of the complement genes but downregulating the rest. These analyses also show that the majority of the cancer types (but not all) overexpress the complement regulators, supporting the notion that this is an escape mechanism to counteract complement attack. The C1q genes are overexpressed in the majority of the tumour types, which might correlate with the infiltration of immunosuppressive macrophages in the TME[32].

The prognostic impact of complement expression in patients with cancer. Next, we evaluated the impact of the expression of genes encoding components of the classical and alternative pathways on overall survival of patients with different malignancies, utilizing data available in the database of TCGA[92] (FIG. 5). Four groups of cancers could be defined. The first contains tumour types for which stronger expression of genes encoding components of the classical and alternative pathways are associated with good prognosis, that is, with longer overall survival. This group of cancers with 'protective complement' includes PRAD, mesothelioma (MESO), sarcoma (SARC) and SKCM. Hepatocellular carcinoma (HCC) belongs to this group as well, but is not presented in FIG. 5 to be consistent with FIG. 4. A second group in which C3 expression correlates with longer overall survival ('protective C3') comprises KICH, ACC and THCA, although significance is not reached in the latter. The third group contains cancers in which high expression of classical and alternative pathway genes correlates with poor prognosis. This group of 'aggressive complement' tumours includes UVM, LGG, GBM, KIRC and LUSC as the tumour types most significantly

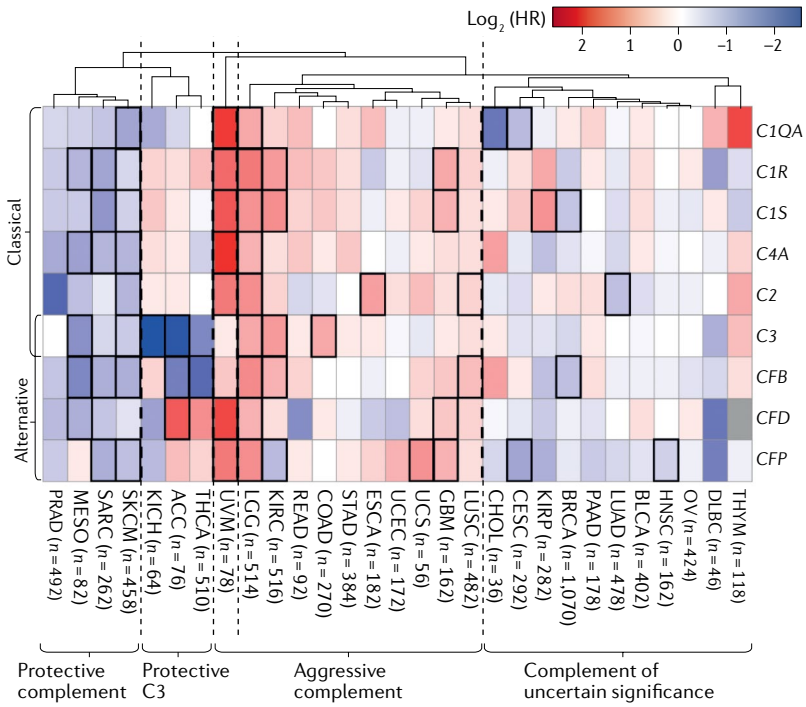


Fig. 5 | **Impact of the expression level of complement genes on the survival of patients with cancer.** Survival analysis was performed using the Gene Expression Profiling Interactive Analysis (GEPIA) tool[144]. Overall survival analysis based on gene expression with a median cut-off was used to calculate hazards ratios (HRs) based on the Cox proportional-hazards model and log-rank *P* value. The median cut-off was used to avoid any prior knowledge of the distributions of gene expression profiles. Given the very low number of events, the testicular germ cell tumours (TGCT) cohort was excluded from the survival analysis. The heatmap representing the $\log_2$ HR was generated using the R package 'pheatmap' and the clustering was done with the Euclidian distance and 'ward.D2' linkage criterion. The heatmap enables the visualization of the $\log_2$ HR with a scale centred at 0 for each complement gene in the different tumour types. Blue colours correspond to a protective effect of complement gene expression, and red colours to an increased risk for tumour progression when the complement gene is overexpressed. The bold outlined boxes correspond to log-rank $P < 0.05$. Four groups of cancers could be defined. The 'protective complement' group contains tumour types for which stronger expression of genes encoding components of the classical and alternative pathways are associated with favourable prognosis. The 'protective C3' group comprises cancer types for which only the C3 overexpression was associated with good prognosis. The 'aggressive complement' group encompasses cancers for which the high expression of complement genes indicates negative prognostic impact. The fourth group most often shows a lack of significant prognostic impact of the complement genes and is hence named 'complement of uncertain significance'. ACC, adrenocortical carcinoma; BLCA, bladder carcinoma; BRCA, invasive breast carcinoma; CESC, cervical squamous carcinoma; CF, complement factor; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, diffuse large B cell lymphoma; ESCA, oesophageal carcinoma; GBM, glioblastoma; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LGG, lower grade glioma; LUAD, lung adenocarcinoma; LUSC, lung squamous carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

impacted. Although significance was not reached for most of the genes, the group also includes digestive tract cancers such as READ, COAD, STAD and ESCA, as well as uterine cancers, such as UCEC and UCS. The fourth group encompasses a large number of tumour types in which the gene expression analysis did not reveal any clear clinical impact; therefore, this group has been named 'complement of uncertain significance'. This group comprises CHOL, CESC, KIRP, BRCA, PAAD, LUAD, BLCA, HNSC, OV, DLBC and THYM.

It is striking that SARC is one of the cancer types within the 'protective complement' group, as the density of B cells and expression of B cell-associated transcriptomic signatures have been associated with longer survival and response to immune checkpoint blockade in soft tissue sarcoma (W.H.F., unpublished results). Hence, it is tempting to speculate that, in this case, complement is involved in the antitumoural effect of B lymphocytes via its activation on intratumoural immune complexes. Indeed, early studies from the 1960s had already noted a potential link between the presence of sarcoma antibodies and an antitumoural effect of complement in mice[101] and, later, that immune complexes could be detected in sera from patients with sarcoma[102]. Furthermore, high expression of C5, produced mainly by tumour cells, correlated with better event-free and overall survival in Ewing sarcoma[103] (not included in the data set of TCGA). In this case, C5aR1 was predominantly detected on tumour cells in situ.

Despite abundant data related to the role of complement in mice grafted with melanoma cell lines (yielding often contradictory conclusions)[43–45,58], little is known about complement activation in human melanoma and its association with clinical parameters in patients' cohorts.

In addition, very limited data related to complement and human prostate cancer are available. Sublytic complement C5b-9 protects prostate cancer cell lines from TNF-induced cell death in vitro[104]. Interestingly, proteolysis of iC3b and C5 by the serine protease prostate-specific antigen (PSA) in prostatic fluid of patients with prostate cancer was detected, which would serve to inhibit the terminal pathway[105].

MESO offers an interesting case. The analysis presented here includes only 82 patients with heart, mediastinum and pleural MESO, and shows an overall tendency for good prognosis in association with high expression of classical and alternative complement pathway genes. Experimental evidence for the role of complement is available only for pleural MESO. Evaluation of C4d and C1q immunohistochemical staining in a patient cohort with malignant pleural MESO revealed an absence of C1q expression in the majority of tumours (only a few infiltrating immune cells stained positively) and an absence of C4d deposits on malignant cells[106]. Membranous C4d deposits were found only in tertiary lymphoid structures and this staining was associated with poor prognosis. Patients with low C4d plasma levels at diagnosis had a significantly better overall survival. In another patient cohort, C1q staining of tumour cells and infiltrating myeloid cells was strong[107]. In this context, C1q was shown to bind to hyaluronic acid and promote human pleural MESO cell adhesion and proliferation in a complement cascade-independent manner in vitro.

Further studies are needed to clarify the potential link between complement and cancer progression for the tumour types within the 'protective complement' group.

Tertiary lymphoid structures
Ectopic lymphoid aggregates that reflect lymphoid neogenesis occurring in tissues at sites of inflammation. These structures are detected in tumours where they orchestrate local and systemic antitumour responses.

These will be needed to provide experimental validation of whether the concerted expression of these proteins indeed occurs and whether complement is activated in situ. Immunohistochemical staining of large cohorts of patients for C1q, C4d, C3d and C5b-9 is necessary to determine the real impact of complement on cancer progression and prognosis.

With respect to the 'protective C3' group, cellular deposits of IgG and complement factors C3d, C4d and C5 were previously shown in up to 80% of patients with THCA (in a cohort of 59 patients), but data relating to prognostic impact were not reported[108]. This cancer type, in general, has a particularly good prognosis, which might account for the lack of prognostic impact of complement found in our analysis of THCA. No data from the literature are available to verify the impact of in situ complement in KICH and ACC, for which high expression of the C3 gene is associated with particularly good prognosis in our analysis. In these cancer types, the protective role of C3 could potentially be driven by non-canonical functions.

Of the cancer types within the 'aggressive complement' group, our analysis reveals a remarkably strong negative prognostic impact of the complement genes in UVM. A high expression level of complement regulators has been reported for this cancer type[109], but no data are available for the status of complement activation in situ. Further studies are warranted to test whether complement expression and/or deposits do indeed impact prognosis in UVM and to evaluate whether patients with this cancer type are likely to benefit from complement blocking therapy.

We propose that gliomas should be classified as cancer types with 'aggressive complement'. In a previous report, the complement components *C1QA*, *C1S*, *C2* and *C7* were found to be upregulated in patients with high-risk relative to low-risk glioma (a glioma cohort comprising GBM and LGG)[110]. The activation of B cells around high-risk gliomas is also likely, as indicated by the enrichment of a B cell-related gene set in high-risk compared with low-risk glioma[110]. Other studies also suggest that complement is activated in GBM, but the deleterious impact on prognosis remains to be proved by in situ analyses[64,111].

To date, the best examples of 'aggressive complement' tumour types for which gene expression and bioinformatics analyses are in agreement with the assessment of complement components at the protein level in situ are KIRC and NSCLC. The classical pathway requires a trigger. C1q can bind over 100 different targets, but the major ones are the IgG and IgM-containing immune complexes[2]. Indeed, IgM and IgG antibodies have been detected in several tumour types such as NSCLC[112], ovarian cancer[113,114], KIRC[32] and breast cancer[115]. These antibodies may come from the circulation or be produced at the tumour site[69,112,114], potentially initiating the complement cascade. The recognized antigens are frequently unknown. Further studies are needed to identify them and to determine whether they are recognized through shaping of the antigen-binding site by genetic processes of recombination and mutation or through unconventional strategies for diversification of the repertoire of antigen specificities[116]. In KIRC[32] and NSCLC[117], C1q-mediated classical pathway activation has been detected, leading to C4d deposits. In both cases, high levels of intratumoural C4d staining were associated with poor prognosis[32,117]. In addition, C4d levels in plasma were increased in patients with NSCLC and associated with poor prognosis[117,118]. Data in the literature do not discriminate between the two subtypes of NSCLC: LUSC and LUAD. In our analysis, although LUSC falls into the 'aggressive complement' group, for LUAD the significance of complement expression is uncertain.

The assessment of complement gene expression in different tumours strongly suggests that when complement is activated in situ, this occurs owing to locally produced complement proteins. Whilst data are lacking for the majority of cancer types, we evaluated the complement status at the protein level in patients with KIRC[32], a cancer type which according to our analysis is part of the 'aggressive complement' group. We found that there is an in situ orchestrated production of C1q by TAMs and of C1r, C1s, C4 and C3 by tumour cells, concomitant with IgG deposits. This enables C1 complex assembly and complement activation. Interestingly, what conferred the poor prognosis in patients with KIRC was the presence of intratumoural C1q-producing TAMs, as well as the concomitant local production and deposition of C4 activation fragments on the tumour cells. A surprising finding was that the local production of C3 by tumour cells indicated poor prognosis, whereas C3d-positive deposits were not associated with prognostic impact. This, together with the clear context-dependent impact of C3 in different tumour mouse models (TABLES 1,2), suggests it will be important to evaluate the full spectrum of deposits of C3 activation fragments in human tumours. Indeed, the antibodies used in our study to evaluate protein expression lacked specificity, with the C3d antibody recognizing C3, C3b and iC3b as well as the C3dg and C3d fragments, each of which have different functions[2,32]. However, in another patient cohort, positive C5a (indistinguishably marking the presence of the anaphylatoxin C5a and the intact C5) and C5aR1 staining was correlated with poor prognosis[41,119]. Therefore, it appears that the malignant cells in KIRC hijack macrophage-produced C1q to supplement the remaining components made by the cancer cells themselves, to promote tumour growth. Moreover, the production of C1q by TAMs and its negative prognostic impact in KIRC was confirmed by another study[87]. The proposed mechanism of action in tumours with 'aggressive complement' is depicted in FIG. 6, based largely on the data from patients with KIRC and NSCLC as well as in vivo and in vitro data. Further studies are needed to evaluate to what extent this mode of action of complement is valid for other cancer types.

The 'complement of uncertain significance' group encompasses a large number of different cancer types, for which either significance associated with prognostic impact was achieved for some complement genes and not others or no significance was found at all, using the median cut-off. Interestingly, in PAAD, tumour cells release exosomes which harbour B cell targets and bind antitumoural IgG to exert decoy function against potential

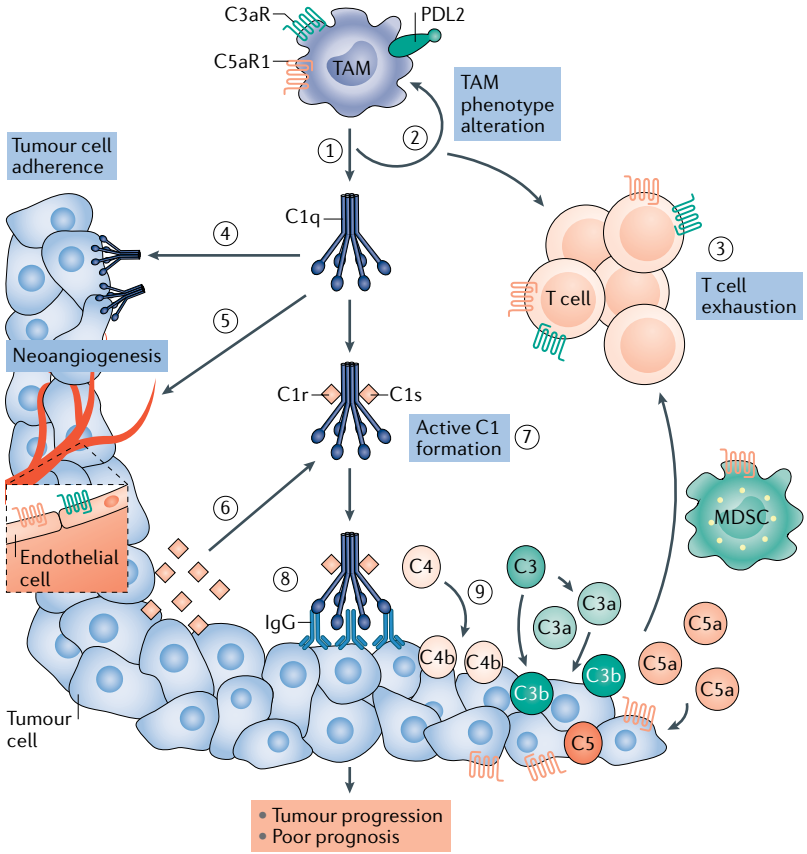


Fig. 6 | **Proposed mechanism of classical complement pathway activation and its consequences on tumour progression in tumours with 'aggressive complement'.** C1q is produced by tumour-associated macrophages (TAMs) (step 1) and contributes to a tumour-promoting phenotype of these cells (step 2) and T cell exhaustion (step 3). Secreted C1q promotes adherence of tumour cells to the extracellular matrix (step 4) and neoangiogenesis (step 5). A particular feature of clear cell renal cell carcinoma (also known as kidney renal clear cell carcinoma (KIRC)) is that the tumour cells produce C1r and C1s (step 6) and allow formation of a functionally active C1 complex (step 7), capable of activating the classical pathway. Moreover, immunoglobulin G (IgG) deposits on tumour cells serve as C1 ligands (step 8) to initiate the cascade. The tumour cells also produce the subsequent components of the complement cascade, which enable C4 and C3 activation fragment deposition (C4b, C4d and C3b, iC3b, C3d; note that not all of these are shown on the schematic for simplicity) (step 9). Anaphylatoxins C3a and C5a are released, exerting their action on the tumour cells and on the microenvironment. The ensemble of these processes contributes to tumour progression and poor prognosis for patients with cancer. This model is based on the data for KIRC and is potentially applicable to other tumour types within the 'aggressive complement' group. C3aR, C3a receptor; C5aR1, C5a receptor 1; MDSC, myeloid-derived suppressor cell; PDL2, programmed cell death 1 ligand 2.

complement-mediated cytotoxicity[120]. This could explain why classical and alternative pathway genes and proteins in PAAD do not seem to be associated with prognosis, despite their high expression[121]. Complement may not be efficiently activated in situ, when IgG is subverted from the tumour cells and targeted to exosomes.

Cascade-independent impact of complement in human cancers. As evidenced from in vitro and mouse models, most of the complement proteins have functions outside the cascade. They can operate alone and/or in parallel with the cascade in any of the tumour groups described herein. An indication of such phenomena could be that one gene or expressed and/or deposited protein is associated with either good or poor prognosis, whereas the remaining members of the same pathway are not. Another indication might be that the protein present within the cell is associated with a prognostic impact, whereas its deposits are not.

The cascade-independent impact has been illustrated with C1q-producing TAMs in patients with KIRC. We found that these TAMs were a robust marker for poor prognosis in three independent cohorts. As C1q was produced by an M2-like subtype of TAMs, the question arises of whether in addition to its role in complement activation, the negative impact of this TAM subtype was a reflection of the well-known pro-tumoural function of the immunosuppressive and pro-angiogenic M2 TAMs[122–124] with C1q being an additional biomarker for this population or whether C1q itself plays a role in the balance between TAM phenotypes, influencing their mode of action. Interestingly, a recent in-depth immune profiling study revealed that TAMs in human KIRC (but likely in other cancers as well) represent a heterogeneous cell population[125]. In particular, a subset called M-5 was shown to be associated with T cell exhaustion[125]. TAMs of the M-5 subset express higher levels of C1q genes as well as C1q receptors and C3aR, making them responsive to C1q and C3a[32]. In addition, they overexpress programmed cell death 1 ligand 2 (PDL2). Both M-5 and $C1q^+$ TAMs were associated with T cell exhaustion[32,125]. As the immunosuppressive action of C1q on T cells has already been described[22,126,127], it is tempting to speculate that M-5 macrophages exert their immunosuppressive activity at least in part via C1q[32].

Another example is the expression of C1r and C1s in human cutaneous squamous cell carcinoma cells, which promote tumour growth in mice as well as ERK1 or ERK2–AKT signal transduction in vitro in the absence of C1q[128]. FH and FI are also produced by this cancer type and promote tumour growth in vivo without evidence of complement activation[76,77].

Caveats associated with transcriptomic data to predict patient outcome. Gene expression analyses may inform on the potential production level of encoded proteins. However, in the complement cascade the generation of effectors is attributed to protein activation and cleavage, which are not detectable at the transcript level. Although transcriptomic analyses are useful to pinpoint associations between complement components and clinical outcome, only in situ analyses of the complement proteins, their activation fragment deposits (C4d, C3b, iC3b and C3d), the anaphylatoxins (C3a and C5a), their receptors (C3aR, C5aR1 and C5aR2) and their regulators will enable us to fully understand how complement modulates tumour cells and the TME, resulting in control or progression of cancers.

A clear example of such discrepancy is the modest prognostic impact of *C3AR* and *C5AR1* gene expression, which reaches statistical significance at the median cut-off only for four malignancies each (data not shown). Nevertheless, C5aR1 seems to be a key effector of the pro-tumoural impact of complement, even when C5a is generated by cascade-independent proteases[16,17,31,42,45,129]. Another example relates to *C1S*.

A clear negative prognostic impact was demonstrated for C1s overexpression in BLCA at the protein level[130]. Detailed gene expression analyses of the cohort for TCGA revealed that *C1S* was one of the most significantly upregulated genes both in urothelial carcinoma of the upper tract and in urinary bladder cancer[130]. This upregulation correlated with a panel of disease markers, but the gene expression failed to reach significance in terms of prognostic impact, contrary to the immunohistochemical staining. This case illustrates the importance of performing immunohistochemical staining to determine the prognostic impact of a given protein.

Another hurdle with transcriptomic data is that different activation fragments of the complement proteins have different biological functions. Therefore, the exact deposited activation fragment has to be distinguished in situ. Well-characterized and validated antibodies with known fragment specificity should be used. Detection of complement and its activation fragments is performed routinely in pathology laboratories and the difficulties of working with paraffin-embedded tissues are well documented[131]. Nevertheless, reliable protocols nowadays exist for immunohistochemical staining of complement components and activation fragments in paraffin-embedded tumour tissue[32], with antibodies validated by competition tests with purified intact proteins or fragments. With the current state of imaging analyses, distinction between intracellular production and deposits with automated algorithms is tricky. This therefore requires experienced observers to stratify the patients. Moreover, proteomic analyses (such as high spatial resolution mass spectrometry imaging or cytometry by time of flight (CyTOF)) enable spatially resolved profiling of the proteins within different tumour regions. Exploration of the role of complement in cancer will benefit from the advent of these technologies, which will ultimately enable better evaluation of complement production and deposits in situ. In turn, this will leverage complement biomarker discovery and validation in patients with cancer. Furthermore, this could open up avenues for personalized therapeutic approaches, selecting for patients who might benefit most from complement-targeting therapy.

A further challenge, when a complement protein is produced both by malignant cells and infiltrating host cells, is to ascribe the pro-tumoural or antitumoural effect to this particular complement protein and its canonical and/or non-canonical functions, or to the presence of the cell itself, as in the case for $C1q^+$ TAMs[32].

Indeed, our prognostic data presented here need to be interpreted within the limits of using median gene expression as a cut-off to stratify patients. Even if the median cut-off did not reveal significant prognostic impact of complement genes in the 'complement of uncertain significance' group of patients, use of statistically defined optimal cut-offs may have revealed significant correlations.

Therapeutic perspectives

Our analysis of a large panel of cancers (FIG. 4) revealed simultaneous intratumoural expression of genes coding for proteins involved in complement activation (C1q, C1s, C1r and C3) or sensing activation products (C3aR and C5aR) as well as in complement regulation (C1inh, FH, FI and CD59). Taken together, the local production and increased activation of complement in the TME is associated with dampening of the antitumour immune responses and promotion of cancer progression in a large number of different cancer types. Although further studies are needed, current evidence suggests that liver-derived complement seems to be less important in tumour biology compared with locally and/or intracellularly derived complement. It is now important to define which cells produce which components at what time points and in which cancer types. This will guide the design of future therapeutic strategies, targeting the right complement component spatially and temporally in the appropriate type of cancer.

Despite the heterogeneity of the data reported in the literature, it can be concluded that, in the majority of tumour types, the anaphylatoxin receptors C3aR and C5aR1 can be considered a novel class of immune checkpoints that could be targeted for cancer immunotherapy. Indeed, C5aR1 blockade by small-molecule inhibitor or antibody led to decreased tumour growth in various tumour mouse models either alone[6] or in combination with immune checkpoint inhibitors (anti-programmed cell death 1 (PD1) or anti-PDL1)[18,45,132]. Nevertheless, mouse data should be interpreted with caution, as substantial differences exist between rodent and human complement regulators and receptors. However, the in vitro experiments with human cells validated the pro-tumoural role of the C5a–C5aR1 axis identified in mouse models. This subsequently promoted a phase I clinical trial for administration of a C5aR1 monoclonal antibody (IPH5401) in combination with the anti-PDL1 therapy durvalumab in patients with advanced solid tumours, which is now recruiting (STELLAR-001, NCT03665129)[133]. C5aR1 blockade has also been shown to improve the efficacy of chemotherapy. Indeed, C5aR1 targeting with the small-molecule inhibitor PMX-53 improved the efficacy of paclitaxel chemotherapy, by promoting an antitumoural T cell response in a mouse model of squamous carcinogenesis[16]. Recent evidence for the role of the interaction of iC3b with CR3 in generating an immunosuppressive phenotype of intratumoural neutrophils suggests that blockade at the level of C3 might be another promising target, at least for ovarian cancer[48].

Many complement-targeting molecules are in the pipeline for various disease indications[134,135] and could be adapted for cancer therapy, acting at different steps of the cascade[49]. Nevertheless, any therapeutic combinations with standard-of-care therapies have to take into account the large variety of functions of complement proteins, such as in the context of radiotherapy, where C3a and C5a are crucial to the antitumour immune response[136]. Moreover, newly described connections between intracellular complement and cellular metabolism, discovered in T cells[19], will likely turn out to shape the TME, affecting therapeutic outcome.

Cancer vaccines are a promising approach to stimulate the immune system to efficiently recognize and kill tumour cells. Endothelial quiescence prevents tumour-specific T cell homing. This endothelial quiescence was

Endothelial quiescence
The resting state of the endothelium, enabling it to exert its barrier functions, preventing thrombosis and inflammation. In the context of cancer, quiescent endothelium establishes a barrier that prevents T cells from efficiently penetrating the tumour.

reversed by cytokine-mediated activation of the tumour vasculature followed by upregulation of C3 and local generation of C5a in the TC-1 mouse model, inoculated with primed T cells. These T cells were derived from mice and primed with human papilloma virus (HPV) antigens expressed by the TC-1 cell line[137]. The C5a-dependent upregulation of endothelial adhesion molecules resulted in efficient T cell extravasation, infiltration into the tumour and malignant cell killing. These results highlight once again the context-dependent action of complement. In the same model, complement activation is pro-tumoural[31,32], but it becomes antitumoural during therapy to elicit a robust antitumour immune response[137]. Indeed, these data suggest that when effector T cells are present, complement facilitates tumour rejection, whereas it may promote inflammation and tumorigenesis when other immune cell types predominate over antitumour T cells[137].

Big efforts are now focused on the design of anticancer monoclonal antibodies with enhanced complement-mediated cytotoxicity in order to kill the tumour cells to which they are directed. The recent discovery of the potentiation of the C1q binding and complement activation by IgG hexamerization prompted the development of a new generation of therapeutic antibodies[138–140]. If given in the right context, these antibodies might provide benefit in the eradication of tumours. Although limited, the data from the literature suggest that the impact of tumour cell-binding IgG on tumour growth is context dependent. In an immunostimulatory context, such antibodies can induce powerful antitumour immunity that can potentially be harnessed for the treatment of patients with cancer. The potential benefit-to-harm ratio has to be evaluated for each subset of patients even within one tumour type to avoid potential enhancement of the pro-tumoural impact of complement. Intervention strategies[11], such as blocking or silencing the membrane complement regulatory proteins, inhibiting the extracellular enzymes that interfere with complement activation or inhibiting the intracellular pathways that support tumour cell resistance and recovery, have to be established to overcome the resistance of tumour cells to complement-mediated killing, which will improve the efficacy of these therapeutic antibodies.

Conclusions

In order to design efficient complement-targeted therapeutics for cancer we have to better understand the mechanisms by which these complement proteins contribute to tumour development. This will enable us to tip the finely tuned balance of the complement reaction to favour tumour rejection and to decide whether the best strategy going forward would be to block the activation, prevent the regulation and/or act on the functions of these proteins of the complement system to treat patients with cancer.

Note added in proof

While this review was in proof, Aykut et al. reported that the binding of MBL to glycans of fungal walls and lectin pathway activation was required for oncogenic progression in pancreatic cancer. Deletion of MBL or C3 in the extratumoural compartment or knockdown of *C3ar* in tumour cells were both protective against tumour growth in mouse models[148].

Published online 30 October 2019

1. Fridman, W. H., Zitvogel, L., Sautes-Fridman, C. & Kroemer, G. The immune contexture in cancer prognosis and treatment. *Nat. Rev. Clin. Oncol.* **14**, 717–734 (2017).
2. Merle, N. S., Church, S. E., Fremeaux-Bacchi, V. & Roumenina, L. T. Complement system part I—molecular mechanisms of activation and regulation. *Front. Immunol.* **6**, 262 (2015).
3. Merle, N. S., Noe, R., Halbwachs-Mecarelli, L., Fremeaux-Bacchi, V. & Roumenina, L. T. Complement system part II: role in immunity. *Front. Immunol.* **6**, 257 (2015).
4. Kemper, C. & Kohl, J. Back to the future—non-canonical functions of complement. *Semin. Immunol.* **37**, 1–3 (2018).
5. Kolev, M., Le Friec, G. & Kemper, C. Complement—tapping into new sites and effector systems. *Nat. Rev. Immunol.* **14**, 811–820 (2014).
6. Reis, E. S., Mastellos, D. C., Ricklin, D., Mantovani, A. & Lambris, J. D. Complement in cancer: untangling an intricate relationship. *Nat. Rev. Immunol.* **18**, 5–18 (2018).
7. Gros, P., Milder, F. J. & Janssen, B. J. Complement driven by conformational changes. *Nat. Rev. Immunol.* **8**, 48–58 (2008).
8. Lalli, P. N. et al. Locally produced C5a binds to T cell-expressed C5aR to enhance effector T-cell expansion by limiting antigen-induced apoptosis. *Blood* **112**, 1759–1766 (2008).
9. Strainic, M. G. et al. Locally produced complement fragments C5a and C3a provide both costimulatory and survival signals to naive $CD4^+$ T cells. *Immunity* **28**, 425–435 (2008).
10. Morgan, B. P., Walters, D., Serna, M. & Bubeck, D. Terminal complexes of the complement system: new structural insights and their relevance to function. *Immunol. Rev.* **274**, 141–151 (2016).
11. Fishelson, Z. & Kirschfink, M. Complement C5b-9 and cancer: mechanisms of cell damage, cancer counteractions, and approaches for intervention. *Front. Immunol.* **10**, 752 (2019).
12. Rozenberg, P., Ziporen, L., Gancz, D., Saar-Ray, M. & Fishelson, Z. Cooperation between Hsp90 and mortalin/GRP75 in resistance to cell death induced by complement C5b-9. *Cell Death Dis.* **9**, 150 (2018).
13. Huber-Lang, M. et al. Generation of C5a in the absence of C3: a new complement activation pathway. *Nat. Med.* **12**, 682–687 (2006).
14. Bekassy, Z. D. et al. Aliskiren inhibits renin-mediated complement activation. *Kidney Int.* **94**, 689–700 (2018).
15. Krisinger, M. J. et al. Thrombin generates previously unidentified C5 products that support the terminal complement activation pathway. *Blood* **120**, 1717–1725 (2012).
16. Medler, T. R. et al. Complement C5a fosters squamous carcinogenesis and limits T cell response to chemotherapy. *Cancer Cell* **34**, 561–578.e6 (2018).
This study demonstrates the pro-tumoural role of C5a, generated independently of the complement cascade in a mouse model.
17. Nitta, H. et al. Cancer cells release anaphylatoxin C5a from C5 by serine protease to enhance invasiveness. *Oncol. Rep.* **32**, 1715–1719 (2014).
18. Zha, H. et al. Intracellular activation of complement C3 leads to PD-L1 antibody treatment resistance by modulating tumor-associated macrophages. *Cancer Immunol.Res* **7**, 193–207 (2019).
19. West, E. E., Kolev, M. & Kemper, C. Complement and the regulation of T cell responses. *Annu. Rev. Immunol.* **36**, 309–338 (2018).
20. Hess, C. & Kemper, C. Complement-mediated regulation of metabolism and basic cellular processes. *Immunity* **45**, 240–254 (2016).
21. Hajishengallis, G., Reis, E. S., Mastellos, D. C., Ricklin, D. & Lambris, J. D. Novel mechanisms and functions of complement. *Nat. Immunol.* **18**, 1288–1298 (2017).
22. Ling, G. S. et al. C1q restrains autoimmunity and viral infection by regulating $CD8^+$ T cell metabolism. *Science* **360**, 558–563 (2018).
23. Kremlitzka, M. et al. Interaction of serum-derived and internalized C3 with DNA in human B cells — a potential involvement in regulation of gene transcription. *Front. Immunol.* **10**, 493 (2019).
24. Kolev, M. et al. Complement regulates nutrient influx and metabolic reprogramming during Th1 cell responses. *Immunity* **42**, 1033–1047 (2015).
25. Arbore, G. et al. Complement receptor CD46 co-stimulates optimal human $CD8^+$ T cell effector function via fatty acid metabolism. *Nat. Commun.* **9**, 4186 (2018).
26. Arbore, G. et al. T helper 1 immunity requires complement-driven NLRP3 inflammasome activity in $CD4^+$ T cells. *Science* **352**, aad1210 (2016).
27. Liszewski, M. K. et al. Intracellular complement activation sustains T cell homeostasis and mediates effector differentiation. *Immunity* **39**, 1143–1157 (2013).
28. Elvington, M., Liszewski, M. K., Bertram, P., Kulkarni, H. S. & Atkinson, J. P. A C3(H_2O) recycling pathway is a component of the intracellular complement system. *J. Clin. Invest.* **127**, 970–981 (2017).
29. Satyam, A. et al. Intracellular activation of complement 3 is responsible for intestinal tissue damage during mesenteric ischemia. *J. Immunol.* **198**, 788–797 (2017).
30. King, B. C. et al. Complement component C3 is highly expressed in human pancreatic islets and prevents β cell death via ATG16L1 interaction and autophagy regulation. *Cell Metab.* **29**, 202–210 e6 (2019).
31. Markiewski, M. M. et al. Modulation of the antitumor immune response by complement. *Nat. Immunol.* **9**, 1225–1235 (2008).
This paper describes the discovery of the pro-tumoural role of C5a, through its effects on MDSCs.
32. Roumenina, L. T. et al. Tumor cells highjack macrophage-produced complement C1q to promote tumor growth. *Cancer Immunol. Res.* **7**, 1091–1105 (2019).
This study demonstrates the tumour-promoting role of the classical complement pathway in renal

cancer and the use of C1q and C4d staining as a prognostic marker.
33. Kwak, J. W. et al. Complement activation via a C3a receptor pathway alters $CD4^+$ T lymphocytes and mediates lung cancer progression. *Cancer Res.* **78**, 143–156 (2018).
34. Arshad, A. et al. Restoration of mannose-binding lectin complement activity is associated with improved outcome in patients with advanced pancreatic cancer treated with gemcitabine and intravenous omega-3 fish oil. *JPEN J. Parenter. Enter. Nutr.* **38**, 214–219 (2014).
35. Ytting, H., Jensenius, J. C., Christensen, I. J., Thiel, S. & Nielsen, H. J. Increased activity of the mannan-binding lectin complement activation pathway in patients with colorectal cancer. *Scand. J. Gastroenterol.* **39**, 674–679 (2004).
36. Frade, R. et al. Procathepsin-L, a proteinase that cleaves human C3 (the third component of complement), confers high tumorigenic and metastatic properties to human melanoma cells. *Cancer Res.* **58**, 2733–2736 (1998).
37. Jean, D. et al. A cysteine proteinase, which cleaves human C3, the third component of complement, is involved in tumorigenicity and metastasis of human melanoma. *Cancer Res.* **56**, 254–258 (1996).
38. Reddel, C. J., Tan, C. W. & Chen, V. M. Thrombin generation and cancer: contributors and consequences. *Cancers* **11**, E100 (2019).
39. Cho, M. S. et al. Complement component 3 is regulated by TWIST1 and mediates epithelial–mesenchymal transition. *J. Immunol.* **196**, 1412–1418 (2016).
40. Cho, M. S. et al. Autocrine effects of tumor-derived complement. *Cell Rep.* **6**, 1085–1095 (2014).
41. Xi, W. et al. High level of anaphylatoxin C5a predicts poor clinical outcome in patients with clear cell renal cell carcinoma. *Sci. Rep.* **6**, 29177 (2016).
42. Bonavita, E. et al. PTX3 is an extrinsic oncosuppressor regulating complement-dependent inflammation in cancer. *Cell* **160**, 700–714 (2015).
This paper shows the impact of C5a on the macrophage phenotype in sarcoma mouse models.
43. Nabizadeh, J. A. et al. The complement C3a receptor contributes to melanoma tumorigenesis by inhibiting neutrophil and $CD4^+$ T cell responses. *J. Immunol.* **196**, 4783–4792 (2016).
44. Janelle, V. et al. Transient complement inhibition promotes a tumor-specific immune response through the implication of natural killer cells. *Cancer Immunol. Res* **2**, 200–206 (2014).
45. Wang, Y. et al. Autocrine complement inhibits IL10-dependent T-cell-mediated antitumor immunity to promote tumor progression. *Cancer Discov.* **6**, 1022–1035 (2016).
46. Guglietta, S. et al. Coagulation induced by C3aR-dependent NETosis drives protumorigenic neutrophils during small intestinal tumorigenesis. *Nat. Commun.* **7**, 11037 (2016).
47. Gunn, L. et al. Opposing roles for complement component C5a in tumor progression and the tumor microenvironment. *J. Immunol.* **189**, 2985–2994 (2012).
48. Singel, K. L. et al. Mature neutrophils suppress T cell immunity in ovarian cancer microenvironment. *JCI Insight* **4**, 122311 (2019).
49. Pio, R., Ajona, D., Ortiz-Espinosa, S., Mantovani, A. & Lambris, J. D. Complementing the cancer-immunity cycle. *Front. Immunol.* **10**, 774 (2019).
50. Kroemer, G., Galluzzi, L., Kepp, O. & Zitvogel, L. Immunogenic cell death in cancer therapy. *Annu. Rev. Immunol.* **31**, 51–72 (2013).
51. Schmidt, J., Klempp, C., Buchler, M. W. & Marten, A. Release of iC3b from apoptotic tumor cells induces tolerance by binding to immature dendritic cells in vitro and in vivo. *Cancer Immunol. Immunother.* **55**, 31–38 (2006).
52. Liu, C. F. et al. Complement receptor 3 has negative impact on tumor surveillance through suppression of natural killer cell function. *Front. Immunol.* **8**, 1602 (2017).
53. Fridman, W. H., Pages, F., Sautes-Fridman, C. & Galon, J. The immune contexture in human tumours: impact on clinical outcome. *Nat. Rev. Cancer* **12**, 298–306 (2012).
54. Kurihara, R. et al. C5a promotes migration, proliferation, and vessel formation in endothelial cells. *Inflamm. Res.* **59**, 659–666 (2010).
55. Corrales, L. et al. Anaphylatoxin C5a creates a favorable microenvironment for lung cancer progression. *J. Immunol.* **189**, 4674–4683 (2012).
56. Nunez-Cruz, S. et al. Genetic and pharmacologic inhibition of complement impairs endothelial cell function and ablates ovarian cancer neovascularization. *Neoplasia* **14**, 994–1004 (2012).
57. Bandini, S. et al. Early onset and enhanced growth of autochthonous mammary carcinomas in C3-deficient Her2/neu transgenic mice. *Oncoimmunology* **2**, e26137 (2013).
58. Bulla, R. et al. C1q acts in the tumour microenvironment as a cancer-promoting factor independently of complement activation. *Nat. Commun.* **7**, 10346 (2016).
59. Singh, J., Ahmed, A. & Girardi, G. Role of complement component C1q in the onset of preeclampsia in mice. *Hypertension* **58**, 716–724 (2011).
60. Bandini, S. et al. The non-inflammatory role of C1q during Her2/neu-driven mammary carcinogenesis. *Oncoimmunology* **5**, e1253653 (2016).
61. Jourde-Chiche, N. et al. Endothelium structure and function in kidney health and disease. *Nat. Rev. Nephrol.* **15**, 87–108 (2019).
62. Roumenina, L. T., Rayes, J., Frimat, M. & Fremeaux-Bacchi, V. Endothelial cells: source, barrier, and target of defensive mediators. *Immunol. Rev.* **274**, 307–329 (2016).
63. Doerner, S. K. et al. High-fat diet-induced complement activation mediates intestinal inflammation and neoplasia, independent of obesity. *Mol. Cancer Res.* **14**, 953–965 (2016).
64. Bouwens van der Vlis, T. A. M. et al. The complement system in glioblastoma multiforme. *Acta Neuropathol. Commun.* **6**, 91 (2018).
65. Hu, W. H. et al. C5a receptor enhances hepatocellular carcinoma cell invasiveness via activating ERK1/2-mediated epithelial-mesenchymal transition. *Exp. Mol. Pathol.* **100**, 101–108 (2016).
66. Kaida, T. et al. C5a receptor (CD88) promotes motility and invasiveness of gastric cancer by activating RhoA. *Oncotarget* **7**, 84798–84809 (2016).
67. Fan, Z., Qin, J., Wang, D. & Geng, S. Complement C3a promotes proliferation, migration and stemness in cutaneous squamous cell carcinoma. *J. Cell Mol. Med.* **23**, 3097–3107 (2019).
68. Vlaicu, S. I. et al. Role of C5b-9 complement complex and response gene to complement-32 (RGC-32) in cancer. *Immunol. Res.* **56**, 109–121 (2013).
69. Ajona, D., Ortiz-Espinosa, S., Pio, R. & Lecanda, F. Complement in metastasis: a comp in the camp. *Front. Immunol.* **10**, 669 (2019).
70. Ajona, D. et al. Blockade of the complement C5a/C5aR1 axis impairs lung cancer bone metastasis by CXCL16-mediated effects. *Am. J Respir. Crit. Care Med.* **197**, 1164–1176 (2018).
71. Vadrevu, S. K. et al. Complement C5a receptor facilitates cancer metastasis by altering T-cell responses in the metastatic niche. *Cancer Res.* **74**, 3454–3465 (2014).
72. Nitta, H. et al. Expression of the anaphylatoxin C5a receptor in gastric cancer: implications for vascular invasion and patient outcomes. *Med. Oncol.* **33**, 118 (2016).
73. Boire, A. et al. Complement component 3 adapts the cerebrospinal fluid for leptomeningeal metastasis. *Cell* **168**, 1101–1113.e13 (2017).
74. Oostindie, S. C. et al. CD20 and CD37 antibodies synergize to activate complement by Fc-mediated clustering. *Haematologica* **104**, 1841–1852 (2019).
75. Ajona, D. et al. Expression of complement factor H by lung cancer cells: effects on the activation of the alternative pathway of complement. *Cancer Res.* **64**, 6310–6318 (2004).
76. Riihila, P. et al. Complement factor I promotes progression of cutaneous squamous cell carcinoma. *J. Invest. Dermatol.* **135**, 579–588 (2015).
77. Riihila, P. M. et al. Complement factor H: a biomarker for progression of cutaneous squamous cell carcinoma. *J. Invest. Dermatol.* **134**, 498–506 (2014).
78. Spiller, O. B., Criado-Garcia, O., Rodriguez De Cordoba, S. & Morgan, B. P. Cytokine-mediated up-regulation of CD55 and CD59 protects human hepatoma cells from complement attack. *Clin. Exp. Immunol.* **121**, 234–241 (2000).
79. Ajona, D., Hsu, Y. F., Corrales, L., Montuenga, L. M. & Pio, R. Down-regulation of human complement factor H sensitizes non-small cell lung cancer cells to complement attack and reduces in vivo tumor growth. *J. Immunol.* **178**, 5991–5998 (2007).
80. Bjorge, L. et al. Ascitic complement system in ovarian cancer. *Br. J. Cancer* **92**, 895–905 (2005).
81. Kapka-Skrzypczak, L. et al. CD55, CD59, factor H and factor H-like 1 gene expression analysis in tumors of the ovary and corpus uteri origin. *Immunol. Lett.* **167**, 67–71 (2015).
82. Okroj, M., Hsu, Y. F., Ajona, D., Pio, R. & Blom, A. M. Non-small cell lung cancer cells produce a functional set of complement factor I and its soluble cofactors. *Mol. Immunol.* **45**, 169–179 (2008).
83. Sakai, N. et al. Tumorigenicity of BALB3T3 A31 cells transfected with hamster-complement-C1s cDNA. *Int. J. Cancer* **58**, 309–313 (1994).
84. Block, I. et al. CFP suppresses breast cancer cell growth by TES-mediated upregulation of the transcription factor DDIT3. *Oncogene* **38**, 4560–4573 (2019).
85. Kaur, A. et al. Human C1q induces apoptosis in an ovarian cancer cell line via tumor necrosis factor pathway. *Front. Immunol.* **7**, 599 (2016).
86. Hong, Q. et al. Complement C1q activates tumor suppressor WWOX to induce apoptosis in prostate cancer cells. *PLOS ONE* **4**, e5755 (2009).
87. Mangogna, A. et al. Is the complement protein C1q a pro- or anti-tumorigenic factor? Bioinformatics analysis involving human carcinomas. *Front. Immunol.* **10**, 865 (2019).
88. Lubbers, R., van Essen, M. F., van Kooten, C. & Trouw, L. A. Production of complement components by cells of the immune system. *Clin. Exp. Immunol.* **188**, 183–194 (2017).
89. Beatty, G. L. & Gladney, W. L. Immune escape mechanisms as a guide for cancer immunotherapy. *Clin. Cancer Res.* **21**, 687–692 (2015).
90. Mohme, M., Riethdorf, S. & Pantel, K. Circulating and disseminated tumour cells—mechanisms of immune surveillance and escape. *Nat. Rev. Clin. Oncol.* **14**, 155–167 (2017).
91. Blok, V. T. et al. A possible role of CD46 for the protection in vivo of human renal tumor cells from complement-mediated damage. *Lab Invest.* **80**, 335–344 (2000).
92. Cancer Genome Atlas Research Network et al. The Cancer Genome Atlas Pan-Cancer analysis project. *Nat. Genet.* **45**, 1113–1120 (2013).
93. Uhlen, M. et al. A pathology atlas of the human cancer transcriptome. *Science* **357**, eaan2507 (2017).
94. Buettner, R. et al. Activated signal transducers and activators of transcription 3 signaling induces CD46 expression and protects human cancer cells from complement-dependent cytotoxicity. *Mol. Cancer Res.* **5**, 823–832 (2007).
95. Ravindranath, N. M. & Shuler, C. Expression of complement restriction factors (CD46, CD55 & CD59) in head and neck squamous cell carcinomas. *J. Oral Pathol. Med.* **35**, 560–567 (2006).
96. Junnikkala, S. et al. Secretion of soluble complement inhibitors factor H and factor H-like protein (FHL-1) by ovarian tumour cells. *Br. J. Cancer* **87**, 1119–1127 (2002).
97. Surowiak, P. et al. CD46 expression is indicative of shorter revival-free survival for ovarian cancer patients. *Anticancer. Res.* **26**, 4943–4948 (2006).
98. Wilczek, E. et al. The possible role of factor H in colon cancer resistance to complement attack. *Int. J. Cancer* **122**, 2030–2037 (2008).
99. Olcina, M. M. et al. Mutations in an innate immunity pathway are associated with poor overall survival outcomes and hypoxic signaling in cancer. *Cell Rep.* **25**, 3721–3732.e6 (2018).
100. Okroj, M., Corrales, L., Stokowska, A., Pio, R. & Blom, A. M. Hypoxia increases susceptibility of non-small cell lung cancer cells to complement attack. *Cancer Immunol. Immunother.* **58**, 1771–1780 (2009).
101. Phillips, M. E., Rother, U. & Rother, K. Serum complement in the rejection of sarcoma I ascites tumor grafts. *J. Immunol.* **100**, 493–500 (1968).
102. Eilber, F. R. & Morton, D. L. Demonstration in sarcoma patients of anti-tumor antibodies which fix only human complement. *Nature* **225**, 1137–1138 (1970).
103. Savola, S. et al. High expression of complement component 5 (C5) at tumor site associates with superior survival in Ewing's sarcoma family of tumour patients. *ISRN Oncol.* **2011**, 168712 (2011).
104. Liu, L., Li, W., Li, Z. & Kirschfink, M. Sublytic complement protects prostate cancer cells from tumour necrosis factor-α-induced cell death. *Clin. Exp. Immunol.* **169**, 100–108 (2012).
105. Manning, M. L., Williams, S. A., Jelinek, C. A., Kostova, M. B. & Denmeade, S. R. Proteolysis of complement factors iC3b and C5 by the serine protease prostate-specific antigen in prostatic fluid and seminal plasma. *J. Immunol.* **190**, 2567–2574 (2013).

106. Klikovits, T. et al. Circulating complement component 4d (C4d) correlates with tumor volume, chemotherapeutic response and survival in patients with malignant pleural mesothelioma. *Sci. Rep.* **7**, 16456 (2017).
107. Agostinis, C. et al. Complement protein C1q binds to hyaluronic acid in the malignant pleural mesothelioma microenvironment and promotes tumor growth. *Front. Immunol.* **8**, 1559 (2017).
108. Lucas, S. D. et al. Tumor-specific deposition of immunoglobulin G and complement in papillary thyroid carcinoma. *Hum. Pathol.* **27**, 1329–1335 (1996).
109. Goslings, W. R. et al. Membrane-bound regulators of complement activation in uveal melanomas. CD46, CD55, and CD59 in uveal melanomas. *Invest. Ophthalmol. Vis. Sci.* **37**, 1884–1891 (1996).
110. Bacolod, M. D. et al. Immune infiltration, glioma stratification, and therapeutic implications. *Transl. Cancer Res.* **5**, S652–S656 (2016).
111. Bouwens, T. A. et al. Complement activation in Glioblastoma multiforme pathophysiology: evidence from serum levels and presence of complement activation products in tumor tissue. *J. NeuroImmunol.* **278**, 271–276 (2015).
112. Germain, C. et al. Presence of B cells in tertiary lymphoid structures is associated with a protective immunity in patients with lung cancer. *Am. J. Respir. Crit. Care Med.* **189**, 832–844 (2014).
This study demonstrates that the density of B cell follicles inside tertiary lymphoid structures correlates with favourable prognosis in NSCLC and that activated intratumoural B cells produce antibodies to tumour-associated antigens.
113. Montfort, A. et al. A strong B-cell response is part of the immune landscape in human high-grade serous ovarian metastases. *Clin. Cancer Res.* **23**, 250–262 (2017).
This study demonstrates that B cells produce antitumoural antibodies in ovarian cancer.
114. Kroeger, D. R., Milne, K. & Nelson, B. H. Tumor-infiltrating plasma cells are associated with tertiary lymphoid structures, cytolytic T-cell responses, and superior prognosis in ovarian cancer. *Clin. Cancer Res.* **22**, 3005–3015 (2016).
115. Yeong, J. et al. High densities of tumor-associated plasma cells predict improved prognosis in triple negative breast cancer. *Front. Immunol.* **9**, 1209 (2018).
116. Kanyavuz, A., Marey-Jarossay, A., Lacroix-Desmazes, S. & Dimitrov, J. D. Breaking the law: unconventional strategies for antibody diversification. *Nat. Rev. Immunol.* **19**, 355–368 (2019).
117. Ajona, D. et al. Investigation of complement activation product C4d as a diagnostic and prognostic biomarker for lung cancer. *J. Natl Cancer Inst.* **105**, 1385–1393 (2013).
This study demonstrates that the complement activation fragment C4d can serve as a diagnostic and prognostic marker in lung cancer.
118. Ajona, D. et al. Complement C4d-specific antibodies for the diagnosis of lung cancer. *Oncotarget* **9**, 6346–6355 (2018).
119. Xi, W. et al. Enrichment of C5a–C5aR axis predicts poor postoperative prognosis of patients with clear cell renal cell carcinoma. *Oncotarget* **7**, 80925–80934 (2016).
120. Capello, M. et al. Exosomes harbor B cell targets in pancreatic adenocarcinoma and exert decoy function against complement-mediated cytotoxicity. *Nat. Commun.* **10**, 254 (2019).
121. Chen, J. et al. Expression and clinical significance of complement C3, complement C4b1 and apolipoprotein E in pancreatic cancer. *Oncol. Lett.* **6**, 43–48 (2013).
122. Ginhoux, F. & Guilliams, M. Tissue-resident macrophage ontogeny and homeostasis. *Immunity* **44**, 439–449 (2016).
123. Komohara, Y. et al. Macrophage infiltration and its prognostic relevance in clear cell renal cell carcinoma. *Cancer Sci.* **102**, 1424–1431 (2011).
124. Mantovani, A., Sozzani, S., Locati, M., Allavena, P. & Sica, A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol.* **23**, 549–555 (2002).
125. Chevrier, S. et al. An immune atlas of clear cell renal cell carcinoma. *Cell* **169**, 736–749 e18 (2017).
This article presents comprehensive analyses at the single-cell level of immune cells infiltrating renal tumours.
126. Clarke, E. V., Weist, B. M., Walsh, C. M. & Tenner, A. J. Complement protein C1q bound to apoptotic cells suppresses human macrophage and dendritic cell-mediated Th17 and Th1 T cell subset proliferation. *J. Leukoc. Biol.* **97**, 147–160 (2015).
127. Ghebrehiwet, B. et al. Evidence that the two C1q binding membrane proteins, gC1q-R and cC1q-R, associate to form a complex. *J. Immunol.* **159**, 1429–1436 (1997).
128. Riihila, P. et al. Tumour-cell-derived complement components C1r and C1s promote growth of cutaneous squamous cell carcinoma. *Br. J. Dermatol.* https://doi.org/10.1111/bjd.18095 (2019).
129. Imamura, T. et al. Influence of the C5a–C5a receptor system on breast cancer progression and patient prognosis. *Breast Cancer* **23**, 876–885 (2016).
130. Chang, I. W. et al. Complement component 1, s subcomponent overexpression is an independent poor prognostic indicator in patients with urothelial carcinomas of the upper urinary tract and urinary bladder. *J. Cancer* **7**, 1396–1405 (2016).
131. Nasr, S. H., Fidler, M. E. & Said, S. M. Paraffin immunofluorescence: a valuable ancillary technique in renal pathology. *Kidney Int. Rep.* **3**, 1260–1266 (2018).
132. Ajona, D. et al. A combined PD-1/C5a blockade synergistically protects against lung cancer growth and metastasis. *Cancer Discov.* **7**, 694–703 (2017).
This study demonstrates that combined blockade of C5a signalling and PD1 can synergize to protect against mouse lung cancer.
133. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT03665129 (2018).
134. Ricklin, D., Mastellos, D. C., Reis, E. S. & Lambris, J. D. The renaissance of complement therapeutics. *Nat. Rev. Nephrol* **14**, 26–47 (2018).
135. Mastellos, D. C., Ricklin, D. & Lambris, J. D. Clinical promise of next-generation complement therapeutics. *Nat. Rev. Drug Discovery* **18**, 707–729 (2019).
136. Surace, L. et al. Complement is a central mediator of radiotherapy-induced tumor-specific immunity and clinical response. *Immunity* **42**, 767–777 (2015).
137. Facciabene, A. et al. Local endothelial complement activation reverses endothelial quiescence, enabling T-cell homing, and tumor control during T-cell immunotherapy. *Oncoimmunology* **6**, e1326442 (2017).
138. Diebolder, C. A. et al. Complement is activated by IgG hexamers assembled at the cell surface. *Science* **343**, 1260–1263 (2014).
This study demonstrates that the most efficient C1q binding and complement activation can be achieved if IgG hexamerizes upon binding to antigen. This concept is being used as an emerging novel strategy for antibodies engaging complement as an anticancer therapy.
139. de Jong, R. N. et al. A novel platform for the potentiation of therapeutic antibodies based on antigen-dependent formation of IGg hexamers at the cell surface. *PLOS Biol* **14**, e1002344 (2016).
140. Lee, C. H. et al. IgG Fc domains that bind C1q but not effector Fcγ receptors delineate the importance of complement-mediated effector functions. *Nat. Immunol.* **18**, 889–898 (2017).
141. Bohlson, S. S., Garred, P., Kemper, C. & Tenner, A. J. Complement nomenclature-deconvoluted. *Front. Immunol.* **10**, 1308 (2019).
142. Hoadley, K. A. et al. Cell-of-origin patterns dominate the molecular classification of 10,000 tumors from 33 types of cancer. *Cell* **173**, 291–304.e6 (2018).
143. Cerami, E. et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov.* **2**, 401–404 (2012).
144. Tang, Z. et al. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res.* **45**, W98–W102 (2017).
145. Piao, C. et al. Complement 5a stimulates macrophage polarization and contributes to tumor metastases of colon cancer. *Exp. Cell Res.* **366**, 127–138 (2018).
146. Magrini, E. & Garlanda, C. Pro-tumoral role of complement activation in murine sarcoma models. Presented at the 12th International Conference on Complement Therapeutics (2019).
147. Riihilä et al. Complement component C3 and complement factor B promote growth of cutaneous squamous cell carcinoma. *Am. J. Pathol.* **1871**, 1186–1197 (2017).
148. Aykut, B. et al. The fungal mycobiome promotes pancreatic oncogenesis via activation of MBL. *Nature* **574**, 264–267 (2019).

Acknowledgements
This work was supported by grants from Pierre Fabre Research Institute (to W.H.F. and L.T.R.); the Association pour la Recherche sur le Cancer (ARC), the Cartes d'Identité des Tumeurs (CIT) programme from the Ligue Nationale Contre le Cancer (RS19/75-111) and Cancer Research for Personalized Medicine (CARPEM) (to L.T.R.); and the Institut National du Cancer (INCa) (HTE Plan Cancer (C1608DS) and PRTK G26 NIVOREN) and the Association pour la recherche de thérapeutiques innovantes en cancérologie (ARTIC, BioniKK programme) (to C.S.-F.). This work was also supported by INSERM, University of Paris, Sorbonne University, CARPEM T8 and the Labex Immuno-Oncology Excellence Program. M.V.D. received a PhD fellowship from ARC, and F.P. received a doctoral fellowship from CARPEM.

Author contributions
L.T.R., M.V.D. and F.P. researched data for the article. M.V.D. performed the transcriptomic analyses and designed the figures. L.T.R., M.V.D., C.S.-F. and W.H.F. provided a substantial contribution to discussions of the content. L.T.R., M.V.D. and W.H.F. contributed to writing the article and all authors contributed to reviewing and editing the manuscript before submission.

Competing interests
L.T.R and W.H.F received research funding from the Research Institute of Pierre Fabre. M.V.D, F.P. and C.S-F declare no competing interests.

Peer review information
Nature Reviews Cancer thanks V. Afshar-Khargan, C. Kemper and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Supplementary information
Supplementary information is available for this paper at https://doi.org/10.1038/s41568-019-0210-0.