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Abstract

The omics era made possible the quest for efficient markers for cancer progression and revealed that macrophage populations are much more complex than just the M1/M2 dichotomy. Complement C1q pops up as a marker of a tolerogenic and immunosupressive macrophage populations in both healthy and tumor tissues, but the specific role of C1q+ tumor associated macrophages is poorly understood. C1q is co-expressed in healthy and tumor macrophages with HLA-DR, APOE and MRC1 (CD206), suggesting a resident origin of this population. Tumor associated macrophages expressing C1q correlate with T cell exhaustion and poor prognosis in numerous cancers. Herein, we discuss the plural roles of C1q in these macrophages and how it could drive cancer progression.

Keywords: C1q, tumor-associated macrophages, T cells exhaustion, complement system

Highlights

- Complement component C1q is a marker of a particular sub-population of tissue-resident macrophages and tumor associated macrophages (TAM), often expressing CD206, HLA-DR, SEPP1, FOLR2, APOE but not SPP1, as revealed by scRNAseq in different normal tissues and tumor types.
- In cancer presence of C1q+ TAM often correlates with poor prognosis.
- Presence of C1q+ TAM correlates with T cell exhaustion in cancer and immune tolerance induction in healthy tissue.
- C1q is the recognition molecule of the classical complement pathway, binding to immune complexes, pentraxins or other activators in the tumor microenvironment.
- C1q directly controls macrophage phenotype by interacting with surface receptors.
- C1q directly controls T cell phenotype be internalization, binding to mitochondria and regulation of mitochondrial metabolism.
- C1q is likely not only a biomarker of a TAM subpopulations but a driver of cancer progression.

Outstanding Questions Box

- Which mechanism does C1q regulate macrophage and T cell phenotypes in normal tissue and in tumor microenvironment? Does C1q act intracellularly in the producing macrophages or in an autocrine manner, at the cell surface?
- What is the exact origin of C1q+ macrophages?
- Could C1q, produced by other cells such as fibroblasts, regulate macrophages and T cells phenotype?
- What is the interplay between the complement cascade-mediated functions of C1q and its functions outside the cascade?
- What parallels can we draw between the break in immune tolerance and autoimmunity, driven by the congenital C1q deficiency and the immunosuppressive tumor microenvironment of the tumors that is rich in C1q+ macrophages?
- Can tumor-promoting C1q+ macrophages turn into an ally during immunotherapy with anti-checkpoint inhibitors?

C1q+ TAM in the light of scRNAseq

Searching for effective biomarkers to predict cancer progression is a holy grail in oncology. Big data is useful in this respect, but it is still challenging to find new biomarkers or fish out the most robust ones. Single cell RNA-seq can characterize the transcriptional state of individual cell types and allows one to define rare populations, otherwise lost in the bulk RNA-seq or undetectable by flow cytometry or CyTOF due to lack of prior knowledge of their existence. For a long time, macrophages had been divided into M1 (proinflammatory) or M2 (anti-inflammatory) populations. Such dichotomy seems too simplistic and outdated. Macrophages appear as a continuous spectrum of phenotypes between these two extreme populations. Thanks to single cell RNAseq analysis, scientists started to explore the macrophage universe, and two molecules have emerged: TREM2, a marker more often expressed by tumor infiltrating macrophages and associated with pro-tumorigenic actions[1], and FOLR2, a marker that suggests tissue residency[2]. Numerous studies are now detailing another marker that is expressed on macrophages, Complement component 1q (C1q). C1Q+ macrophage populations in both healthy and tumor tissues (Figure 1) have been observed for many years, but a deeper understanding of its function is still lacking.

Recent studies suggest that C1q could be used as a marker of poor prognosis for various cancers. Transcriptomic data[3] and protein staining on tumor sections showed that a high presence of C1q+ macrophages[3,4,5] is associated with higher post-surgical recurrence in clear cell renal cell carcinoma (ccRCC)[5] as well as in hepatocellular carcinoma[6], and breast cancer[7]. In osteosarcoma, the expression of C1Q, mostly by macrophages, negatively correlates with patient survival[8]. In pancreatic ductal adenocarcinoma (PDAC), C1q expression in primary tumors and hepatic metastasis is higher compared to normal tissue, and the presence of C1Q+M2-like macrophages is associated with worse prognosis[9].

Despite its potential role as a biomarker, recent studies also suggest that C1q could drive tumor progression. Here we discuss recent evidence that suggests C1q+ macrophages play a major role in tumor immunity and cancer progression.

C1q+ TAM – a bunch of gene correlations

In healthy tissue, the C1Q+ macrophage population is characterized by the expression of C1QA, C1QB, C1QC, HLA-DRB1 and MRC1 (gene coding for the CD206 molecule)[10]. Different cancer types exhibit an increased C1Q+ Tumor Associated Macrophages (TAM) population expressing CD206, HLA-DR, SEPP1 and FOLR2[11,12,4]. C1Q+ macrophages also express APOE in renal cancer[3], breast cancer[7], and liver metastasis from colorectal cancer [13]. Nevertheless, there are exceptions. In renal cancer the C1Q+ TAM do not express FOLR2 but TREM2[3], which is known to be associated with immunosuppression and poor prognosis in several cancers. Similarly, C1Q+ TAMs express TREM2 in liver metastases in patients and in mice with PDAC [14]. The C1Q+ TAM population has a strict mutually exclusive relationship with SPP1+ TAM[15,16] (Figure 2). This dichotomy is nearly perfect, although some studies have shown that C1Q+/SPP1+ associates with FOLR2+ and/or TREM2+ TAM[17,7]. In the colon, C1Q+ macrophages can be found in both healthy and tumor tissue, whereas SPP1+ macrophages are only found in tumor tissue[12]. These macrophages can be deciphered by the transcriptional factors they express: C1Q+ TAM express mostly MAF/MAFB, while SPP1+ TAM express FOS/JUN for and CEBPB/ZEB2[12]. Although these cells likely originate from the same precursor, they have a very different evolutionary path (Box 1). C1q+ TAM may be linked to sex, as females with non-small cell lung cancer present with a higher number of C1Q+ TAMs, whereas male have higher number of SPP1+ macrphages[16].

The ensemble of these gene expression correlations (positive correlation with APOE, HLA-DR, MRC1, FOLR2 or TREM2, and negative correlation with SPP1) draws a portrait of the C1Q+ macrophages as a distinct immunosuppressive population. Indeed, in melanoma and basal cell skin carcinoma, C1Q+TAM are enriched in non-responders to immune checkpoint therapy[17], suggesting that they play a role in regulating anti-tumor immunity. As such, single cell RNA sequencing (scRNA-seq) is unveiling the interplay between C1Q+ macrophages and other immune cells that might be responsible for the poor overall survival in cancer patients and lack of response to immunotherapies.

C1q correlates with T cells exhaustion

The presence of C1Q+ macrophages correlates with exhausted T cells, forming a dysfunctional immune circuit in ccRCC[3–5]. In colorectal cancer, C1Q+ TAM interact with T cell subsets. Analysis of ligand-receptors pairs revealed a significant enrichment of CXCL10-CXCR3 axis in

C1Q+ TAM, suggesting that production of CXCL10 by C1Q+ TAM binds to its receptor CXCR3, which is mostly present at the T cell surface. This finding highlights the potential role of C1Q+ TAM in the recruitment and activation of the Th1 response[12] (Figure 3 point 1). In lung cancer a similar increase in CXCL-10 was described in C1Q+ TAM, in association with an enrichment of the transcription factors IRF1, IRF7 and STAT1[16]. IRF1 correlates with STAT1, HLA-DR, PD-1 and LAG-3 in metastases of colorectal cancer[18]. Moreover, in ccRCC tumors, C1q+ cell density correlated with expression of inhibitory receptors PD-1 and LAG3 at the CD8+ T cells surface[5], and these C1Q+ macrophages express additional immune checkpoint ligands, such as PD-L1 and PDL-2[4,5]. In cervical cancer, patients with C1Q+ TAM also express high levels of immune checkpoint inhibitors including CD40L, CTLA4, LAG3, PD-1, and TIGIT[19] (Figure 3 point 2). Moreover, in mouse models of cancer, C1Q+ macrophages specifically express EBI3, a subunit of the IL-35 cytokine, which allows their cross talk with intratumoral T cells and leads to their dysfunction when combined with the p35 subunit of IL-12[20]. This gene was already described as a promotor of CD8+ T cell exhaustion, when it is expressed by Tregs[21]. Interestingly, Tregs are also found at a higher proportion within tumors with C1Q+ TAM as compared with normal samples [3].

The maturation of dendritic cells can impact on the expression levels of C1q, ultimately affecting T cell function. Indeed, immature dendritic cells express large amounts of C1q, but during dendritic cell maturation, which is driven by CXCL4, C1q gene is hyper-methylated and its expression decreases[22]. A murine model of subcutaneous injection of various murine tumor cell lines (lung cancer (LLC), colorectal cancer (MC38) or melanoma cancer (B16-F10)), showed that reduced C1q methylation (i.e. high C1q) promotes CD8+ T cell dysfunction and tumor progression[20]. In lung cancer and idiopathic pulmonary fibrosis, the methylation status of C1q decreases as compared to healthy tissue, leading to an increase in tumor associated C1Q expression, which is associated with poor prognosis[23].

In cervical cancer, tumors with a gene signature of C1Q+ TAM are more infiltrated by immune cells and express more immune-checkpoint markers than tumors with a gene signature of SPP1+ TAM[19]. However, it is unclear which cells express these immune checkpoints. Compared to SPP1+ TAM, C1Q+ TAM also express higher levels of HLA-DR[12], which could help C1Q+ TAM interact with immune cells.

Taken together, these lines of evidence show a clear correlation between C1q+ macrophages and the activation status of T cells, but is C1q a driver or a passenger in this process?

C1q in TAM… So what?

Are these C1q+ macrophages accompanying other factors responsible for poor prognosis or driving recurrence? Is C1q only a "marker" of these macrophages or does it play a direct role in their pro-tumoral effect? As C1q is a major factor involved in the complement system, recent studies are unveiling the role of the complement cascade in regulating tumor progression. However, C1q is a versatile molecule and has functions extending beyond the borders of the complement cascade. Below we describe emerging mechanisms by which C1q regulates tumor progression.

C1q in the complement system

C1q is the initiating protein of the complement cascade[24]. Complement is a part of the innate immune system and its best-known function is to defend the host against invading pathogens. C1q can trigger the classical pathway of the cascade when it binds to immune complexes, pentraxins or one of its other over 100 different ligands. The plasma source of the majority of complement proteins is the liver, but C1q is an exception, being secreted by tissue-resident macrophages[24]. To function within the cascade, C1q needs to associate with two serine proteases – C1r and C1s, which trigger the proteolytic cascade that results in the generation of proinflammatory anaphylatoxins C3a and C5a, the membrane attack complex C5b-9 and the opsonization of target cells. How could this cascade function in the context of cancer and what is the role of C1q in this scenario?

Transcriptomic analysis across different tumor types has revealed that cells of the tumor microenvironment or tumor cells themselves express components of the classical and alternative complement pathways, including C1q (Figure 1)[24]. It appears that their co-regulated overexpression is context-dependent, and their prognostic value is either favorable or poor or of undetermined significance in particular types of cancers. ccRCC falls in the "aggressive complement" group, where overexpression of these genes correlates with worse survival. *In situ* staining, scRNAseq analysis and *ex vivo* experiments revealed that the ccRCC tumors have a complement-rich environment, where some tumor cells produce C1r, C1s, C4

and C3 but need macrophage-derived C1q to activate the classical pathway on intratumoral IgG-containing immune complexes[5,9] (Figure 3 point 3). While complement is activated on most tumor cells and promotes cell death, it does not result in cell killing and even promotes tumor progression in some cancer types. This finding can be explained by the low intratumoral expression of the components of the terminal pathway[3] and the limited formation of a membrane attack complex due to expression of specific inhibitors at the tumor cell surface[5,9,25]. Thus, chronic inflammation mediated by C5a favors an immunosuppressive microenvironment and facilitates T cells exhaustion[3,4] (Figure 3 point 4). C1Q+ macrophages also expressed ApoE - a protein able to bind C1q and to activate the complement system[26]. Therefore, C1Q+ positive macrophages can induce tumor progression by triggering the complement cascade.

C1q and neoangiogenesis

C1q can interact directly with endothelial cells (EC) to promote neoangiogenesis, via still unknown cell surface receptors or heparan sulfate [27,5,28]. C1q deposits can be found at the surface of EC in the absence of other complement factors such as C3 or C4. C1q can induce adhesion, spreading and expression of adhesion molecules by directly binding EC[29]. In addition, in a lesioned area, EC start to express C1q, which induces EC permeability, proliferation, migration and endothelial tube formation *in vitro*[28]. *In vivo*, C1q-/- mice show a disordered vascular network in subcutaneously implanted tumors. Other *in vitro* studies also indicate that the interaction of C1q with melanoma and PDAC cells promotes proliferation, migration and invasion of the tumor cells[9,27] (Figure 3 point 5).

C1q, immune tolerance, and T cell exhaustion

In physiology C1q regulates human macrophage polarization via interactions with LAIR1, as a switch towards inflammation resolution to avoid autoimmunity[30–32] (Figure 3 point 6). Indeed, complete C1q deficiency, although very rare, is the strongest genetic predisposing factor to systemic autoimmunity[33]. C1q opsonizes apoptotic cells, enhances their uptake by macrophages and immature dendritic cells, modulates cytokine release, and promotes immune tolerance[34–37] Figure 3 point 7). In this context macrophages and immature dendritic cells produce and secrete C1q, which will act in an autocrine manner[38]. In addition, C1Q+ cells

have been found to associate with tolerance in the fetal-maternal interface during pregnancy, which is reminiscent of that seen in cancer. An HLA-DR^{high} group of cells, characterized by high expression of C1Q, APOE, various genes of lipid metabolism, EBI3, IDO 1 and 2 (inducers of cell tolerance) and the immune-checkpoint PD-L1, limits T-cell expansion driven by fetal alloantigen and establishes an immune-tolerance to fetal allotransplant[39]. These processes are not well studied in cancer, but it is tempting to speculate that uptake of C1q-opsonized dying cancer cells may be perceived in a tolerogenic manner by the TAM, and that this C1q will re-orient their phenotype to hamper the immune response against tumor neo-antigens.

C1q and cell metabolism

Another function of C1q that is unrelated to the complement cascade is to play as a rheostat of the mitochondrial metabolism of CD8 T cells[40] (Figure 3 point 8). Extracellular C1q is internalize by CD8+ Tcells and is found at the surface of mitochondria. Intracellularly C1q is involved in the upregulation of mitochondria biogenesis genes, leading to the differentiation of CD8+ Tcells into memory T cells and not effector cells. By C1q its presence at the mitochondrial surface C1q dampens CD8+ T cell responses to self-antigens. Congenital deficiency of C1q is rare in humans, but it results in enhanced CD8+ T cell responses, becoming the strongest genetic predisposing factor for autoimmunity. C1q is not produced by T cells, but it is internalized from the extracellular milieu. Therefore, it is tempting to speculate that C1q, secreted by the C1Q+ macrophages in ccRCC downregulate the capacity of adjacent intratumoral CD8 T cells to respond to stimulation, thereby contributing to their exhausted phenotype. The intracellular role of C1q within macrophages has not been studied, but again, we speculate that it may affect their metabolism, and functional orientation towards an immunosuppressive phenotype, thereby inducing T cell exhaustion In the context of atherosclerosis, C1q can modulate the cytokine expression of macrophages while they digest lipid proteins, leading to an M2-like polarization[41]. The MafB transcription factor, which is present in C1Q+TAM in colorectal cancer[12], was also described to promote M2 polarization in atherosclerosis[42].

These results raise the question whether pro-tumoral M2-like macrophages start to express C1q or does C1q allow the polarization of these cells into pro-tumoral macrophages?

C1q in CAF, another actor of this story

Like macrophages, recent studies have begun to distinguish different sub-types of fibroblasts and especially cancer-associated fibroblasts (CAF). They notably, showed that in breast and pancreatic cancer a subpopulation of CAF MHC-II+ led to an immunosuppressive tumor micro-environment[43,44]. In breast cancer, mesenchymal stem cells (MSC) can produce exosomes containing TGF-β and C1q[45]. Fibroblasts are similar to MSC and can be considered as old MSC[46]. The exosomes from MSC but not from tumor cells drive the polarization of Monocytic myeloid derived suppressor cells (M-MDSC) into M2-macrophages overexpressing CD206, PD-L1 and MHC-II[45] (Figure 3 point 9). In contrast, in lung cancer, MHC-II+ CAF can activate the TCR of CD4+ T cells and rescue them from apoptosis via the C1q/C1qbp axis. Indeed, these CAFs have the ability to produce C1q that will be released and will interact with its receptor C1qbp present at the surface of CD4+ T cells[47] (Figure 3 point 10). These recent studies suggest that C1q excreted by CAF influence the tumor immune microenvironment, by both directly acting on T cells and on macrophage polarization. More studies are necessary to determine the role of CAF upstream of macrophage actions.

Concluding remarks and future perspectives

Analyses of scRNAseq data and the growing literature indicate that C1q+ TAM are key players in the tumor microenvironment, but many questions remain unanswered (see Outstanding Questions). Although formal proof is needed, it seems that C1q+ TAM are drivers of cancer progression with a direct pro-tumoral effect in the absence of immunotherapy. Interestingly, ccRCC tumors with mature tertiary lymphoid structures have IgG deposition on tumor cells and respond better to anti-checkpoint inhibitors[48]. The tertiary lymphoid structure signature contained C1q genes and APOE, and the macrophages likely contributed to the elimination of tumor cells and the mounting of an anti-tumoral immune response. Further studies are needed to determine how C1q and complement contribute to this process. It is still poorly understood if C1q acts in an autocrine manner, on the cell surface of the macrophages or intracellularly or as deposits on tumor cells. It is also unclear which cells C1q impacts on and how it controls immune activation vs tolerance and exhaustion. Understanding the mechanisms of action of C1q in the modulation of macrophages phenotype in health and

disease and whether C1q can be harnessed as a therapeutic target in combination with anti-cancer checkpoint inhibitors are perspectives for the future.

Beyond C1q, other complement proteins also impact on the TAM phenotype. Factor H differentiates CD14+ human monocytes into immunosuppressive macrophages in the context of breast but not renal cancer[49,50]. C5aR1 is also overexpressed on TAM, which exhibit an M2-like functional profile. C3aR deficiency is associated with reduced accumulation and functional skewing of TAM, increased T cell activation and response to anti-PD-1 therapy[51] in mouse models of sarcoma. In models of squamous carcinogenesis, C5aR1 inhibition improves chemotherapy efficacy by reprogramming macrophages to recruit cytotoxic CD8+ T cells[52]. Similar effects were observed in a mouse model of renal cancer[53]. Future studies should address how other complement proteins and activation fragments, such as FH or C3a and C5a impact on C1q+ TAM vs TAM lacking C1q expression.

Acknowledgements

This work was supported by grants from: Les Entreprises contre le Cancer, Ligue Regionale Contre le Cancer (GEFLUC) IDF; Institut National Du Cancer INCa_16096; Agence Nationale de la Recherche ANR-21-CE14-0066-02; Comité de Paris de la Ligue contre le cancer RS22/75-37 and l'Idex Sorbonne Université (Programmes Investissements d'Avenir Émergences 2021-2022) to LTR. Equipe labellisée Ligue contre le Cancer, 2021-2 to CSF and WHF. This work is also supported by The Labex Immuno-Oncology Excellence Program, INSERM, Université de Paris and Sorbonne Université.

The authors declare no competing interests.

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Box 1. C1q TAM = tissue resident macrophages?

The origins of C1Q+ TAM is not well understood, but a Single Cell RNAseq study across species (mouse, rat, pig and human) found a conserved gene signature of kidney resident macrophages composed of CD81, CD74 (coding for HLA-DR-gamma), APOE and C1QC[54]. A similar study in lung found conserved genes between human and mice macrophages, including APOE, MRC1, C1QA, C1QB and C1QC[55]. These data agree with the ontogenic study of multiple tumor types, describing a high similarity between C1Q+ and FOLR2+ macrophages. FOLR2+ is associated with an embryonic origin of macrophages[9,10,15]. In healthy lung tissue, two types of macrophages can be distinguished, alveolar or interstitial. A sub-type of alveolar macrophages expresses both C1Q and APOE[56]. C1Q+ macrophages are larger in size and are identified as resident in patients with colorectal cancer liver metastasis[13] and in the peritoneal cavity of mice[57]. These large C1Q+ TAM confer poor prognosis[13], and they have features of foamy cells, overexpress genes of cholesterol metabolism, scavenger receptors, as well as C1QA and C1QB.

C1Q+ TAM express, in a conserved way in multiple cancer types, HLA-DR, APOE and MRC1, suggesting an embryonic, tissue resident origin. However, a recent study showed that C1Q and APOE are commonly expressed by TAM in breast cancer[7] and resident TAM express FOLR2+, while infiltrating TAM express TREM2+, opening new questions on the origin of the C1q+ TAM in particular contexts.

A transcriptional trajectory study using the example of colorectal cancer[12] shows that C1Q+ TAM and SPP1+ TAM populations could arise from a common precursor: CD14-expressing monocytes, which differentiate towards FCN1+ monocyte-like cells and different macrophage populations. One of them are the SPP1+, while the other overexpresses IL-1β+ and gives rise to the sub-population of C1q+ TAM. In healthy tissue, C1q regulates the macrophages polarization during the uptake of apoptotic cells by inhibiting NLRP3 gene expression, that suppresses the IL-1 β cleavage[36]. It is interesting to note that the FCN1 is defense collagen and a close relative to C1q, called ficolin 1, activator of the lectin complement pathway[24]. Another complement protein, C5aR1, regulate the IL-1 β production un macrophages[58]. Whether a cross-talk exist between these complement proteins/pathways in macrophages, being they TRM or TAM, is still unknown.

Finally, future work will show whether there is a difference in the C1q+ macrophages from normal tissue (the ones that generate the plasma C1q pool, https://www.proteinatlas.org/ENSG00000173372-C1QA/celltype) and the ones in tumors.

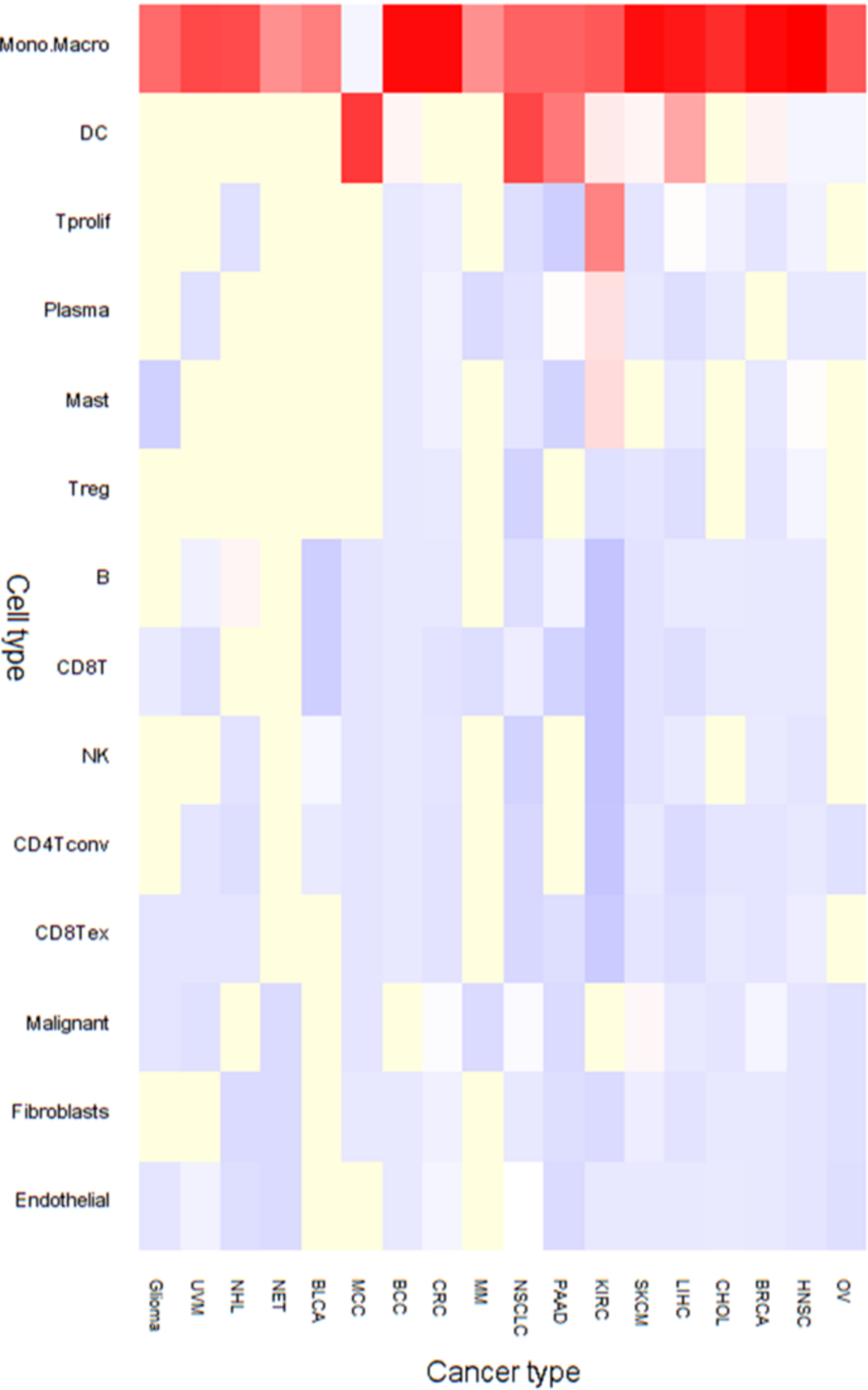
Figure legends

Figure 1: Heatmap representing the expression of C1QB gene in different immune or non-immune cells depending on the cancer type. Data is retrieved from the Tumor Immune Single-cell Hub (TISCH) scRNA-seq database[59]. In light yellow are indicated positions for which data is not available, in blue low gene expression of C1QB and in red high gene expression of C1QB. Similar data were obtained for C1QA and C1QC.

Figure 2: Single Cell Analysis from the TISCH database. Gene expression of SPP1, C1QA, C1QB and C1QC of Colorectal Carcinoma tumor (accession number GSE146771). On the left, the cell characterization of the Single Cell RNAseq analysis. The upper panel is a large view of the gene expression. The lower panel is a zoom in on the Monocytes/Macrophages population.

Figure 3: Proposed mechanisms by which C1q+ TAM drive cancer progression. C1q is produced by sub-populations of TAM and TRM. 1) The C1Q+ TAM secrete the chemokine CXCL-10 that binds to its receptor CXCR3 at the surface of CD8+ and CD4+ T cells, especially Th1 T cells. This binding will activate and recruit T cells inside the tumor. 2) By its expression of immune checkpoint inhibitors, C1Q+ TAM interact with T cell immune checkpoints, favoring T cell exhaustion. 3) C1q impacts on tumor progression by activating the complement cascade in the extracellular space, which will generate the anaphylatoxins C3a and C5a, and 4) promote chronic inflammation. 5) The C1q molecule can interact directly with endothelial cells to promote neo-angiogenesis needed for the tumor growth. 6) In healthy tissue, C1q is produced by TRM and immature dendritic cells (imDC) to maintain homeostasis and opsonize apoptotic cells. 7) The C1q molecule can also act in an autocrine way on macrophages, by interacting with LAIR1 to regulate their polarization. 8) CD8+ T cells can internalize C1q, which will interact with mitochondria to control the CD8 metabolism. 9) The secretion of C1q-containing exosomes by fibroblasts leads to M2 polarization of macrophages. 10) MHC-II+ cancer

associated fibroblasts also produce C1q, which binds to its receptor C1qbp at the surface of CD4+ T cells to activate and to rescue them from apoptosis. Figure generated with biorender.com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Malignant
Fibroblasts
Endothelial
Cell type
Glioma
UVM
NHL
NET
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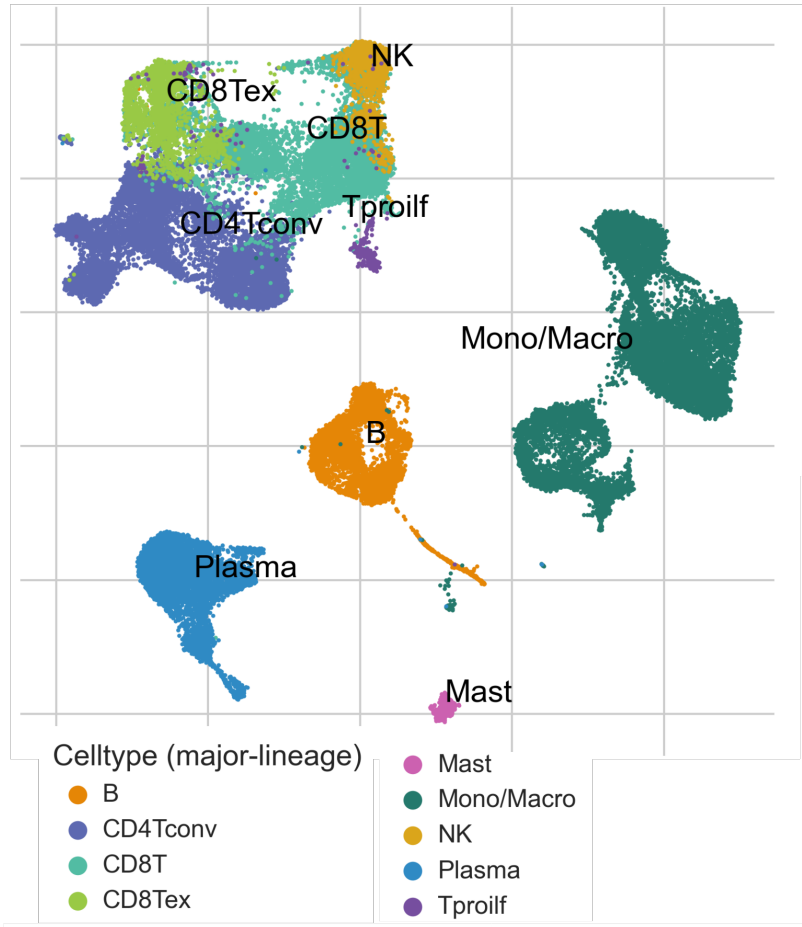
Cancer type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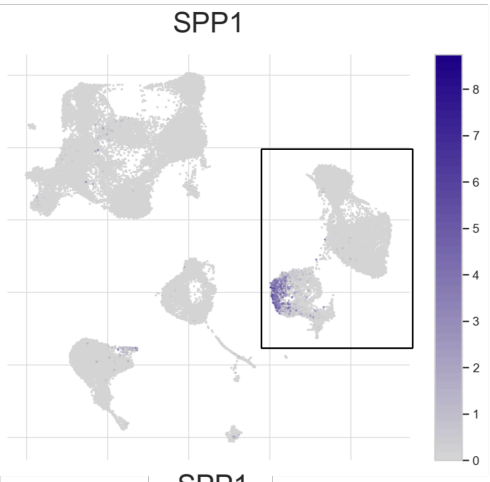
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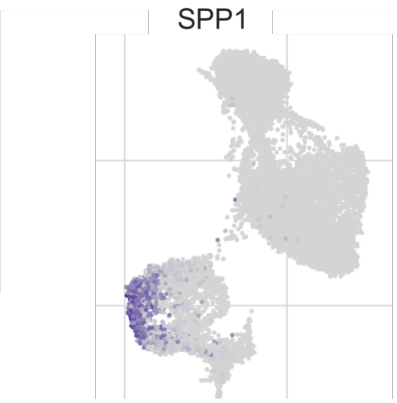
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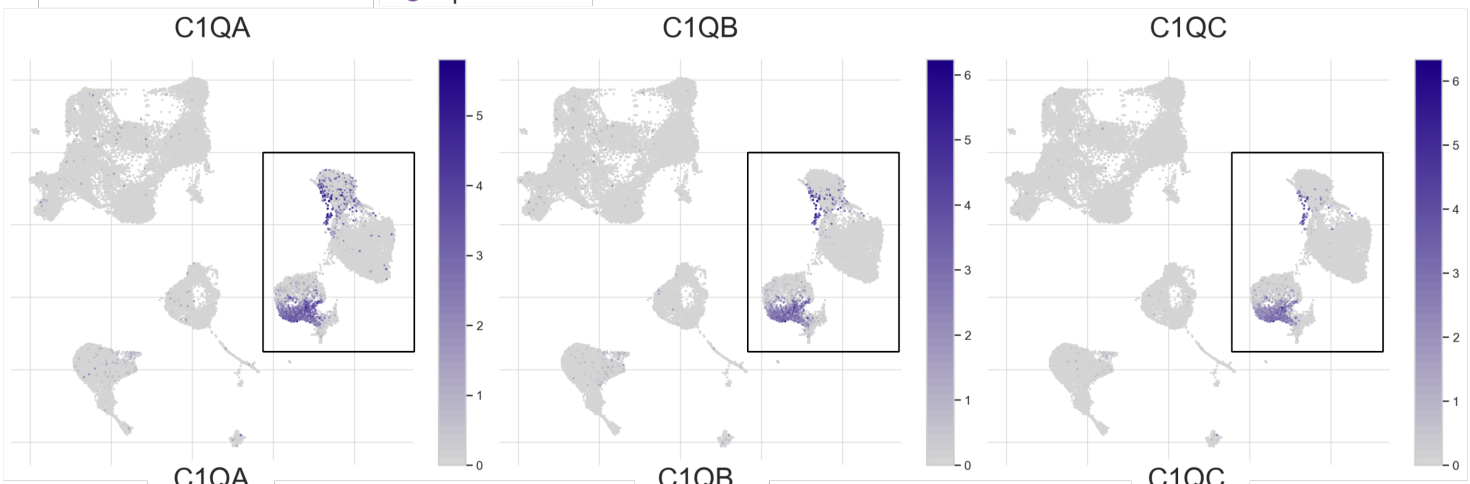
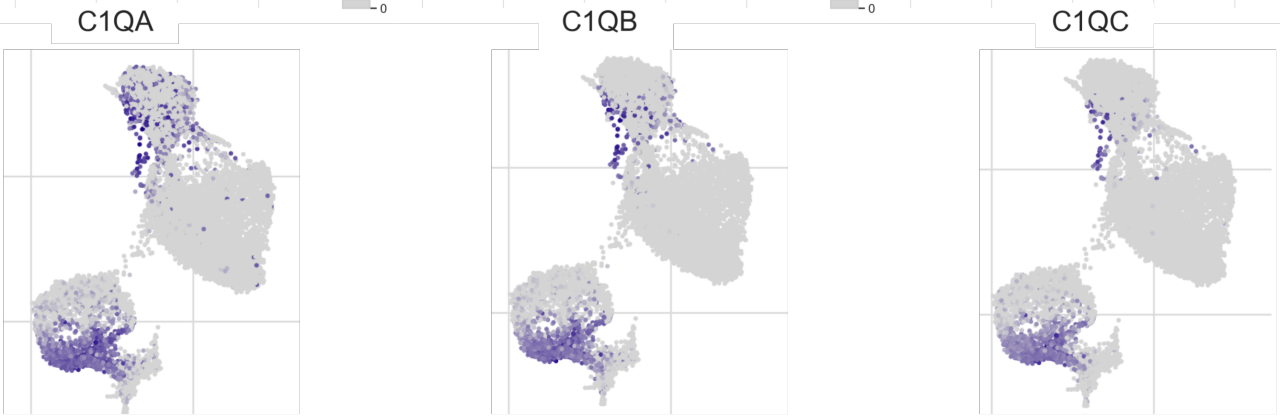

C1QA
C1QB
C1QC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C1QB
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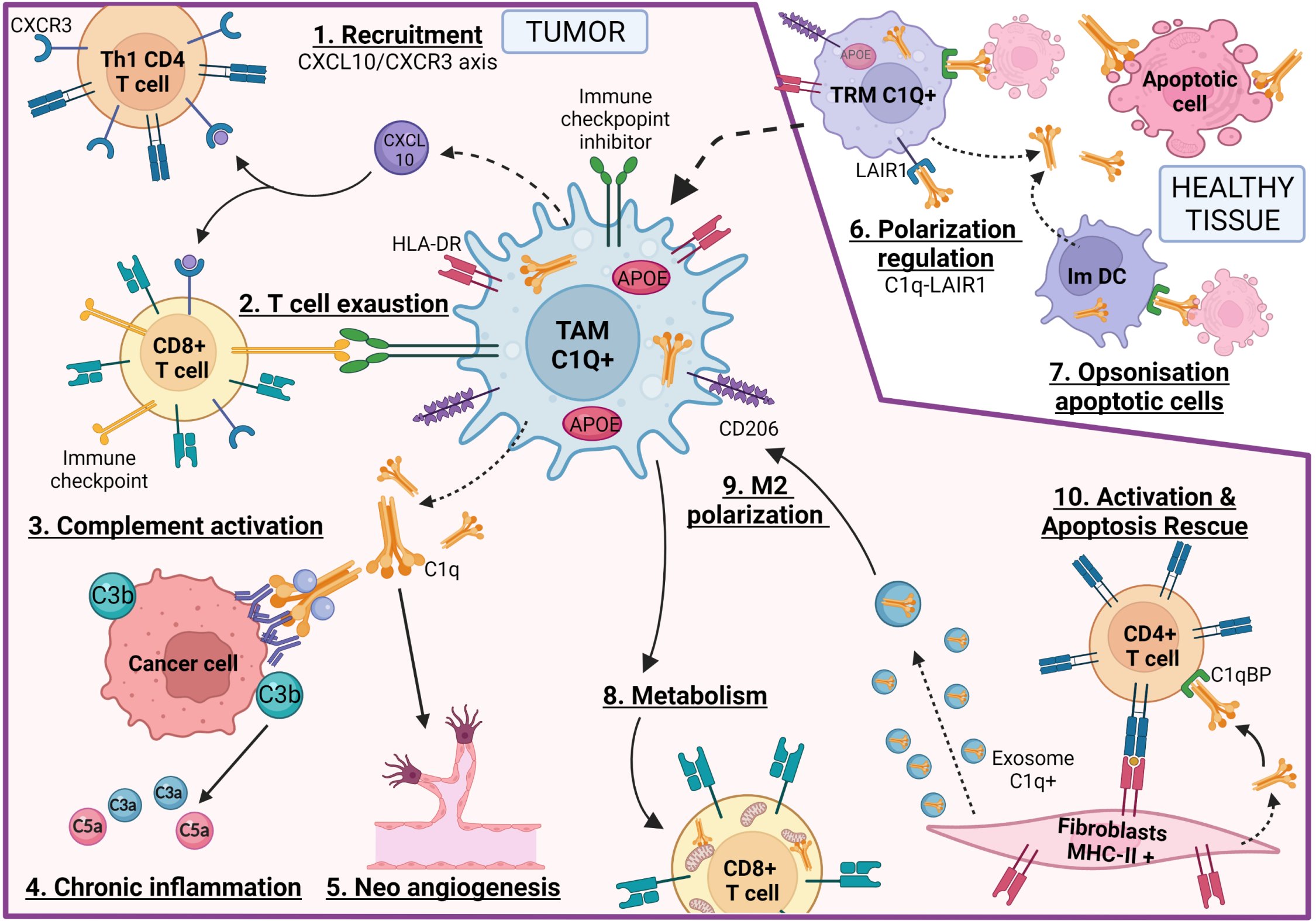
CXCR3
Th1 CD4 T cell
1. Recruitment
CXCL10/CXCR3 axis
TUMOR
CXCL 10
Immune checkpopint inhibitor
HLA-DR
APOE
TAM C1Q+
APOE
CD206
CD8+ T cell
2. T cell exaustion
Immune checkpoint
3. Complement activation
C1q
C3b
Cancer cell
C3b
C3a
C3a
C5a
C5a
4. Chronic inflammation
5. Neo angiogenesis
8. Metabolism
CD8+ T cell
9. M2 polarization
APOE
TRM C1Q+
LAIR1
6. Polarization regulation
C1q-LAIR1
Apoptotic cell
HEALTHY TISSUE
Im DC
7. Opsonisation apoptotic cells
10. Activation & Apoptosis Rescue
CD4+ T cell
C1qBP
Exosome C1q+
Fibroblasts MHC-II +