The acidic tumour microenvironment: manipulating the immune response to elicit escape

Abbreviated title: Immune escape in the tumour microenvironment

Catherine M. Worsley [1,2,3] *, Rob B. Veale [4] and Elizabeth S. Mayne [2,5,6]

[1] Department of Immunology, Faculty of Health Sciences, University of Pretoria, South Africa

[2] Department of Haematology and Molecular Medicine, Faculty of Health Sciences, University of the Witwatersrand, South Africa

[3] National Health Laboratory Service, South Africa

[4] School of Molecular and Cell Biology, Faculty of Science, University of the Witwatersrand, South Africa

[5] Department of Immunology Faculty of Health Sciences, University of the Witwatersrand, South Africa

[6] Division of Immunology, Department of Pathology, Faculty of Health Sciences, University of Cape Town

* Corresponding author: Catherine M. Worsley

      Email address: u05116602@up.ac.za

      Physical address: Department of Immunology Room 5-42, Pathology Building, 5 Bophelo Road, University of Pretoria, City of Tshwane, 0001, South Africa

      Phone: +27 (0)12 319 2322

Abstract

The success of cancer treatment relies on the composition of the tumour microenvironment which is comprised of tumour cells, blood vessels, stromal cells, immune cells, and extracellular matrix components. Barriers to effective cancer treatment need to be overcome, and the acidic microenvironment of the tumour provides a key target for treatment. This review intends to provide an overview of the effects that low extracellular pH has on components of the tumour microenvironment and how they contribute to immune escape. Further, potential therapeutic targets will be discussed.

Keywords

Tumour microenvironment; acidosis; tolerance; immune escape; T cells

Abbreviations

ACE – acetazolamide

ACE-NP- acetazolamide nanoparticle

AKT – a serine/threonine-specific protein kinase

APC – antigen presenting cell

ATPase - adenosine triphosphatase

Breg – regulatory B cell

CA – carbonic anhydrase

CA II – carbonic anhydrase II

CA IX – carbonic anhydrase 9

CA XII – carbonic anhydrase 12

CAF – cancer-associated fibroblast

CCL – chemokine (C-C motif) ligand

CCL2 – chemokine (C-C motif) ligand 2

CCL7 – chemokine (C-C motif) ligand 7

CCL8 – chemokine (C-C motif) ligand 8

CCL22 – chemokine (C-C motif) ligand 22

CD – cluster of differentiation

CO_2 – carbon dioxide

CSF-1 – colony stimulating factor 1

CTL – cytotoxic T-lymphocyte

CTLA-4 – cytotoxic T-lymphocyte-associated protein 4

CXCL – chemokine (C-X-C motif) ligand

CXCL1 – chemokine (C-X-C motif) ligand 1

CXCL8 - chemokine (C-X-C motif) ligand 8

CXCL12 - chemokine (C-X-C motif) ligand 12

CXCL13 - chemokine (C-X-C motif) ligand 13

CXCL16 – chemokine (C-X-C motif) ligand 16

DAMP – damage-associated molecular patterns

DC – dendritic cell

DNA – deoxyribonucleic acid

EC – endothelial cell

ECM – extracellular matrix

EGF – epidermal growth factor

EGFR – epidermal growth factor receptor

EMT – epithelial-mesenchymal transition

EPO – erythropoietin

ERK – extracellular signal-regulated kinase

EV – extracellular vesicle

FAK – focal adhesion kinase

FGF – fibroblast growth factor

FGF2 – fibroblast growth factor 2

FoxP3 – forkhead box protein P3

GAPDH – glyceraldehyde 3-phosphate dehydrogenase

GLUT – glucose transporter

GLUT-1 - glucose transporter-1

GLUT-3 - glucose transporter-3

GTPase – guanosine triphosphatase

H – hydrogen

H^+ ATPase – hydrogen adenosine triphosphatase

HIF – hypoxia-inducible factor

HIF-α – hypoxia-inducible factor alpha

HIF-1 – hypoxia-inducible factor-1

HLA – human leukocyte antigen

HNSCC – head and neck squamous cell carcinoma

HRE – hypoxia response element

ICAM-1 – intercellular cell adhesion molecule 1

IDO - indoleamine 2,3-dioxygenase

IFN-γ – interferon gamma

IL – interleukin

IL-1α – interleukin-1 alpha

IL-1β – interleukin-1 beta

IL-10 – interleukin-10

IL-12 – interleukin-12

IL-13 – interleukin13

IL-2 – interleukin-2

IL-35 – interleukin-35

IL-4 – interleukin-4

IL-6 – interleukin-6

IL-8 – interleukin-8

iNOS – inducible nitric oxide synthase

JNK – c-Jun N-terminal kinase

K – potassium

K^+ ATPase – potassium adenosine triphosphatase

LD - lipid droplet

MCT – monocarboxylate transporter

MCT-1 – monocarboxylate transporter 1

MCT-4 – monocarboxylate transporter 4

MDSC – myeloid-derived suppressor cell

MHC – major histocompatibility complex

MMP – matrix metalloproteinase

MMP-8 - matrix metalloproteinase 8

MMP-9 - matrix metalloproteinase 9

MPO - myeloperoxidase

MV - microvesicle

Na – sodium

NAD^+ - nicotinamide adenine dinucleotide

NADH - nicotinamide adenine dinucleotide plus hydrogen

NE – neutrophil elastase

NET – neutrophil extracellular trap

NFAT – nuclear factor of activated T cells

NF-κB – nuclear factor kappa B

NHE – sodium-hydrogen exchanger

NHE1 – sodium-hydrogen exchanger 1

NK – natural killer

NKG2D - natural killer group 2D

NO – nitric oxide

NP - nanoparticle

PD-1 - programmed cell death protein-1

PDGF – platelet derived growth factor

PD-L1 – programmed death ligand-1

PD-L2 - programmed death ligand-2

pHe – extracellular pH

pHi – intracellular pH

PPARα – peroxisome proliferator-activated receptor alpha

PPI – proton pump inhibitor

ROS – reactive oxygen species

STAT5 – signal transducer and activator of transcription 5

TAM – tumour associated macrophage

TCR – T cell receptor

TGF-β – transforming growth factor beta

TGF-β1 – transforming growth factor beta1

Th – T helper

Th1 – T helper 1

Th2 – T helper 2

Th17 – T helper 17

TIL – tumour infiltrating lymphocyte

TLR – Toll-like receptor

TME – tumour microenvironment

TNF-α – tumour necrosis factor alpha

TRAIL/Apo-2L – TNF-related apoptosis-inducing ligand

Treg – regulatory T cell

V-ATPase – vacuolar-type H^+ adenosine triphosphatase

VCAM-1 – vascular cell adhesion molecule 1

VEGF – vascular endothelial growth factor

VGSC - voltage gated sodium channels

α – alpha

β – beta

γ – gamma

κ – kappa

ζ - zeta

1. <u>Introduction</u>

Understanding the effects of low extracellular pH (pHe) on tumour biology is vital to treating and curing cancer. The tumour microenvironment (TME) is the physical niche in which a cell develops into a tumour cell. This environment influences different stages of oncogenesis by inducing angiogenesis, promoting sustained and uncontrolled clonal proliferation, stimulating invasion and metastasis, and by assisting tumour cells in evading checkpoint regulation and avoiding cell death [1].

The TME contains a number of different cell types including fibroblasts, innate and adaptive immune cells, adipocytes, and endothelial cells (ECs), as well as extracellular matrix (ECM) components, signalling molecules, and antibodies [2, 3]. These components within the TME, controlled and manipulated by the tumour itself, co-operate in a series of complex interactions to create an environment in which tumour cells adapt, survive, and spread to other tissues. As tumour cells proliferate and tumour size increases, molecular and cellular changes occur in the TME [4].

Tumour cells use a number of strategies to evade the immune response including impaired antigen presentation, the release of immunosuppressive molecules and extracellular vesicles (EVs) (containing multiple humoral factors), and overexpression of checkpoint molecules [5, 6]. Checkpoint molecules, such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed-death cell protein 1 (PD-1), are instrumental in preventing T cell activation. The T cell receptor (TCR) binds to major histocompatibility complex (MHC) molecules presenting antigen, and requires a second signal for activation. CD28 on the T cell binds to B7 molecules (CD80/CD86) on antigen-presenting cells (APCs) (e.g. dendritic cells, macrophages) leading to stimulation of the T cell [7]. CTLA-4 is a CD28 homolog that competes with a higher affinity for B7. It inhibits T cell stimulation, and causes decreased interaction of T cells with APCs [7]. PD-1 also regulates T cell activation by binding to its ligands programmed death ligand-1 (PD-L1) or PD-L2. This interaction inhibits T cell proliferation, the production of interferon gamma (IFN-γ), tumour necrosis factor alpha (TNF-α) and interleukin-2 (IL-2), and decreased T cell survival. Many tumour cells express high levels of PD-L1, which is associated with more tumour infiltrating lymphocytes (TILs) and worse prognosis [7].

Evolution of the TME is strongly influenced by tumour cell metabolism which causes hypoxia, glucose and nutrient depletion, waste accumulation, oxidative stress, lactate accumulation, and increased extracellular acidosis [4, 8]. This acidic TME is incompatible with normal cell survival, with low pHe promoting cancer development in all stages from dysplasia to malignant disease [8]. Tumour cells adapt to survive acid-mediated toxicity, evade the immune system, and escape therapeutic intervention [9]. These strategies enable the fittest clones to survive in the acidic TME. These factors, combined with the acidic microenvironment can affect both treatment pharmacokinetics and effectiveness. This review focusses on how the acidic TME directly impacts tumour cells, and how it manipulates both stromal and immune cells to drive immune escape. This has implications for targeting specific processes in the acidic TME to improve therapeutic intervention and patient outcomes.

1.1 Tumour cell metabolism lowers extracellular pH in the TME

The development of low pHe in tumours is associated with hypoxia. Although tumours promote angiogenesis, the resulting vascular network is often chaotic and inefficient leading to reduced oxygen and nutrient transport, and removal of metabolic waste [10]. Cancer cells have a high demand for glucose, and use anaerobic glycolysis to maintain intracellular energy levels under hypoxic conditions [3, 10]. This causes accumulation of H^+ ions and lactic acid in the TME. In aggressive cancers, oncogenes drive aerobic glycolysis even under normoxic conditions, known as the Warburg effect [11].

Glycolysis increases the production of both lactic acid and H^+ ions that cause the intracellular pH (pHi) of the tumour cell to decrease [10]. A slightly alkaline pHi (7.0-7.4) is required to maintain many cellular processes including cell adhesion, proliferation, metabolism, and apoptosis, while intracellular acidification can block cell cycle progression [3, 10]. Histone acetylation, required to allow access of chromatin to transcription factors, is negatively affected by decreasing pHi [10, 12].

To mitigate the effects of intracellular acidification, the pHi of cells is tightly controlled by the hypoxia-inducible factor (HIF) family of transcription factors which regulate the expression of genes

involved in lactate and hydrogen ion removal [10] (Figure 1). HIF proteins induce the transcription of over 70 genes in tumours via the hypoxia response element (HRE) in their promoter regions, including glucose transporters such as glucose transporter-1 (GLUT-1) and GLUT-3, and net acid extruders such as monocarboxylate transporters (MCTs), hydrogen/potassium adenosine triphosphatases (H^+/K^+ ATPases), vacuolar-type H^+-ATPases (V-ATPases), carbonic anhydrases (CAs), and sodium-hydrogen exchangers (NHEs) [12] (Figure 1). Maintaining an alkaline pHi during glycolysis results in extrusion of more H^+ ions from the cell with a concomitant decrease in extracellular pH [12].
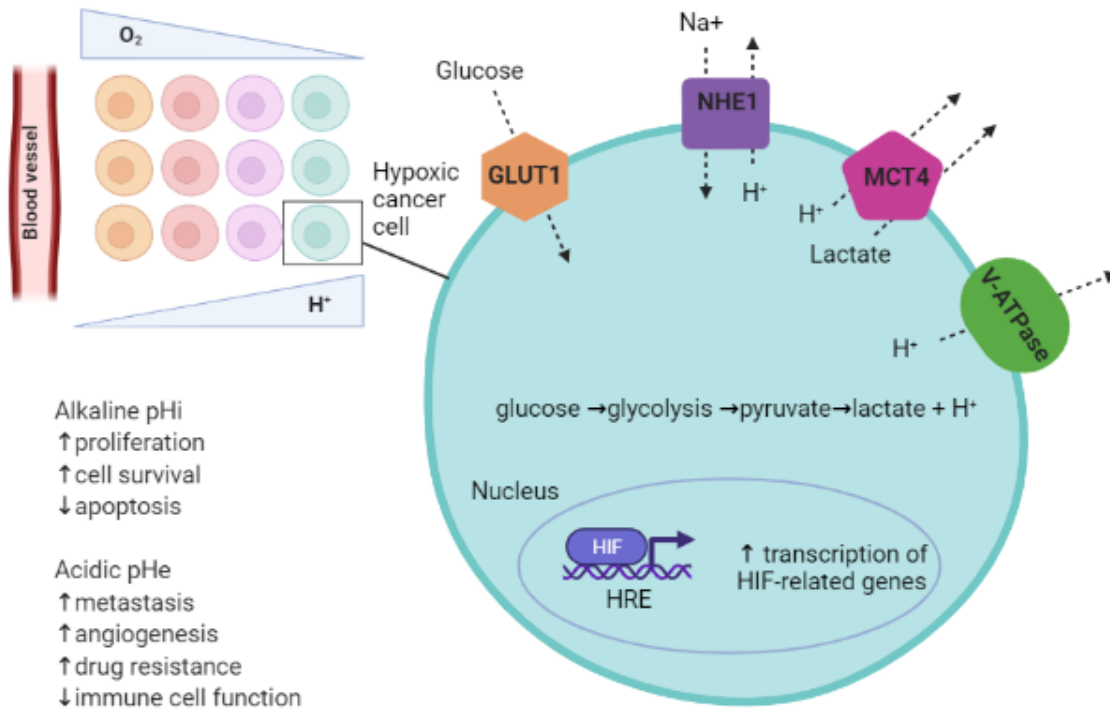

Figure 1. The HIF family regulate lactate and hydrogen ion removal. As cells are positioned further away from adequate blood supply, oxygen levels decrease to produce a hypoxic environment. HIF-1 promotes the expression of glucose transporters GLUT-1, GLUT-3 which increase glycolysis, and expression of net acid extruders. These are responsible for regulating pHi by transporting H+ and lactate out of the cell. These include monocarboxylate transporters (MCTs), Vacuolar-type H+-ATPases (V-ATPases), and sodium-hydrogen exchangers (NHEs). The resultant decrease in pHe contributes to metastasis and immune evasion.

Lactate extruded by tumour cells lowers the pH of the TME, however, lactate itself also has direct immunosuppressive functions. Increased levels of lactate are sensed by both tumour and immune cells in the TME, and impacts on the behaviour and function of these cells. Lactate efflux from immune cells depends on the concentration gradient between the extracellular and intracellular environment, and is prevented by the abundance of tumour-secreted lactate in the TME. The accumulation of lactate in $CD8^+$ cytotoxic T lymphocytes (CTLs) impairs cytotoxicity by inhibiting nuclear factor of activated T cells (NFAT), causing decreased IFN-γ production. It promotes CTL and natural killer (NK) cell apoptosis by increasing CTLA-4 and PD-L1 expression [13]. T cell polarization is

also affected by lactate, with a decrease in anti-tumoural T helper 1 (Th1) cells with concomitant increases in regulatory T cells (Tregs) [14]. Increased lactate causes a reduction of nicotinamide adenine dinucleotide (NAD$^+$) to NADH, leading to depletion of post-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) glycolytic intermediates and serine which are vital for T cell proliferation [15]. Lactate also impacts on antigen processing and presentation, differentiation and activation of dendritic cells (DCs), and induces a tolerogenic M2 tumour-associated macrophage (TAM) phenotype [13].

1.2 The survival of adaptive cancer clones is promoted by the acidic TME

Normal mammalian cells cannot survive long in a microenvironment with an pHe of less than 7.2 [3]. The pHe of cancer cells is usually between 6.7-7.1, but can drop as low as 6.0 [10]. Heterogeneous cell clones within the TME compete for space and resources, providing a selective survival advantage over suboptimal clones. In addition, the presence of the proton extruder channels shown in Figure 1 provide an adaptive advantage. The acidic TME drives the evolution of cancer cells with more malignant phenotypes adapted to survive the acidic TME [8]. Depending on the tumour type and microenvironmental conditions, cancer cells have to adapt their metabolic strategies [8]. Cells adapted to an acidic environment metabolize glucose more rapidly at low pH, and may adapt alternate strategies in the hypoglycaemic TME including the metabolism of fatty acids [16].

Extracellular acidity also drives epithelial-mesenchymal transition (EMT), induces genetic instability and DNA damage, redistribution of lysosomes, and promotes ECM remodelling and invasiveness [8]. During EMT, dynamic cytoskeletal reorganization is needed to enable morphology change, migration, and invasive capabilities of cancer cells [17]. The acidic pHe regulates cytoskeletal changes by promoting focal adhesion maturation, activating Rho-GTPases, and regulating microfilament reorganization through β1-integrin-activated focal adhesion kinase (FAK) signalling [17]. Reorganization of cytoskeletal proteins such as actin, tubulin, and vimentin, contributes to acidic pHe-triggered morphology change and cell migration [17]. Genetic instability is induced by extracellular acidosis which causes double-stranded DNA breaks through reactive oxygen species (ROS) production, and prevention of damage repair [8]. This allows for the mutation of clones, and selection of clones with survival advantages.

Extracellular acidity promotes autocrine transforming growth factor beta 2 (TGF-β2) signalling in cancer cells, which enhances lipid droplet (LD) formation enabling storage of energy to resist apoptosis [18]. Acidosis-induced TGF-β2 activation promotes EMT as well as fatty acid metabolism [18]. Another advantage stimulated by chronic acidosis is the increase in distribution of lysosomes to the plasma membrane where they protect the plasma membrane against acid-mediated damage [19]. Lysosomes containing cathepsins are also stimulated, increasing ECM remodelling [20].

The acidic pHe shifts tumour cell metabolism. Human breast cancer cells under acute acidic pH exposure show reduced proliferation, G1 cell cycle arrest, and increased cytoplasmic vacuolization [21]. These cells reduce their glucose metabolism, while increasing their metabolism of both glutamine and fatty acids [22, 23]. The acidic TME stimulates autophagy in which organelles and proteins are self-digested to maintain metabolism and promote cell survival [21]. Cells that were exposed to low pH for months had restored proliferative ability, however, autophagy markers LC3-II

and double-membrane markers were maintained [21]. Thus autophagy may be used as an adaptive strategy to maintain survival in an acidic TME.

Acidity also promotes release of EVs by cancer cells, with increasing numbers of EVs released as the pHe drops from 7.4 to 6.5 [6]. EVs include microvesicles (MVs), exosomes, and apoptotic bodies, which have different origins, properties, and functions [24]. Their main function is cell-cell communication between local and distant cells. EVs can contain different lipids, nucleic acids, and proteins, that are selectively taken up by stromal or tumour cells. They can confer molecules that promote tumour cell survival and metastasis, angiogenesis, and suppression of immune cells [6, 24].

While the intrinsic effects of acidity are important for understanding tumour behaviour, acidity has larger influence beyond the tumour itself. Here we describe the relationship between the acidic TME and stromal cells, the influence of the acidic TME in mediating and manipulating immune evasion, and potential therapeutic targets requiring investigation.

1.3 Stromal cells within the TME promote tumourigenesis

In solid tumours, cancer cells are embedded in stromal connective tissue that is also affected by the acidity of the TME [8]. Numerous bi-directional interactions occur between cancer and stromal cells, and the acidic TME can alter the composition and function of the stromal cells present [8]. Fibroblasts are abundant in the TME and play important roles in tumour growth, survival, proliferation, migration and invasion [25, 26]. Fibroblasts are usually inactive, however through interactions with tumour cells and other TME components, they are stimulated to become cancer-associated fibroblasts (CAFs) that promote tumour growth. Although it remains to be seen whether the acidic pHe plays a direct role in the activation of CAFs, the acidic TME promotes tumour cells to release a number of factors which promote their activation including cytokines such as IL-6 and TGF-β, and growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), and fibroblast growth factor 2 (FGF2) (Table 1) [1]. CAFs secrete matrix proteins and proteolytic enzymes that degrade the ECM, chemokines and growth factors that promote cell growth, proliferation and malignancy, as well as various cytokines that recruit immune cells to the TME (Table 1) [1, 25]. CAFs can also contribute to extracellular acidification by secreting carbonic anhydrase 9 (CA IX) into the TME[8]. In head and neck squamous cell carcinomas (HNSCC), CAFs have been shown to have high glucose uptake, generate high levels of lactate, and have increased expression of GLUT-1 and monocarboxylate transporter 4 (MCT-4) [27]. They therefore further contribute to the acidity of the TME.

Endothelial cells line blood vessels in the TME, and are responsible for critical functions such as angiogenesis, transportation of immune cells, and intra- and extravasation of tumour cells during metastasis [28]. They adapt to survive low pHe, maintaining their pHi by extruding lactate via carbonic anhydrase II (CA II) [29]. Tumour cells release EVs that transfer the EGF receptor (EGFR) to ECs, activating the autocrine vascular endothelial growth factor (VEGF) pathway in ECs to promote angiogenesis [30]. Induction of VEGF expression by ECs also downregulate the expression of adhesion markers, such as intercellular cell adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), on blood vessels in the TME. This prevents CTLs from infiltrating the tumour

[31]. VEGF also stimulates PD-L1 expression, inhibiting CTLs [31]. Tumour-associated ECs also preferentially attract immunosuppressive Tregs to the TME (Table 1) [31].

Table 1. The effects of acidic pHe on cells in the tumour microenvironment

Functional group	Cell type	Effect of acidic TME	References
Stromal cells	Fibroblasts	↑ CCL7 and MMP secretion to enhance cell migration and invasion ↑ CXCL1 secretion to promote tumour cell proliferation ↑ CXCL12 and FGF to promote tumour growth ↑ CXCL16 ↑ erythropoietin (EPO), FGF, and VEGF to promote angiogenesis ↑ TGF-β to suppress T and NK cells ↑ IL-1β, IL-6, IL-8 production ↑ IL-6, TGF-β, IL-1α, EGF, PDGF, FGF2 stimulates activation of CAFs	[1, 2, 25, 26]
	Endothelial cells	↑ expression of VEGF and FGF to promote angiogenesis and cell migration ↑ secretion of IL-6 and CSF-1 ↑ recruitment of innate immune cells to create an immunosuppressive environment ↑ PD-L1 expression to disable T cells ↑ Treg recruitment ↑ polyunsaturated fats to support tumour growth ↓ apoptosis ↓ ICAM-1 and VCAM-1	[28, 31]
Phagocytic and antigen-presenting cells	Myeloid-derived suppressor cells (MDSC)	↑ Recruitment to tumour site ↑ MDSC secretion of arginase and iNOS ↑ immunosuppression of NK and T cells	[28, 33, 35, 36]
	Neutrophils	Switch to N2 phenotype ↑ NETs release ↑ release of MMP-8, MMP-9 and NE to promote ECM remodelling ↓ Cytotoxic killing of tumour cells ↑ Hydrogen peroxide and MPO release ↑ H+ATPase release to block NK and T cell activity	[44, 46, 47, 49]

Functional group	Cell type	Effect of acidic TME	References
	Macrophages	Switch to M2 phenotype ↑ Th2 cytokine release e.g. IL-10 and TGF-β to suppress T cells ↑ iNOS and ROS ↑ Prostaglandin release ↑ release of EGF, VEGF, CXCL8, IL-6 to promote tumour cell survival and angiogenesis ↑ MMP secretion to promote invasion ↑ oncogenic EV release ↓ Antigen presentation	[2, 28, 39, 40, 42]
	Dendritic cells	↑ anti-inflammatory cytokines e.g. IL-10 ↓ Maturation and migration to lymph nodes ↓ HLA class II expression and antigen presentation ↓ co-stimulatory molecule expression (CD40, CD80, CD86) ↓ Pro-inflammatory cytokines e.g. IL-12 ↑PD-L1 and CTLA-4	[10, 28, 38]
Natural Killer cells	Natural Killer cells	↑ Anergy and apoptosis ↓ Cytotoxic activity (perforin and granzyme release) ↓ NKG2D expression and activation ↓ IFN-γ production ↓ Degranulation ↓ metabolic activity Inhibited by TGF-β, IL-10	[33, 50-53]
T lymphocytes	CD8$^+$ (cytotoxic) and CD4$^+$ (helper) T cells	↑ CTLA-4 ↑ Anergy and apoptosis ↑iNOS and arginase depletes arginine needed to TCR function ↓ CD3 ζ chain expression ↓ release of cytokines e.g. IL-2, IFN-γ, TNF-α ↓ cytotoxic activity (perforin and granzyme release) ↓ STAT5, ERK, AKT, JNK, c-Jun and p38 phosphorylation ↓ NFAT activation ↓ Metabolism, chemotaxis and proliferation of T cells ↓TCR activation - blocked by IDO converting tryptophan to kynurenine	[11, 54, 55, 58-66]
	Regulatory T cells (Tregs)	↑ numbers of Tregs recruited by IDO, TGF-β, and CCL22 Lactic acid in TME supports Treg metabolism ↑ Immunosuppression of DCs, B Cells, T cells and NK cells through release of TGF-β, IL-10 and IL-35 ↑ VEGF to promote angiogenesis	[2, 33, 43, 68]
B cells	B lymphocytes	↓ recruitment to the TME Different B cell subsets exert different effects in different cancer types	[69, 70, 73]

Functional group	Cell type	Effect of acidic TME	References
	Regulatory B cells (Bregs)	Tumour metabolites and IDO recruit Bregs to tumour site ↑ IL-10, IL-35 and TGB-β production to inhibit T cells and stimulate FoxP3 expression in Tregs ↑ PD-1, PD-L1 and CTLA-4 expression to block T and NK cells ↑ adenosine production to suppress T cells Impairs Th17 response Suppresses IFN-γ release by CTLs	[69, 71, 75, 77, 78, 80]

1.4 Acidosis in the TME drives immune escape by inhibiting effector cells while promoting immunosuppressive phenotypes

Immune cells comprise a large component of the TME, although their anti-tumour effects are mostly downregulated. The immune cell infiltrate is comprised of innate immune cells (macrophages, neutrophils, dendritic cells, innate lymphoid cells, myeloid-derived suppressor cells, and natural killer cells), as well as adaptive immune cells (T cells and B cells). The presence of effector cells including CD4 or CD8 T cells, and NK cells, is associated with good disease prognosis, while the presence of regulatory immune cells such as monocyte derived suppressor cells (MDSCs), Tregs, and regulatory B cells (Bregs) are linked to poor outcomes. During the early stages of tumour development, cancer cells in the TME stimulate a weak immune response. Over time these cells become resistant to the innate immune response, and go on to undermine and manipulate the adaptive immune response.

1.4.1 Monocyte derived suppressor cells

Monocyte derived suppressor cells comprise a heterogeneous group of immature, poorly differentiated myelomonocytic cells including monocytes, dendritic cells, macrophages, and neutrophils [32]. Tumour exosomes can drive monocyte differentiation to immunosuppressive MDSCs, and tumour cells secrete chemokine (C-X-C) motif ligand 1 (CXCL1) to recruit MDSCs to the TME [28, 33]. In the acidic TME, MDSCs are activated by a number of cancer-derived signals including IL-1β, IL-4, IL-6, IL-13, toll-like receptors (TLRs), VEGF, and TGF-β [32, 34]. MDSCs upregulate expression of arginase and inducible nitric oxide synthase (iNOS), thereby blocking activation of NK cells and T cells (Table 1) [35, 36]. The numbers of circulating and TME-infiltrating MDSCs correlate with tumour progression and poor patient outcomes [37].

1.4.2 Dendritic cells

The acidic pHe inhibits DC maturation, allowing them to become tolerogenic. Tolerogenic DCs have reduced levels of co-stimulatory molecules such as CD80 and CD86, and increased levels of PD-L1 and CTLA-4 [28]. They also show reduced capacity to present antigen to T cells [10]. In mouse models, extracellular acidosis causes damage to DC cytoskeletal structure, with reduced DC

migration and altered membrane charges and membrane fluidity of DCs [38]. This may reduce DC migration to lymph nodes and antigen presentation. Treatment of DCs with pH 6.5 medium also impaired DC ability to stimulate T cells [38]. Tolerogenic DCs release immunosuppressive molecules such as indoleamine 2,3-dioxygenase (IDO) and nitric oxide (NO) which suppress T cells, and immunosuppressive cytokines TGF-β and IL-10 which recruit Tregs (Table 1) [28].

1.4.3 Macrophages

Macrophages are actively recruited to tumours in response to growth factors such as VEGF, colony-stimulating factor 1 (CSF-1), and granulocyte-macrophage colony-stimulating factor (GM-CSF), cytokines (IL-6, IL-10 and TGF-β) and chemokines such as chemokine (C-C) motif ligand 2 (CCL2) and CCL8 released by tumour and stromal cells in the TME [2, 28, 39]. Lactic acid in the TME drives macrophage polarization towards the M2 phenotype, with the release of anti-inflammatory cytokines, ROS production, and release of prostaglandins [2, 40, 41]. M2-like TAMs have poor antigen-presenting capabilities as they downregulate human leukocyte antigen (HLA) class II expression, and suppress T cells by releasing IL-10 and TGF-β [40].They release growth factors, cytokines and matrix metalloproteinases (MMPs) which further promote angiogenesis, tumour survival, invasion and metastasis (Table 1) [2, 39, 40, 42]. High numbers of TAMs are found in many tumour types and are correlated with poor prognosis [40]. TAMs also release chemokines such as CCL22, which recruit Tregs to the TME [43].

1.4.4 Neutrophils

Neutrophils migrate to the tumour site in response to damage-associated molecular patterns (DAMPs), chemokines and cytokines, although their role in the TME is controversial. Environmental stimuli can drive neutrophil polarization towards either an anti-tumourigenic N1, or pro-tumourigenic N2 phenotype. Acidic extracellular pH was shown to polarize neutrophils to an N2 phenotype *in vitro* [44]. In murine models, the absence of TGF-β can induce anti-tumourigenic neutrophils (N1), that may recruit and stimulate activation of CD8^{+} CTLs [45]. As the acidic TME stimulates release of TGF-β by tumour cells and certain immune cells, this may favour the development of the immunosuppressive N2 neutrophils. In addition to polarization, neutrophil function is influenced by acidic extracellular pH. Studies conducted *in vitro* show that acidic pH medium causes neutrophils to upregulate hydrogen peroxide production and myeloperoxidase (MPO) release [46]. Extracellular acidosis also delays neutrophil apoptosis, allowing the neutrophils to sustain an inflammatory response [46]. Neutrophils release neutrophil extracellular traps (NETs) that consist of nuclear or mitochondrial DNA, histones, and proteases [47]. The NETS stimulate the nuclear factor kappa B (NF-κB) pathway, block apoptosis, and stimulate cell proliferation. They promote ECM remodelling through release of proteolytic granules containing neutrophil elastase (NE), MMP-8 and MMP-9, and MPO [47, 48]. Neutrophils also further contribute to acidosis in TME by the release of granular H^{+} ATPases, which block NK and T cell activity (Table 1) [49].

1.4.5 Natural Killer Cells

T and B lymphocytes as well as NK cells are present at the tumour site. NK cells are vital for curtailing tumour growth, as they are able to directly kill tumour cells by using death receptor-mediated apoptosis, and by releasing perforin and granzyme [40]. Tumour cells escape NK cytotoxicity by downregulating their surface antigens. Natural killer group 2D (NKG2D) receptors that are vital for NK cell function are blocked by NKG2D ligands on tumour-derived exosomes [50]. NK cells are inhibited in the acidic TME. Acidic pH and lactate accumulation in the TME results in decreased IFN-γ production by NK cells [51]. Within the acidic TME, NK cells are also less efficient at producing pro-inflammatory cytokines, and their function is inhibited by cytokines secreted by tumour cells and other immune cells such as TGF-β and IL-10 [52]. When exposed to tumour-conditioned media of low pH, NK cells undergo apoptosis caused by decreasing intracellular pH and mitochondrial stress [53].

1.4.6 T cells (CD4, CD8 and Treg)

The intratumoural T cell population is heterogeneous and includes naïve, effector, memory, or regulatory T cells. CD8+ CTLs recognize tumour antigens presented on HLA molecules, and then release perforin-containing granules that damage the tumour cell membrane, and granzyme which causes apoptosis by damaging tumour cell DNA [54]. Once the CTL is activated, maturation and clonal expansion occur with the release of immuno-stimulatory cytokines including IL-2, IFN-γ, and TNF-α+ [54]. The release of these factors is important in sustaining anti-tumour immunity, and in developing immunological memory [54].

CTL activation is sustained by the presence of $CD4^+$ T cells, particularly Th1 cells, which provide essential cytokine support. Tumours evade a Th1 response through downregulation of HLA molecules, release of EVs and immunosuppressive cytokines, and loss of cell adhesion molecules such as ICAM-1. The acidic microenvironment however drives T cell differentiation towards Th2, Th17, or Treg polarisation [55]. Th2 and Th17 cells promote angiogenesis and tumour growth, and inhibit Th1 immunity [56]. Some T cells are sensitive to changes in pH and reduced glucose availability and are thus inhibited in the acidic TME [57].

The acidic TME affects T cell metabolism, as elevated glycolysis in cancer cells themselves can limit the availability of glucose to other cells [57, 58]. T cells increase their glucose uptake when they encounter a stimulatory antigen in order to proliferate and support effector functions [58]. Owing to poor vasculature within the TME, there is tough competition between cells for nutrients [59]. Although tumour cells can become quiescent in the absence of glucose or switch to fatty acid metabolism, activated T cells cannot survive or expand without glucose [58]. Lactate, released as a waste product by tumour cells, downregulates T cell metabolism and blocks lactate export from T cells [59]. Lactic acidosis impairs TCR-triggered phosphorylation of c-Jun N-terminal kinase (JNK), c-Jun and p38 [60]. Acidosis impairs CTL function, blocks signalling through NFAT, reduces IL-2, IFN-γ, perforin and granzyme production, and increases anergy and apoptosis [58, 61-63]. In the presence of lactic acid, CTL motility is reduced and contact with tumour cells is prolonged. This prolonged

contact means that the number of tumour cells they interact with is reduced, and this slows down the rate of serial cytotoxic killing, allowing tumour cells to escape death [64].

T cells require both arginine and tryptophan to function. Tumour cells release IDO into the TME which converts tryptophan into kynurenine. This blocks T cell activation and proliferation, and promotes T cell apoptosis. A lack of tryptophan in the TME also causes downregulation of the TCR ζ-chain in cytotoxic T cells, which impairs their function [65]. The acidic TME also contains elevated levels of arginase 1 and (iNOS which deplete arginine [11, 65]. Arginine is crucial to T cell function as it is also required for the expression of the TCR ζ-chain [35], and depleting it blocks T cell proliferation, activation, glycolysis, and cytokine production [11, 66]. The acidic TME also reduces CTL motility and prolongs cell-CTL contact which reduces the number of CTL-tumour cell interactions and hence tumour cytolysis [64].

In contrast to its impact on T effector cells, the acidic TME is supportive of Treg presence and activity. Their presence correlates with poor outcomes in many tumour types. Tregs are recruited to the TME by stimuli such as hypoxia, the secretion of factors such as VEGF, HIF-α and IDO by tumours, and by tumour-associated chemokines such as TGF-β and CCL22 [43, 67]. Studies in murine models show that unlike T effector cells, Tregs thrive under glucose restriction [68]. Instead of metabolizing glucose, tumour-associated Tregs upregulate pathways that metabolize lactic acid which is abundant in the TME. This increases Treg survival and proliferation, supporting the idea that the acidic TME provides metabolic support for Tregs [68]. Tregs contribute to the immunosuppressive TME by releasing additional TGF-β, IL-10 and IL-35, which suppress DCs, B cells, NK cells, and T cells [2, 67] (Table 1). Tregs express granzyme B and perforin, allowing them to directly kill CTLs and NK cells [43]. They also express CTLA-4 and compete with T cells for CD28 binding, and downregulate CD80 and CD86 expression in APCs [43].

1.4.7 B cells and Bregs

The TME recruits B cells and controls the localization of the different B cell subsets [69, 70]. Phenotypically distinct B cell subsets play different functional roles in tumour responses, and can be either pro- or anti-tumourigenic [71, 72]. The B cell chemoattractant CXCL13, secreted by tumour cells and dendritic cells, recruits B cells to the tumour site [69]. Antigens released by tumour cells trigger a humoral response. These antigens can be expressed as a result of mutations, gene overexpression, or expression of aberrant markers not common to that cell type [72]. Some tumour-associated antigens are recognized by B cells, leading to the production of antibodies that aid in killing tumour cells through activation of the complement pathway and stimulating phagocytosis by macrophages, or by activating NK cells [72]. B cells also stimulate T cell responses such as T cell activation in response to antigen presentation, clonal expansion, and memory T cell formation [69]. In some instances, B cells may also exhibit direct cytotoxic activity, by killing tumour cells via granzyme B or via TNF-related apoptosis-inducing ligand (TRAIL/Apo-2L)-dependent mechanisms [69, 73]. The presence of tumour infiltrating B cells is associated with good prognosis in some cancers (e.g. non-small cell lung carcinoma and melanoma) [70, 71, 74], and contribute to both humoral and cellular immunity [69]. In other cancers, such as head and neck squamous cell carcinomas and ovarian cancers, they have little to no beneficial impact [70]. This may in part be due to the high degree of functional plasticity and the high degree of heterogeneity in B cell subsets [70].

Bregs are a subpopulation of B cells that have immuno-regulatory or suppressive properties. Unlike other cell types, there are a number of different Breg phenotypes depending on the tumour type and location [75], leading to the idea that they are not lineage specific but arise as a result of environmental stimuli [76]. Although the acidic TME recruits and promotes the differentiation of other immunosuppressive cells such as MDSCs, the direct effect of the acidic TME on Breg differentiation is not known. Tumour cell metabolites can induce Bregs via the peroxisome proliferator-activated receptor α (PPARα) [77], and IDO released by MDSCs has been shown to induce Breg differentiation *in vitro* [78]. Bregs inhibit other immune cells by releasing immunosuppressive cytokines such as IL-10, IL-35, and TGF-β [71]. Bregs release TGF-β which converts CD4+ T cells into Tregs [79], IL-35 which promotes Treg differentiation and impairs Th17 responses [75], while IL-10 producing Bregs suppress IFN-γ production by CTLs [80]. They also perform suppressive functions through direct contact with immune cells, and by upregulating PD-L1 and CTLA-4 they block T and NK cell responses [69]. Some Bregs subtypes (CD73$^+$) induce adenosine production, which causes T cell suppression [71]. The release of pro-inflammatory cytokines by DCs is also suppressed by Bregs, enabling Bregs to indirectly block Th1 and Th17 differentiation [76] (Table 1). Thus Bregs present in the TME help facilitate immune escape, and provide a potential therapeutic target for future immunotherapy research.

1.5 The acidic tumour microenvironment is a therapeutic target

Chemotherapy is the leading cancer therapy worldwide, alone or in combination with surgery and/or radiotherapy, but limitations including drug resistance exist, with increased instances of relapse, and increased morbidity and mortality for patients [4].

An acidic microenvironment reduces the effectiveness of anti-tumour immunity [81]. The acidic TME alters drug structure and charge. This reduces their uptake into tumour cells, and affects the delivery and efficacy of anti-cancer drugs, as well as chemotherapy and radiation [9, 10]. This has prompted evaluation of the TME as a therapeutic target. Elements of the TME provide promising targets for therapy, including the ECM, immune cells, and stromal cells. Trials targeting tumour-associated leukocytes have had variable and limited success [82-85].

General buffer therapy is a direct approach to target tumour pH. In a murine model, neutralization of tumour acidity by bicarbonate increased susceptibility to checkpoint inhibitors such as anti-CTLA-4 and anti-PD-1, and improved survival [81]. Research into inhibiting proton extruder systems in humans is being explored (summarized in Table 2), but as these systems are redundant, targeting more than one may be necessary for successful treatment. The use of proton pump-inhibitors (PPIs) and other drugs to prevent extrusion of protons by tumour cells look promising to increase pHe. An important caveat is the impact of these interventions on the pHi and normal cellular processes [86].

Other approaches exploit the acidic environment including use of pH-sensitive drug delivery systems to deliver drugs selectively to the tumour [86]. pH-sensitive nano-carriers including peptides, liposomes, and polymeric nanoparticles (NPs) have been used with some success. These systems remain stable and intact at normal physiological pH, but are released in acidic environments [86]. This targeted therapy reduces systemic toxicity and allows more predictable pharmacokinetics [4, 86]. In mouse models, pH-responsive acetazolamide nanoparticles (ACE-NPs) disintegrate in acidic solution, resulting in rapid release of acetazolamide (ACE) which inhibits expression of CA IX and increases the alkalinity of the pHe [87].

Table 2. Proton extruder system targets

Effector molecular target	Therapeutic treatment	Rationale	References
Vacuolar-ATPase (V-ATPase) proton pump	Proton pump inhibitors (PPIs) e.g. omeprazole	Decrease pHi and prevention of protons being extruded into the TME. Increased T cell infiltration. Decreased cell proliferation Increase effects of chemotherapy	[90, 91]
	Archazolid	Inhibits endocytic activation of the Rho-GTPase Rac1 and prevents metastasis. Induces apoptosis. Inhibits proliferation.	[92, 93]
	Anti-epileptic drugs e.g. Phenytoin	Reduce tumour growth and metastasis	[94, 95]
Sodium-hydrogen exchanger-1 (NHE1)	Amiloride or cariporide	Blocks Na+ extrusion and pHi increase. Impairs growth factor-induced DNA synthesis Decreases proliferation	[86, 96, 97]
Voltage gated sodium channels (VGSCs)	Anti-epileptic drugs partially inhibit VGSCs	Inhibits tumour growth	[96]
	Antibiotics (e.g. clofazimine)	Increase mitochondrial ROS production and stimulate apoptosis	[98]
Monocarboxylate transporters (MCTs)	MCT inhibitor e.g. bioflavonoids	Partially inhibit MCT	[57, 86, 96]
	Ionidamine	Acts on MCT-1 to reduce pHi and sensitize cells to hyperthermia. Inhibits glycolysis and mitochondrial respiration	[99, 100]
	Statins	Partially inhibit MCT-1 and MCT-4	[96, 101]
Carbonic anhydrases (CAs)	CA inhibitor e.g. acetazolamide, sulfonamide	Impair growth of primary tumour. Reduces cell proliferation and metastasis. Stimulates T cell function	[86, 96, 102]
	Coumarins	Inhibits carbonic anhydrase IX and XII Inhibits tumour growth and metastasis	[86, 103]

Effector molecular target	Therapeutic treatment	Rationale	References
	Monoclonal antibody therapy e.g. Imatinib and nilotinib	Inhibit CA and tyrosine kinase activity.	[104]
	Small molecule inhibitor SLC-0111 (targets CA IX and CA XII)	Decrease in metastatic burden Reduced metastasis Inhibit tumour growth	[102]

Some NPs target immune cells within the TME and reduce the effects of hypoxia and acidity. Manganese dioxide NPs reduce hypoxia in cancer spheroids by catalysing degradation of hydrogen peroxide to produce oxygen. This revived NK cell cytotoxic activity [88]. Calcium-assisted NPs promote T cell activity and proliferation and prevent relapse and activity in a breast cancer mouse model [89].

2. Conclusion

Tumour cell metabolism drives an acidic TME, selecting for tumour cells with evolved survival strategies in a hypoxic and hypoglycaemic environment. The acidic TME inhibits immune cell activity and has implications for prognosis and treatment. Despite compelling evidence from *in vitro* and animal studies, few drugs that interfere with tumour pH have reached clinical trials because of redundancy in pH-mediating proteins in humans being targeted. New approaches to therapy should consider the acidic microenvironment. The use of pH-responsive nanoparticles is promising, as these can be used to affect tumour cells directly, impact on the TME, and positively influence the proliferation and activity of immune cells. In combination with other conventional therapies currently used, manipulating the acidic TME may aid in successful treatment of cancer patients.

Conflicts of Interest: The authors declare no conflict of interest

References

1. Boulter L, Bullock E, Mabruk Z, Brunton VG. The fibrotic and immune microenvironments as targetable drivers of metastasis. Br J Cancer. 2021; 124(1): 27-36
2. Ribeiro Franco PI, Rodrigues AP, de Menezes LB, Pacheco Miguel M. Tumor microenvironment components: Allies of cancer progression. Pathol Res Pract. 2020; 216(1): 152729
3. Fais S, Venturi G, Gatenby B. Microenvironmental acidosis in carcinogenesis and metastases: new strategies in prevention and therapy. Cancer Metastasis Rev. 2014; 33(4): 1095-108
4. Roma-Rodrigues C, Mendes R, Baptista PV, Fernandes AR. Targeting Tumor Microenvironment for Cancer Therapy. Int J Mol Sci. 2019; 20(4): 840
5. Cerezo M, Rocchi S. Cancer cell metabolic reprogramming: a keystone for the response to immunotherapy. Cell Death Dis. 2020; 11: 964
6. Logozzi M, Spugnini E, Mizzoni D, Di Raimo R, Fais S. Extracellular acidity and increased exosome release as key phenotypes of malignant tumors. Cancer Metastasis Rev. 2019; 38(1-2): 93-101
7. Buchbinder EI, Desai A. CTLA-4 and PD-1 Pathways: Similarities, Differences, and Implications of Their Inhibition. Am J Clin Oncol. 2016; 39(1): 98-106
8. Boedtkjer E, Pedersen SF. The Acidic Tumor Microenvironment as a Driver of Cancer. Annu Rev Physiol. 2020; 82: 103-26
9. Fais S, Marunaka Y. The Acidic Microenvironment: Is It a Phenotype of All Cancers? A Focus on Multiple Myeloma and Some Analogies with Diabetes Mellitus. Cancers (Basel). 2020; 12(11): 3226
10. Ward C, Meehan J, Gray M, Murray AF, Argyle D, et al. The impact of tumour pH on cancer progression: strategies for clinical intervention. Explor Target Anti-tumor Ther. 2020; 1(2): 71-100
11. Siska PJ, Rathmell JC. T cell metabolic fitness in antitumor immunity. Trends Immunol. 2015; 36(4): 257-64
12. Flinck M, Kramer SH, Pedersen SF. Roles of pH in control of cell proliferation. Acta Physiol (Oxf). 2018; 223(3): e13068
13. Hayes C, Donohoe CL, Davern M, Donlon NE. The oncogenic and clinical implications of lactate induced immunosuppression in the tumour microenvironment. Cancer Lett. 2021; 500: 75-86
14. Comito G, Iscaro A, Bacci M, Morandi A, Ippolito L, et al. Lactate modulates CD4(+) T-cell polarization and induces an immunosuppressive environment, which sustains prostate carcinoma progression via TLR8/miR21 axis. Oncogene. 2019; 38(19): 3681-95
15. Quinn WJ, 3rd, Jiao J, TeSlaa T, Stadanlick J, Wang Z, et al. Lactate Limits T Cell Proliferation via the NAD(H) Redox State. Cell Rep. 2020; 33(11): 108500
16. Corbet C, Feron O. Emerging roles of lipid metabolism in cancer progression. Curr Opin Clin Nutr Metab Care. 2017; 20(4): 254-60
17. Li S, Xiong N, Peng Y, Tang K, Bai H, et al. Acidic pHe regulates cytoskeletal dynamics through conformational integrin beta1 activation and promotes membrane protrusion. Biochim Biophys Acta Mol Basis Dis. 2018; 1864(7): 2395-408
18. Corbet C, Bastien E, Santiago de Jesus JP, Dierge E, Martherus R, et al. TGFbeta2-induced formation of lipid droplets supports acidosis-driven EMT and the metastatic spreading of cancer cells. Nat Commun. 2020; 11(1): 454
19. Damaghi M, Gillies RJ. Lysosomal protein relocation as an adaptation mechanism to extracellular acidosis. Cell Cycle. 2016; 15(13): 1659-60
20. Ibrahim-Hashim A, Estrella V. Acidosis and cancer: from mechanism to neutralization. Cancer Metastasis Rev. 2019; 38(1-2): 149-55
21. Wojtkowiak JW, Rothberg JM, Kumar V, Schramm KJ, Haller E, et al. Chronic autophagy is a cellular adaptation to tumor acidic pH microenvironments. Cancer Res. 2012; 72(16): 3938-47

22.   LaMonte G, Tang X, Chen J, L-Y., Wu J, Ding C-KC, et al. Acidosis induces reprogramming of cellular metabolism to mitigate oxidative stress. Cancer & Metabolism. 2013; 1: 23

23.   Gao J, Guo Z, Cheng J, Sun B, Yang J, et al. Differential metabolic responses in breast cancer cell lines to acidosis and lactic acidosis revealed by stable isotope assisted metabolomics. Sci Rep. 2020; 10(1): 21967

24.   Doyle LM, Wang MZ. Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. Cells. 2019; 8(7): 727

25.   Han Y, Zhang Y, Jia T, Sun Y. Molecular mechanism underlying the tumor-promoting functions of carcinoma-associated fibroblasts. Tumour Biol. 2015; 36(3): 1385-94

26.   da Cunha BR, Domingos C, Stefanini ACB, Henrique T, Polachini GM, et al. Cellular Interactions in the Tumor Microenvironment: The Role of Secretome. J Cancer. 2019; 10(19): 4574-87

27.   Custodio M, Biddle A, Tavassoli M. Portrait of a CAF: The story of cancer-associated fibroblasts in head and neck cancer. Oral Oncol. 2020; 110: 104972

28.   Labani-Motlagh A, Ashja-Mahdavi M, Loskog A. The Tumor Microenvironment: A Milieu Hindering and Obstructing Antitumor Immune Responses. Front Immunol. 2020; 11: 940

29.   Korhonen K, Parkkila AK, Helen P, Valimaki R, Pastorekova S, et al. Carbonic anhydrases in meningiomas: association of endothelial carbonic anhydrase II with aggressive tumor features. J Neurosurg. 2009; 111(3): 472-7

30.   Al-Nedawi K, Meehan B, Kerbel R, Allison A, Rak J. Endothelial expression of autocrine VEGF upon the uptake of tumor-derived microvesicles containing oncogenic EGFR. Proc Natl Acad Sci U S A. 2009; 106(10): 3794-9

31.   Missiaen R, Mazzone M, Bergers G. The reciprocal function and regulation of tumor vessels and immune cells offers new therapeutic opportunities in cancer. Semin Cancer Biol. 2018; 52(Pt 2): 107-16

32.   Gao J, Wu Y, Su Z, Amoah Barnie P, Jiao Z, et al. Infiltration of alternatively activated macrophages in cancer tissue is associated with MDSC and Th2 polarization in patients with esophageal cancer. PLoS One. 2014; 9(8): e104453

33.   Filipazzi P, Burdek M, Villa A, Rivoltini L, Huber V. Recent advances on the role of tumor exosomes in immunosuppression and disease progression. Semin Cancer Biol. 2012; 22(4): 342-9

34.   Liu Q, Liao Q, Zhao Y. Myeloid-derived suppressor cells (MDSC) facilitate distant metastasis of malignancies by shielding circulating tumor cells (CTC) from immune surveillance. Med Hypotheses. 2016; 87: 34-9

35.   Rodriguez P, Quiceno D, Zabaleta J, Ortiz B, Zea A, et al. Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses. Cancer Res. 2004; 64: 5839-49

36.   Stiff A, Trikha P, Mundy-Bosse B, McMichael E, Mace TA, et al. Nitric Oxide Production by Myeloid-Derived Suppressor Cells Plays a Role in Impairing Fc Receptor-Mediated Natural Killer Cell Function. Clin Cancer Res. 2018; 24(8): 1891-904

37.   Maimela NR, Liu S, Zhang Y. Fates of CD8+ T cells in Tumor Microenvironment. Comput Struct Biotechnol J. 2019; 17: 1-13

38.   Tong L, Yue P, Yang Y, Huang J, Zeng Z, et al. Motility and Mechanical Properties of Dendritic Cells Deteriorated by Extracellular Acidosis. Inflammation. 2020; 44(2): 737-45

39.   Chanmee T, Ontong P, Konno K, Itano N. Tumor-associated macrophages as major players in the tumor microenvironment. Cancers (Basel). 2014; 6(3): 1670-90

40.   Hinshaw DC, Shevde LA. The Tumor Microenvironment Innately Modulates Cancer Progression. Cancer Res. 2019; 79(18): 4557-66

41.   Whiteside TL. The tumor microenvironment and its role in promoting tumor growth. Oncogene. 2008; 27(45): 5904-12

42.   Kim J, Bae JS. Tumor-Associated Macrophages and Neutrophils in Tumor Microenvironment. Mediators Inflamm. 2016; 2016: 6058147

43.	Cinier J, Hubert M, Besson L, Di Roio A, Rodriguez C, et al. Recruitment and Expansion of Tregs Cells in the Tumor Environment-How to Target Them? Cancers (Basel). 2021; 13(8): 1850

44.	Ohms M, Moller S, Laskay T. An Attempt to Polarize Human Neutrophils Toward N1 and N2 Phenotypes in vitro. Front Immunol. 2020; 11: 532

45.	Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, et al. Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. Cancer Cell. 2009; 16(3): 183-94

46.	Trevani A, Andonegui G, Giordano M, Lopez D, Gamberale R, et al. Extracellular Acidification induces human neutrophil activation. J Immunol. 1999; 162: 4849-57

47.	Cedervall J, Hamidi A, Olsson AK. Platelets, NETs and cancer. Thromb Res. 2018; 164 Suppl 1: S148-S52

48.	Wu L, Saxena S, Singh RK. Neutrophils in the Tumor Microenvironment. Adv Exp Med Biol. 2020; 1224: 1-20

49.	Mollinedo F. Neutrophil Degranulation, Plasticity, and Cancer Metastasis. Trends Immunol. 2019; 40(3): 228-42

50.	Chen W, Jiang J, Xia W, Huang J. Tumor-Related Exosomes Contribute to Tumor-Promoting Microenvironment: An Immunological Perspective. J Immunol Res. 2017; 2017: 1073947

51.	Potzl J, Roser D, Bankel L, Homberg N, Geishauser A, et al. Reversal of tumor acidosis by systemic buffering reactivates NK cells to express IFN-gamma and induces NK cell-dependent lymphoma control without other immunotherapies. Int J Cancer. 2017; 140(9): 2125-33

52.	Gottfried E, Kreutz M, Mackensen A. Tumor metabolism as modulator of immune response and tumor progression. Semin Cancer Biol. 2012; 22(4): 335-41

53.	Harmon C, Robinson MW, Hand F, Almuaili D, Mentor K, et al. Lactate-Mediated Acidification of Tumor Microenvironment Induces Apoptosis of Liver-Resident NK Cells in Colorectal Liver Metastasis. Cancer Immunol Res. 2019; 7(2): 335-46

54.	Huber V, Camisaschi C, Berzi A, Ferro S, Lugini L, et al. Cancer acidity: An ultimate frontier of tumor immune escape and a novel target of immunomodulation. Semin Cancer Biol. 2017; 43: 74-89

55.	Zhang Z, Liu S, Zhang B, Qiao L, Zhang Y, et al. T Cell Dysfunction and Exhaustion in Cancer. Front Cell Dev Biol. 2020; 8: 17

56.	Feng S, Chen X, Wang J, Xu X. Th17 cells associated cytokines and cancer. Eur Rev Med Pharmacol Sci. 2016; 20: 4032-40

57.	Damgaci S, Ibrahim-Hashim A, Enriquez-Navas PM, Pilon-Thomas S, Guvenis A, et al. Hypoxia and acidosis: immune suppressors and therapeutic targets. Immunology. 2018; 154(3): 354-62

58.	Beckermann KE, Dudzinski SO, Rathmell JC. Dysfunctional T cell metabolism in the tumor microenvironment. Cytokine Growth Factor Rev. 2017; 35: 7-14

59.	Lim AR, Rathmell WK, Rathmell JC. The tumor microenvironment as a metabolic barrier to effector T cells and immunotherapy. Elife. 2020; 9: e55185

60.	Mendler AN, Hu B, Prinz PU, Kreutz M, Gottfried E, et al. Tumor lactic acidosis suppresses CTL function by inhibition of p38 and JNK/c-Jun activation. Int J Cancer. 2012; 131(3): 633-40

61.	Bosticardo M, Ariotti S, Losana G, Bernabei P, Forni G, et al. Biased activation of human T lymphocytes due to low extracellular pH is antagonized by B7/CD28 costimulation. Eur J Immunol. 2001; 31: 2829-38

62.	Chalmin F, Bruchard M, Vegran F, Ghiringhelli F. Regulation of T cell antitumor immune response by tumor induced metabolic stress. Cell Stress. 2018; 3(1): 9-18

63.	Calcinotto A, Filipazzi P, Grioni M, Iero M, De Milito A, et al. Modulation of microenvironment acidity reverses anergy in human and murine tumor-infiltrating T lymphocytes. Cancer Res. 2012; 72(11): 2746-56

64.	Fischbeck AJ, Ruehland S, Ettinger A, Paetzold K, Masouris I, et al. Tumor Lactic Acidosis: Protecting Tumor by Inhibiting Cytotoxic Activity Through Motility Arrest and Bioenergetic Silencing. Front Oncol. 2020; 10: 589434

65.	Wu AA, Drake V, Huang HS, Chiu S, Zheng L. Reprogramming the tumor microenvironment: tumor-induced immunosuppressive factors paralyze T cells. Oncoimmunology. 2015; 4(7): e1016700

66.   Cassim S, Pouyssegur J. Tumor Microenvironment: A Metabolic Player that Shapes the Immune Response. Int J Mol Sci. 2019; 21(1): 157

67.   Bhatia A, Kumar Y. Cellular and molecular mechanisms in cancer immune escape: a comprehensive review. Expert Rev Clin Immunol. 2014; 10(1): 41-62

68.   Watson MJ, Vignali PDA, Mullett SJ, Overacre-Delgoffe AE, Peralta RM, et al. Metabolic support of tumour-infiltrating regulatory T cells by lactic acid. Nature. 2021; 591(7851): 645-51

69.   Wang SS, Liu W, Ly D, Xu H, Qu L, et al. Tumor-infiltrating B cells: their role and application in anti-tumor immunity in lung cancer. Cell Mol Immunol. 2019; 16(1): 6-18

70.   Lechner A, Schlosser HA, Thelen M, Wennhold K, Rothschild SI, et al. Tumor-associated B cells and humoral immune response in head and neck squamous cell carcinoma. Oncoimmunology. 2019; 8(3): 1535293

71.   Zhang Y, Gallastegui N, Rosenblatt JD. Regulatory B cells in anti-tumor immunity. Int Immunol. 2015; 27(10): 521-30

72.   Largeot A, Pagano G, Gonder S, Moussay E, Paggetti J. The B-side of Cancer Immunity: The Underrated Tune. Cells. 2019; 8(5): 449

73.   Sarvaria A, Madrigal JA, Saudemont A. B cell regulation in cancer and anti-tumor immunity. Cell Mol Immunol. 2017; 14(8): 662-74

74.   Zhang Z, Zhu Y, Wang Z, Zhang T, Wu P, et al. Yin-yang effect of tumor infiltrating B cells in breast cancer: From mechanism to immunotherapy. Cancer Lett. 2017; 393: 1-7

75.   Shang J, Zha H, Sun Y. Phenotypes, Functions, and Clinical Relevance of Regulatory B Cells in Cancer. Front Immunol. 2020; 11: 582657

76.   Rosser Elizabeth C, Mauri C. Regulatory B Cells: Origin, Phenotype, and Function. Immunity. 2015; 42(4): 607-12

77.   Wejksza K, Lee-Chang C, Bodogai M, Bonzo J, Gonzalez FJ, et al. Cancer-Produced Metabolites of 5-Lipoxygenase Induce Tumor-Evoked Regulatory B Cells via Peroxisome Proliferator–Activated Receptor α. The Journal of Immunology. 2013; 190(6): 2575-84

78.   Tousif S, Wang Y, Jackson J, Hough KP, Strenkowski JG, et al. Indoleamine 2, 3-Dioxygenase Promotes Aryl Hydrocarbon Receptor-Dependent Differentiation Of Regulatory B Cells in Lung Cancer. Front Immunol. 2021; 12: 747780

79.   Olkhanud PB, Damdinsuren B, Bodogai M, Gress RE, Sen R, et al. Tumor-Evoked Regulatory B Cells Promote Breast Cancer Metastasis by Converting Resting CD4+ T Cells to T-Regulatory Cells. Cancer Research. 2011; 71(10): 3505-15

80.   Wei X, Jin Y, Tian Y, Zhang H, Wu J, et al. Regulatory B cells contribute to the impaired antitumor immunity in ovarian cancer patients. Tumor Biology. 2015; 37(5): 6581-8

81.   Pilon-Thomas S, Kodumudi KN, El-Kenawi AE, Russell S, Weber AM, et al. Neutralization of Tumor Acidity Improves Antitumor Responses to Immunotherapy. Cancer Res. 2016; 76(6): 1381-90

82.   Wang S, Lin Y, Xiong X, Wang L, Guo Y, et al. Low-Dose Metformin Reprograms the Tumor Immune Microenvironment in Human Esophageal Cancer: Results of a Phase II Clinical Trial. Clin Cancer Res. 2020; 26(18): 4921-32

83.   Beatty GL, Chiorean EG, Fishman MP, Saboury B, Teitelbaum UR, et al. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. Science. 2011; 331(6024): 1612-6

84.   Keskin DB, Anandappa AJ, Sun J, Tirosh I, Mathewson ND, et al. Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. Nature. 2019; 565(7738): 234-9

85.   Tada Y, Togashi Y, Kotani D, Kuwata T, Sato E, et al. Targeting VEGFR2 with Ramucirumab strongly impacts effector/ activated regulatory T cells and CD8(+) T cells in the tumor microenvironment. J Immunother Cancer. 2018; 6(1): 106

86.   Corbet C, Feron O. Tumour acidosis: from the passenger to the driver's seat. Nat Rev Cancer. 2017; 17(10): 577-93

87. Liu S, Luo X, Liu S, Xu P, Wang J, et al. Acetazolamide-Loaded pH-Responsive Nanoparticles Alleviating Tumor Acidosis to Enhance Chemotherapy Effects. Macromol Biosci. 2019; 19(2): e1800366

88. Murphy DA, Cheng H, Yang T, Yan X, Adjei IM. Reversing Hypoxia with PLGA-Encapsulated Manganese Dioxide Nanoparticles Improves Natural Killer Cell Response to Tumor Spheroids. Mol Pharm. 2021; 18(8): 2935-46

89. Li Y, Gong S, Pan W, Chen Y, Liu B, et al. A tumor acidity activatable and Ca(2+)-assisted immuno-nanoagent enhances breast cancer therapy and suppresses cancer recurrence. Chem Sci. 2020; 11(28): 7429-37

90. Bellone M, Calcinotto A, Filipazzi P, De Milito A, Fais S, et al. The acidity of the tumor microenvironment is a mechanism of immune escape that can be overcome by proton pump inhibitors. Oncoimmunology. 2013; 2(1): e22058

91. Iessi E, Logozzi M, Mizzoni D, Di Raimo R, Supuran CT, et al. Rethinking the Combination of Proton Exchanger Inhibitors in Cancer Therapy. Metabolites. 2017; 8(1): 2

92. Wiedmann RM, von Schwarzenberg K, Palamidessi A, Schreiner L, Kubisch R, et al. The V-ATPase-inhibitor archazolid abrogates tumor metastasis via inhibition of endocytic activation of the Rho-GTPase Rac1. Cancer Res. 2012; 72(22): 5976-87

93. Bartel K, Winzi M, Ulrich M, Koeberle A, Menche D, et al. V-ATPase inhibition increases cancer cell stiffness and blocks membrane related Ras signaling - a new option for HCC therapy. Oncotarget. 2017; 8(6): 9476-87

94. Nelson M, Yang M, Dowle AA, Thomas JR, Brackenbury WJ. The sodium channel-blocking antiepileptic drug phenytoin inhibits breast tumour growth and metastasis. Mol Cancer. 2015; 14: 13

95. Mohammed FH, Khajah MA, Yang M, Brackenbury WJ, Luqmani YA. Blockade of voltage-gated sodium channels inhibits invasion of endocrine-resistant breast cancer cells. Int J Oncol. 2016; 48(1): 73-83

96. Koltai T. Triple-edged therapy targeting intracellular alkalosis and extracellular acidosis in cancer. Semin Cancer Biol. 2017; 43: 139-46

97. Chen Q, Liu Y, Zhu XL, Feng F, Yang H, et al. Increased NHE1 expression is targeted by specific inhibitor cariporide to sensitize resistant breast cancer cells to doxorubicin in vitro and in vivo. BMC Cancer. 2019; 19(1): 211

98. Zaccagnino A, Managò A, Leanza L, Gontarewitz A, Linder B, et al. Tumor-reducing effect of the clinically used drug clofazimine in a SCID mouse model of pancreatic ductal adenocarcinoma. Oncotarget. 2017; 8(24): 38276-93

99. Coss RA, Storck CW, Wells TC, Kulp KA, Wahl M, et al. Thermal sensitisation by lonidamine of human melanoma cells grown at low extracellular pH. Int J Hyperthermia. 2014; 30(1): 75-8

100. Nancolas B, Guo L, Zhou R, Nath K, Nelson DS, et al. The anti-tumour agent lonidamine is a potent inhibitor of the mitochondrial pyruvate carrier and plasma membrane monocarboxylate transporters. Biochem J. 2016; 473(7): 929-36

101. Mehibel M, Ortiz-Martinez F, Voelxen N, Boyers A, Chadwick A, et al. Statin-induced metabolic reprogramming in head and neck cancer: a biomarker for targeting monocarboxylate transporters. Sci Rep. 2018; 8(1): 16804

102. Supuran CT. Carbonic Anhydrase Inhibition and the Management of Hypoxic Tumors. Metabolites. 2017; 7(3): 48

103. Buran K, Bua S, Poli G, Onen Bayram FE, Tuccinardi T, et al. Novel 8-Substituted Coumarins That Selectively Inhibit Human Carbonic Anhydrase IX and XII. Int J Mol Sci. 2019; 20(5): 1208

104. Parkkila S, Innocenti A, Kallio H, Hilvo M, Scozzafava A, et al. The protein tyrosine kinase inhibitors imatinib and nilotinib strongly inhibit several mammalian alpha-carbonic anhydrase isoforms. Bioorg Med Chem Lett. 2009; 19(15): 4102-6