



# Obstacles for T-lymphocytes in the tumour microenvironment: Therapeutic challenges, advances and opportunities beyond immune checkpoint

Navin Kumar Verma,<sup>a\*</sup> Brandon Han Siang Wong,<sup>a,b</sup> Zhi Sheng Poh,<sup>a</sup> Aiswarya Udayakumar,<sup>a,c</sup> Ritu Verma,<sup>a,d</sup> Ryan Kwang Jin Goh,<sup>a</sup> Shane P. Duggan,<sup>e</sup> Vishalkumar G. Shelat,<sup>a,f</sup> K. George Chandy,<sup>a</sup> and Nicholas Francis Grigoropoulos<sup>g,h\*\*</sup>

<sup>a</sup>Lee Kong Chian School of Medicine, Nanyang Technological University Singapore, Singapore

<sup>b</sup>Interdisciplinary Graduate Programme, NTU Institute for Health Technologies (HealthTech NTU), Nanyang Technological University Singapore, Singapore

<sup>c</sup>Manipal Institute of Technology, Manipal Academy of Higher Education, India

<sup>d</sup>Department of Biotechnology, University Institute of Engineering and Technology, Panjab University, Chandigarh, India

<sup>e</sup>Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada

<sup>f</sup>Department of General Surgery, Tan Tock Seng Hospital, Singapore

<sup>g</sup>Department of Haematology, Singapore General Hospital, Singapore

<sup>h</sup>Duke-NUS Medical School, Singapore

## Summary

The tumour microenvironment (TME) imposes a major obstacle to infiltrating T-lymphocytes and suppresses their function. Several immune checkpoint proteins that interfere with ligand/receptor interactions and impede T-cell anti-tumour responses have been identified. Immunotherapies that block immune checkpoints have revolutionized the treatment paradigm for many patients with advanced-stage tumours. However, metabolic constraints and soluble factors that exist within the TME exacerbate the functional exhaustion of tumour-infiltrating T-cells. Here we review these multifactorial constraints and mechanisms – elevated immunosuppressive metabolites and enzymes, nutrient insufficiency, hypoxia, increased acidity, immense amounts of extracellular ATP and adenosine, dysregulated bioenergetic and purinergic signalling, and ionic imbalance - that operate in the TME and collectively suppress T-cell function. We discuss how scientific advances could help overcome the complex TME obstacles for tumour-infiltrating T-lymphocytes, aiming to stimulate further research for developing new therapeutic strategies by harnessing the full potential of the immune system in combating cancer.

eBioMedicine 2022;83:

104216

Published online xxx

<https://doi.org/10.1016/j.ebiom.2022.104216>

ebiom.2022.104216

**Copyright** © 2022 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

**Keywords:** Immunotherapy; Ionic checkpoint; Tumor-interstitial fluid; Tumor-infiltrating lymphocytes

## Introduction

“Immunosurveillance” is a powerful defense mechanism by which both innate and adaptive arms of the immune system coordinate to protect from tumour development. However, immune defense systems often fail, leading to advanced cancer and demise of the patient. In this context, a 3E theory - “Elimination, Equilibrium, Escape” - that explains cancer immunoediting has been proposed.<sup>1</sup> The ‘elimination’ phase employs

cells and molecules of the immune system to destroy pre-malignant and malignant cells as they emerge. In the next phase, cancerous cells that have endured the elimination phase enter a dynamic survival ‘equilibrium’ by a variety of genomic and epigenomic changes. These changes endow tumour cells with resilience to immune detection, eventually leading to ‘escape’ from immunological containment and subsequent unchecked tumour growth.

The plasticity of tumour cells lies in their aberrant ability to reprogram vital pathways that ensure survival within the dynamic tumour microenvironment (TME). In other words, tumours utilize the concept of “the enemy of my enemy is my friend”, as featured in the 2004 science fiction movie “Alien vs. Predator”, where humans ally with predators to fight Xenomorph aliens. Tumour cells thus shift the balance of hostile vs permissive TME to favour their own survival.<sup>2</sup>

\*Corresponding author at: Lee Kong Chian School of Medicine, Nanyang Technological University Singapore, 11 Mandalay Road, Clinical Sciences Building, 308232 Singapore.

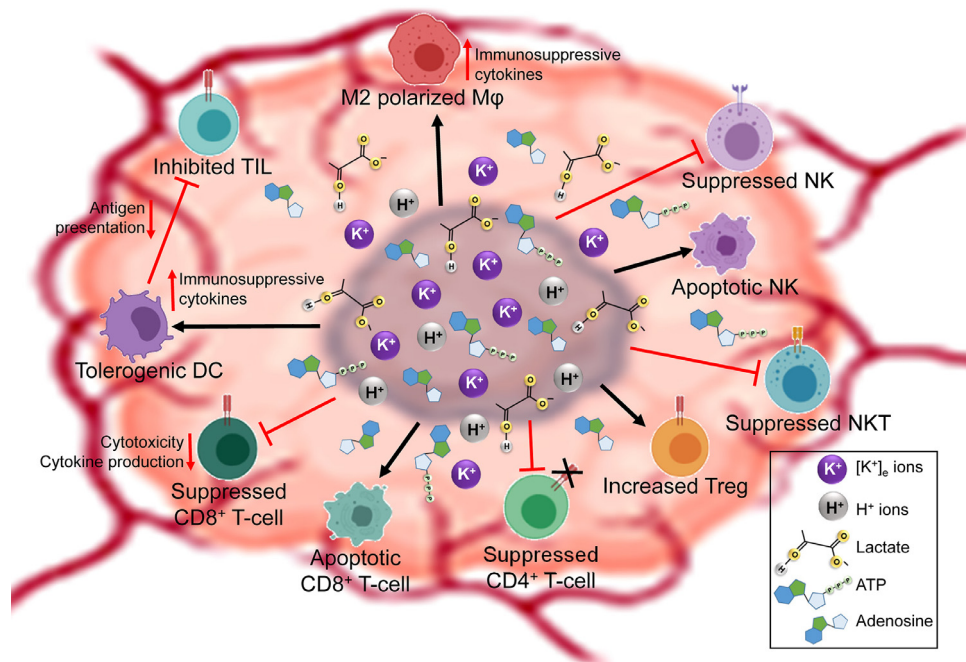
\*\*Corresponding author at: Department of Haematology, Singapore General Hospital, The Academia, 20 College Road, Singapore.

E-mail addresses: [nkverma@ntu.edu.sg](mailto:nkverma@ntu.edu.sg) (N.K. Verma), [nicholas.francis.grigoropoulos@singhealth.com.sg](mailto:nicholas.francis.grigoropoulos@singhealth.com.sg) (N.F. Grigoropoulos).

T-lymphocytes are the host's most potent immune defense weapons. To perform an effective immune surveillance and mount an efficient immune response, circulating and tissue-localized T-cells are armed with a range of immunological safety controls. These controls include T-cell expression of inhibitory proteins such as programmed death-1 (PD-1), lymphocyte activation gene-3 (LAG-3), T-cell immunoglobulin and mucin domain-containing protein-3 (TIM-3), leukocyte-associated immunoglobulin-like receptor-1 (LAIR-1), T-cell immunoreceptor with Ig and ITIM domains (TIGIT), sialic acid binding Ig-like lectins (Siglecs), and cytotoxic T-lymphocyte antigen-4 (CTLA-4), widely known as "immune checkpoints". In addition, regulatory T-cells (Tregs) and other immune cell types, such as M2 polarized macrophages, tolerogenic dendritic cells (DCs), and myeloid-derived suppressor cells (MDSCs) in the TME maintain immune equilibrium by suppressing T-cell function. While main roles of these inhibitory proteins and suppressor cells are to ensure self-tolerance and mitigate potential autoimmunity, they also endow the TME with the capacity to thwart host's anti-tumour responses. Moreover, in many tumours, the TME exposes tumour-infiltrating lymphocytes (TILs) to a spectrum of factors, including dysregulated ion concentrations [e.g., increased potassium ( $K^+$ )], hypoxia, and increased acidity (Figure 1). Altogether, these factors result in T-cell anergy favouring tumour immune escape, growth, and metastasis.

Exciting advances over the past decade have led to new therapies that harness the anti-tumour activities of T-cells by targeting immune checkpoints. Immune checkpoint blocking antibodies against CTLA-4 (ipilimumab), PD-1 (pembrolizumab, nivolumab, and cemiplimab), and PD-1 ligand 1 (PD-L1; atezolizumab, durvalumab, and avelumab) have shown encouraging results in clinical trials and were approved by the US Food and Drug Administration (FDA) to treat several cancer types.<sup>3</sup> However, many tumours are or become resistant to immune checkpoint blockers and relapses remain frequent.<sup>4</sup> These facts led to a realization that cancer cells employ multifaceted mechanisms to reprogram the TME and thus limit T-cells from mounting effective anti-tumour responses.

Major immune checkpoints and their therapeutic interventions have been described in many publications.<sup>3-5</sup> However, beyond immune checkpoints, relatively less well-understood obstacles created by the complex TME that preferentially limit TIL function and their potential targeting for cancer treatment have not been given as much attention in literature. Here we review some of these less-appreciated but extremely important factors and processes in the TME, including elevated immunosuppressive metabolites, nutrient deficiency, hypoxia, lactic acid, dysregulated purinergic and bioenergetic signalling, increased amounts of extracellular ATP ([ATP]<sub>e</sub>), adenosine, and potassium ion ( $K^+$ ) released from dying/necrotic tumour cells, that



**Figure 1.** TME factors that suppress T-cell anti-tumour responses. Dying/necrotic tumour cells release significant amounts of intracellular contents, such as  $K^+$  ions, ATP, and adenosine into the extracellular milieu. In addition, nutrient insufficiency, hypoxia, and altered enzymatic activities increase lactate concentrations creating the TME acidic. These conditions favour the differentiation of suppressive Tregs and tolerogenic DCs; all of which are unfavourable for the anti-tumour functions of infiltrating T-lymphocytes.

collectively suppress T-cell anti-tumour response. We discuss challenges to tackle these secondary checkpoints. Finally, we summarize strategies to integrate increasing knowledge for directing and revitalizing T-lymphocytes into the TME, which are critical to augment the clinical benefit of immunotherapy.

### Nutrient insufficiency, hypoxia, and acidity in the TME impose unique metabolic hurdles to infiltrating T-lymphocytes

The two major metabolic pathways that provide cellular energy (*i.e.*, ATP) are glycolysis and oxidative phosphorylation (OXPHOS). For immunosurveillance, T-cells predominantly use glycolysis that converts glucose into pyruvate *via* a series of oxygen-independent metabolic reactions.<sup>6</sup> Upon encountering specific tumour antigens, activated helper CD4<sup>+</sup> and cytotoxic CD8<sup>+</sup> T-cells undergo extensive clonal proliferation and differentiation into various effector subtypes. These processes utilize anabolic growth programs driven by elevated glycolytic and glutaminolytic pathways, whilst shutting down fatty acid oxidation-related pathways. T-cells that fail to activate these metabolic programs become hyporesponsive. Of note, this immunosuppressive effect may be exacerbated under nutrient inadequacy specifically encountered in the TME.<sup>7</sup>

Tumour cells exhibit high metabolic demands for glucose, amino acids, and fatty acids to sustain their growth and hyperproliferation. In this context, metabolic reprogramming from OXPHOS to aerobic glycolysis (termed as “Warburg effect”) plays a key role in conferring a competitive advantage for energy to cancer cells.<sup>8</sup> Aerobic glycolysis is energetically unfavourable since it generates eighteen times less ATP in comparison to OXPHOS per mole of glucose. However, the kinetics of aerobic glycolysis, which occurs up to hundred times faster than OXPHOS, allows tumours to compete and access energy from glucose at a rapid rate. Although this Warburg effect is reversible and T-cells can adapt to metabolic changes,<sup>9</sup> dysregulated cellular energetics due to the accumulation of oncogenic mutations makes it irreversible in tumour cells.<sup>6</sup>

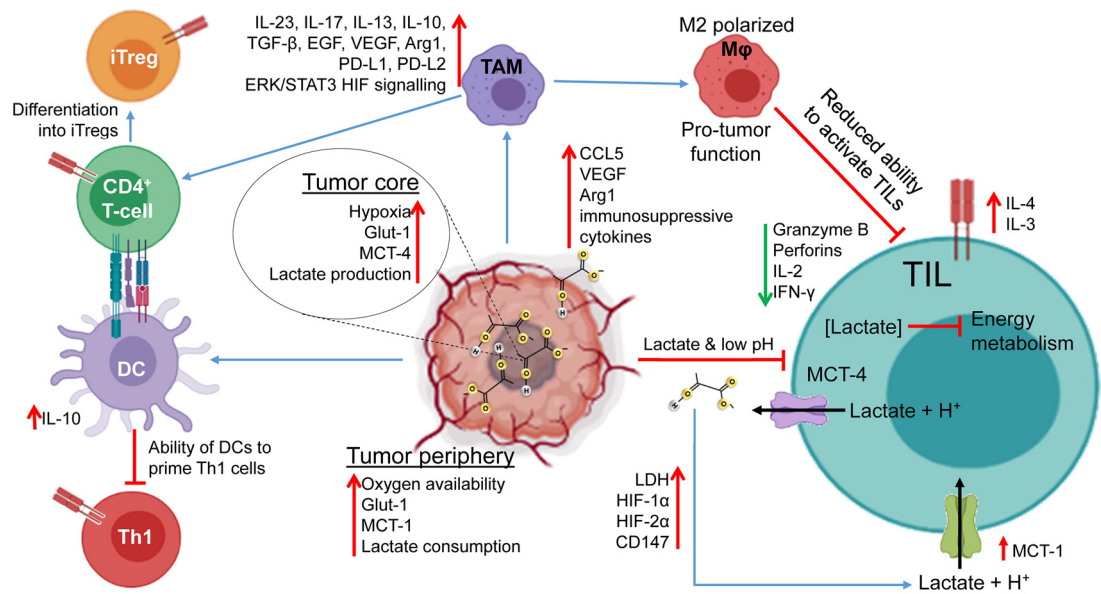
In addition to carbohydrate metabolism, altered amino acid metabolism and elevated cholesterol in the TME cause progressive loss of effector functions of TILs.<sup>10,11</sup> For example, the deficiency of L-arginine and its downstream metabolites, ornithine and citrulline, in the TME arrests T-cell cycle in the G<sub>0</sub>-G<sub>1</sub> phase *via* cyclin D<sub>3</sub> and Cdk4 blockade, impairs T-cell proliferation and cytokine release, and blunts T-cell anti-tumour responses.<sup>12,13</sup> On the other hand, increased amounts of kynurenine in the TME produced from tryptophan catabolism induces immunosuppression.<sup>14</sup> Increased accumulation of cholesterol in the TME and consequently in TILs disrupts lipid metabolism network in

infiltrating T-cells.<sup>11,15</sup> High cholesterol increases endoplasmic reticulum stress and upregulates the expression of immune checkpoint proteins, including PD-1, TIM-3, and LAG-3 leading to CD8<sup>+</sup> T-cell exhaustion.<sup>11,15</sup> In a mouse breast cancer model, the cholesterol metabolite 27-hydroxycholesterol has been found to deplete CD8<sup>+</sup> T-cells within tumours and metastatic lesions.<sup>16</sup>

In the tumour core, aberrant vascularization and impaired blood-flow limit oxygen supply, creating a hypoxic environment. Hypoxia attracts Tregs into the TME, which in turn suppresses the functions of effector T-cells and promotes tumour growth.<sup>17</sup> This condition upregulates the expression of Hypoxia-Inducible Factors (HIFs), including HIF-1 $\alpha$  and HIF-1 $\beta$ . Hypoxia also enhances glucose uptake and intensifies glycolysis by cancer cells, exacerbating glucose paucity in the TME.<sup>17</sup>

Hypoxic stress forces cells to balance their energy requirements by overexpressing the lactate dehydrogenase (LDH). This enzyme induces tumour cells to produce excess lactic acid through over-consumption of glucose.<sup>18</sup> In addition, tumours overexpress lactic acid transporters, such as the lactate-activated G-protein-coupled receptor (GPR81) and the monocarboxylate transporter (MCT) family (*e.g.*, MCT1, MCT4), that mediate lactate efflux, thus preventing feedback inhibition of the glycolytic pathway.<sup>19</sup> MCTs co-transport protons and hence contribute significantly to the acidification of the TME. Increased acidification further activates lactic acid transporters, thus influencing angiogenesis, metabolism, metastasis, and tumour survival.<sup>19</sup> In some tumours, lactate concentrations rise from the physiological 2 mM to as high as 50 mM.<sup>20</sup>

While tumour cells and Tregs can thrive in a lactate-rich environment,<sup>21</sup> increased acidity in the TME suppresses infiltrating cytotoxic T-cells (CTLs) by multiple mechanisms, including inhibition of proliferation, cytokine production and cytotoxicity, motility arrest and bioenergetic silencing.<sup>22</sup> Intracellular accumulation of lactic acid in T-cells acts as a feedback inhibitor for glycolysis by inhibiting phosphofructokinase and downregulating hexokinase-1, which hampers T-cell energy metabolism.<sup>22</sup> Similar to high cholesterol, the highly glycolytic TME upregulates the expression of the immunosuppressive ligands (*e.g.*, PD-L1 and PD-L2) in tumour cells and also PD-1 in Tregs,<sup>18,23</sup> contributing to T-cell anergy. Furthermore, lactic acid epigenetically alters gene expression in tumour-associated macrophages (TAMs), favouring their polarization into the M2 subtype.<sup>24</sup> These M2 TAMs in turn facilitate tumour immune escape by secreting immunosuppressive cytokines, tumour promoting growth factors and enzymes, including IL-13, IL-10, TGF $\beta$ , CCL9, VEGF, EGF, and arginase 1.<sup>24</sup> Impaired differentiation of DCs in the TME impedes tumour antigen presentation to T-cells,<sup>25</sup> which is an essential step in mounting T-cell anti-tumour responses (Figure 2). Increased amounts of lactate in the TME and serum of patients with



**Figure 2.** Lactic acid buildup in the TME impacting immunological pathways resulting in impaired T-cell anti-tumour response. Hypoxia in the tumour core, over-consumption of glucose, and increased expression of LDH and lactate transporters contribute to the TME acidification. Increased acidity in the TME hampers the function of TILs by multiple mechanisms - inhibiting energy metabolism, upregulating inhibitory receptors (e.g., PD-1), disrupting TCR signalling, and producing immunosuppressive cytokines, enzymes, and signalling proteins (e.g., IL-4, IL-10, CCL5, TGFβ, VEGF). It also inhibits the ability of DCs to prime Th1 cells and skews TAM polarization toward the M2 phenotype. These M2-like TAMs lack the ability of phagocytizing tumour cells and help tumour cells escape from the TIL attack.

osteosarcoma and pancreatic cancer have been linked to poor prognosis.<sup>26,27</sup>

Continuous metabolic stress and antigen stimulation, complemented by hypoxia in the TME, repress mitochondrial biogenesis and functions of TILs.<sup>28</sup> Major mitochondrial dysfunctions include defects in the OXPHOS pathway, depolarization, and decreased mitochondrial mass and activity (Figure 3), rendering TILs metabolically inefficient and functionally exhausted.<sup>28</sup>

In comparison to naive T-cells, memory T-cells have larger mitochondrial biomass and higher expression of the mitochondrial enzyme, carnitine palmitoyl transferase,<sup>28</sup> which is a rate limiting enzyme in the fatty acid oxidation process. Lower mitochondrial mass in TILs causes diminished production of cytokines, such as TNF-α and IFN-γ, and the upregulation of co-inhibitory proteins TIM-3, LAG-3, and PD-1.<sup>28</sup> The increased levels of PD-1 in exhausted TILs induce dynamic changes in the mitochondria. These include increased accumulation of depolarized mitochondria with shorter and fewer cristae,<sup>29</sup> which impair T-cell metabolic reprogramming and induce cellular senescence, ultimately causing TILs to be terminally exhausted.<sup>30</sup>

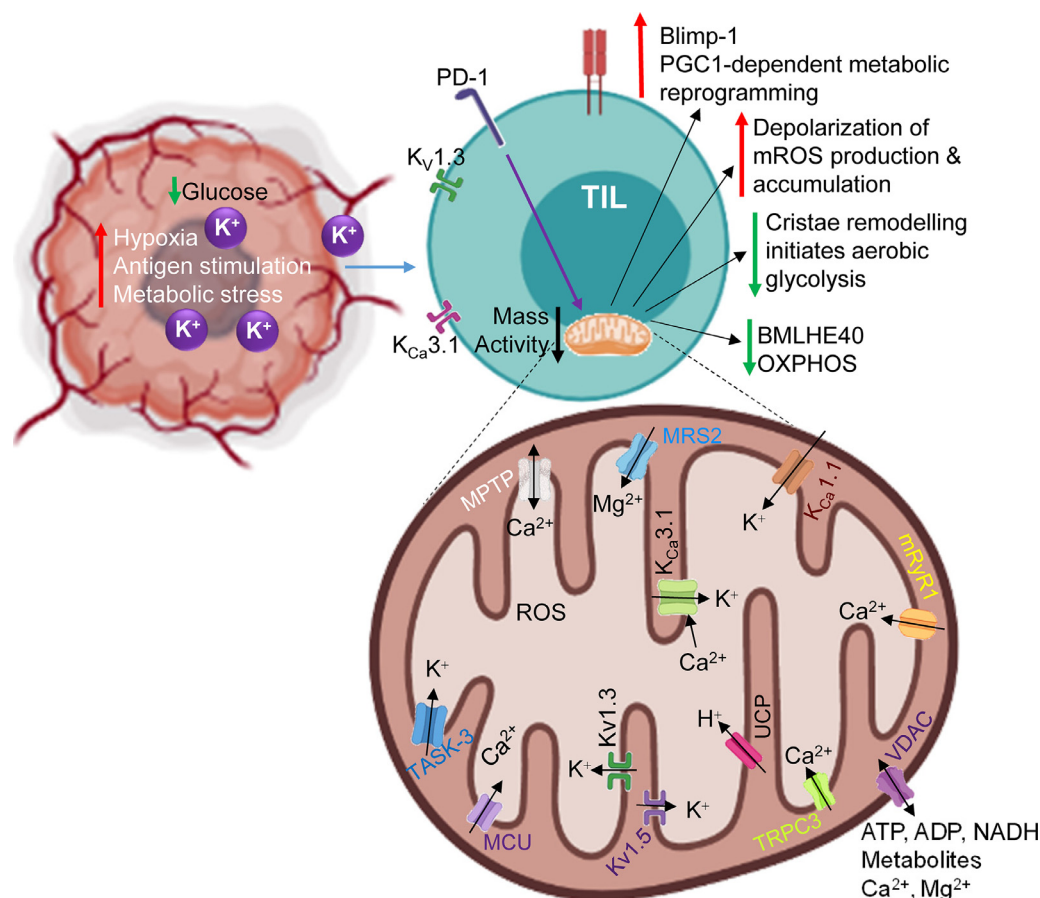
The hypoxic stress and sustained antigen-mediated stimulation within the TME upregulate the expression of the B lymphocyte-induced maturation protein 1 (BLIMP-1) in TILs.<sup>31</sup> This represses the peroxisome proliferator-activated receptor-γ coactivator 1-α (PGC1-α),

which is a master regulator of genes associated with mitochondrial biogenesis.<sup>31</sup> Progressive loss of PGC1-α in antigen-stimulated T-cells induce chronic Akt signalling, which further impairs T-cell metabolic programming and function.<sup>32</sup> In addition, decreased PGC1-α in TILs increases the production of mitochondrial reactive oxygen species (mROS) triggering NFAT signalling, which causes T-cell exhaustion.<sup>28</sup> Dysfunctional mitochondria in T-cells coupled with impaired OXPHOS further limits the proliferation and self-renewal of T-cells exposed to persistent antigens.<sup>33</sup>

PD-1 deficient mice upregulate mitochondrial genes and have larger mitochondrial mass.<sup>34</sup> This suggests an interesting link between mitochondrial function and restoration of exhausted T-cells. Indeed, stronger anti-tumour immune responses are seen in tumour-specific T-cells by reprogramming mitochondrial function through restoring lost PGC1-α expression.<sup>28</sup> Altered metabolic states, hypoxia, and acidity in the TME thus blunt T-cell anti-tumour responses and facilitate immune evasion of rapidly proliferating cancerous cells.

### TME purinergic signalling hinders T-cell anti-tumour responses

As with elevated lactic acid levels discussed above, [ATP]<sub>e</sub> is another oncometabolite present at very high concentrations in the TME. While physiological [ATP]<sub>e</sub>



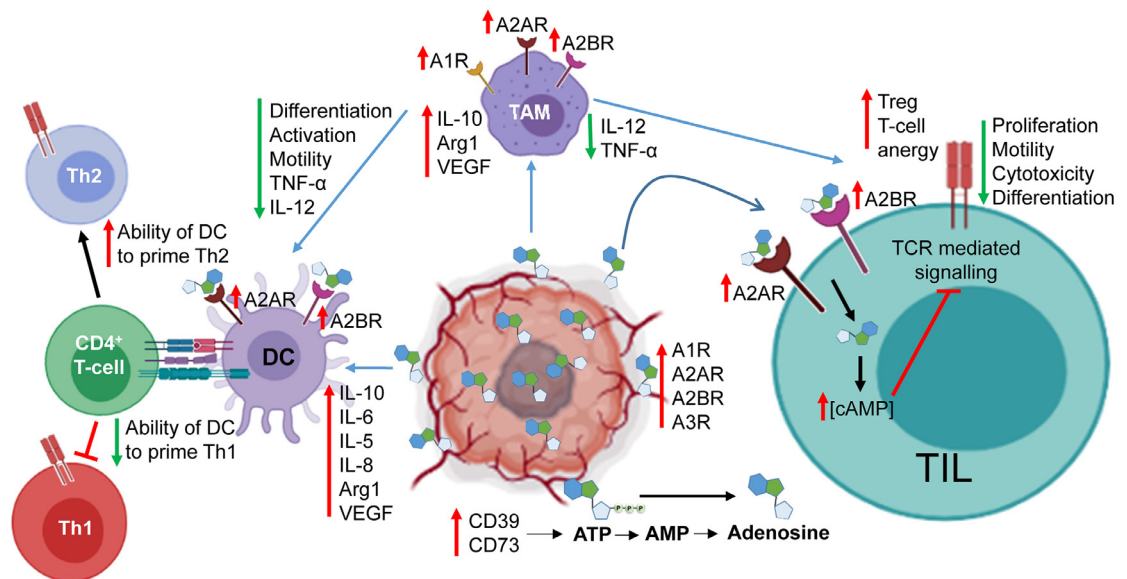
**Figure 3.** TME factors interfering mitochondrial functions of TILs. Continuous hypoxia, metabolic stress and antigen stimulation impede the functional fitness of TIL mitochondria and mitochondrial biogenesis. In addition, they upregulate PD-1, Blimp-1 and PGC1-dependent metabolic reprogramming and depolarize mROS production, while reducing mitochondrial cristae remodelling and OXPHOS pathway. These changes skew bioenergetic signalling, metabolic reprogramming and ion-based pathways rendering TILs exhausted and non-functional.

levels are confined to the nanomolar range,  $[ATP]_e$  released from dying/necrotic cells in the TME increase up to several hundred micromoles,<sup>35</sup> becoming a toxic metabolite or an “energy checkpoint”. The elevated concentrations of  $[ATP]_e$  in the TME depend on multiple factors, including rates of tumour necrosis, hypoxia, and inflammation at the tumour site, all of which disrupt tumour cell membranes causing intracellular ATP leak into the TME.

ATP is metabolized into ADP, AMP, and adenosine, and these metabolic toxics play diverse roles in T-cell function in the context of anti-tumour immunity. Soluble and membrane-bound ectonucleotidases, mainly CD39 and CD73, present on tumour cells and tumour-associated immune cell types such as TAMs, MDSCs, Th17 cells, Tregs, and exhausted TILs, hydrolyze  $[ATP]_e$  resulting in high adenosine levels in the TME.<sup>36</sup> While CD39 facilitates the conversion of ATP and ADP to AMP, CD73 subsequently converts AMP into adenosine.<sup>37</sup> In addition to hypoxic conditions in the TME,

inflammatory cytokines, including IL-2, TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-27 and factors that induce cAMP and Wnt pathways also aid in upregulation of CD39 and CD73.<sup>37</sup> (Figure 4). Elevated expression of CD39 and CD73 is associated with poor therapeutic outcomes in cancer patients.<sup>37</sup>

Accumulation of adenosine in the TME impacts cellular signal transduction through adenosine receptors. P1R and P2R are the two major purinergic receptor categories. P1R has 4 subtypes: A1R, A2AR, A2BR, and A3R, all belonging to the G-protein coupled receptors (GPCR) class. The P2R comprises two receptor families: the ligand-gated ion channel receptor P2XR and the P2YR, which is a GPCR. While P1R shows selectivity towards adenosine, P2R selects ATP and ADP. Adenosine binding to P1R facilitates intracellular accumulation of cAMP.<sup>36</sup> The main effector molecule downstream of the cAMP pathway is the protein kinase A (PKA), which negatively regulates multiple signalling cascades, including JAK3, Raf-1, Csk, Rho-A, SHP-1 and NFAT,



**Figure 4.** Major TME purinergic signalling pathways that impair anti-tumour responses of infiltrating T-lymphocytes. Increased soluble and cell membrane-bound ectonucleotidases (CD39 and CD73) increase adenosine levels within the TME. Adenosine induces the production of immunosuppressive cytokines (e.g., IL-10, IL-6, VEGF). It also inhibits the functioning of TAMs and DCs that not only favour tumour progression but hinder TIL function (such as proliferation, motility, and cytotoxicity).

all of which contributes to T-cell anergy and tumour survival.<sup>36</sup> PKA also increases intracellular  $K^+$  levels by inhibiting the activities of the  $K^+$  efflux channels,  $Kv1.3$  and  $KCa3.1$ , thus dampening T-cell activity.<sup>36,38</sup>

High concentrations of  $[ATP]_e$  in the TME activate P2YR receptors, which promote proliferation, metastasis, and invasiveness of tumour cells.<sup>39</sup> Low intensity stimulation of the P2X7R increases intracellular  $Ca^{+2}$ , which increases mitochondrial OXPHOS and enhances aerobic glycolysis rate, resulting in an excessive lactic acid buildup in the TME. P2X7R blockade in mice has been found to reduce  $[ATP]_e$  concentrations in the TME, resulting in increased tumour infiltration of  $CD4^+$  effector T-cells with their reduced expression of CD39 and CD73.<sup>40</sup>

Apart from a direct impact on T-cells, adenosine exerts its effect on other cells like DCs, TAMs and stromal cells,<sup>36</sup> all of which subsequently influence T-cell functions. For example, adenosine signalling through A2BR abolishes the differentiation of DCs from monocytes, reducing their ability to prime anti-tumour T-cell responses.<sup>41</sup> Complementing to this differentiation shift, adenosine-treated DCs exhibit reduced secretion of pro-inflammatory cytokines (e.g.,  $TNF-\alpha$ , IL-12) and increased secretion of suppressive cytokines (e.g., IL-6, IL-5, IL-10, IL-8,  $TGF-\beta$ ).<sup>42</sup> In summary, the cumulative changes caused by the triggering of the “energy checkpoint” result in a hyporesponsive immune state forming a perfect niche for cancerous cells.

### Enzymatic modulation in the TME selectively impacts T-cell function

In addition to the above described ectoenzymes that metabolize  $[ATP]_e$ , tumour cells express several other immunosuppressive enzymes that catabolize essential amino acids and metabolites such as, tryptophan, arginine, and phenylalanine. For example, TME is rich in indolamine-2,3-dioxygenase (IDO), which is a rate-limiting enzyme involved in tryptophan catabolism by the kynurenine pathway. Kynurenine is toxic to T-cells and feeding into the feedback loop of immunosuppression, IDO expression is upregulated in tumour cells.<sup>43</sup> Notably, increased expression of IDO in breast cancer correlates with enhanced Treg infiltration and lymph node metastasis.<sup>14</sup> Importantly, the immunosuppressive effects of IDO are not restricted to immune cells, but also to other cells in the vicinity, thereby imposing additive metabolic inhibitory effects on TILs.

The ubiquitin-proteasome complex present in the TME degrades proteins tagged by the ubiquitin-activating enzyme E1, the ubiquitin-conjugating enzyme E2, and the ubiquitin ligase E3.<sup>44</sup> For example, the E3 ligase mediates the degradation of promyelocytic leukaemia protein resulting in the production of the ectonucleotidase CD73, which metabolizes AMP to adenosine and hinders T-cell anti-tumour response.<sup>45</sup> The E3 ligase UBR5 has been found to drive the growth and metastasis of triple-negative breast cancer by depleting T-cells.<sup>46</sup> The ubiquitin-proteasome complex also inhibits the expression of

proinflammatory cytokines (such as, IL-2 and IFN- $\gamma$ ), and upregulates immune checkpoint proteins (such as PD-L1), which together contribute to immune suppression and tumour survival.

### TME ionic imbalance suppresses T-cell functions

Interest is rising in understanding the immunomodulatory roles of ionic imbalance in the TME, especially pertaining to T-cell functions.<sup>47</sup> Akin to [ATP]<sub>e</sub>, intracellular K<sup>+</sup> is released by dying/tumour cells into the TME, which accumulates in the tumour milieu. The elevation of extracellular K<sup>+</sup> ([K<sup>+</sup>]<sub>e</sub>) in the tumour-interstitial fluid can often reach levels ten to twelve times higher than those encountered by T-cells in the bloodstream.<sup>48</sup> Bathing T-cells in K<sup>+</sup>-rich tumour-interstitial fluid impairs tumoricidal properties.<sup>48,49</sup> These findings led to a novel paradigm, the “K<sup>+</sup> checkpoint”, explaining how this toxic metabolic element “high-[K<sup>+</sup>]<sub>e</sub>” elicits T-cell suppression within the TME.

Molecular and electrophysiological studies have identified a unique contingent of ion channels in T-cells that play a pivotal role in eliciting and orchestrating TCR activation, influencing Ca<sup>2+</sup> homeostasis, cytokine production, cell proliferation, and clonal expansion.<sup>50</sup> Of particular importance are the voltage-gated K<sub>V1.3</sub> and the calcium-activated K<sub>Ca3.1</sub> K<sup>+</sup> channels. K<sub>V1.3</sub> is specifically upregulated in CCR7<sup>-</sup> effector memory (T<sub>EM</sub>) T-cells, while K<sub>Ca3.1</sub> expression is high in CCR7<sup>+</sup> naïve and central memory (T<sub>CM</sub>) T-cells.<sup>50</sup> Notably, a recent paper developed K<sub>V1.3</sub>-targeting radiotracers that were used to track TIL activation within the TME after immunotherapy.<sup>51</sup>

High-[K<sup>+</sup>]<sub>e</sub> suppresses T-cells mainly through excessive intracellular accumulation of K<sup>+</sup> because of influx exceeding efflux.<sup>49</sup> Moreover, hypoxia within the TME aggravates the situation by reducing the function and expression of K<sub>V1.3</sub>,<sup>52</sup> which would further impair K<sup>+</sup> efflux and elevate intracellular K<sup>+</sup> in tumour-infiltrating T-cells. In the TME, increased levels of adenosine selectively inhibit the action of K<sub>Ca3.1</sub> via the A<sub>2A</sub> receptor. As a result, K<sup>+</sup> efflux by K<sub>V1.3</sub> and K<sub>Ca3.1</sub> is unable to balance the rate of K<sup>+</sup> influx. The excessive amounts of internal K<sup>+</sup> explicitly attenuate TCR-driven phosphorylation of Akt by up-regulating the activity of protein phosphatase enzyme PP2A.<sup>48,49</sup> Reduced Akt phosphorylation inhibits the activities of downstream signalling and metabolic effector proteins, like mTOR kinases. Consequently, an upsurge in [K<sup>+</sup>]<sub>e</sub> promotes intracellular hyperkalemia within T-cells, and as intracellular K<sup>+</sup> rises above the K<sup>+</sup> checkpoint (~40 mM), T-cells become suppressed with an impaired ability to produce IFN- $\gamma$  and IL-2, thus reduced tumour cell clearance.<sup>48,49</sup>

### Harnessing novel paradigms to empower T-cells in the TME

It is evident that while tumour cells and activated T-cells share convergent metabolic reprogramming, divergent

processes employed by cancer cells allow them to out-compete their immune counterparts and thrive unrestricted in the TME. Despite these setbacks, pharmacological manipulation of T-cell metabolism to improve cancer immunotherapies is emerging as an exciting approach (Table 1).

Tumours have high demands for metabolites owing to their high growth and proliferation rates and hence certain semi-essential amino acids, such as arginine, become essential to them. Since *de novo* synthesis becomes insufficient for tumour cells, they require an exogenous source. Moreover, many tumours in patients with small-cell lung cancer, hepatocellular carcinoma, melanoma, and other cancers downregulate the argininosuccinate synthetase 1 (ASS1),<sup>53,54</sup> an enzyme that converts citrulline to arginine. These ASS1-deficient tumours are dependent on exogenous arginine. Based on this knowledge, arginine deprivation is being exploited as a potential therapeutic approach. One such approach is the use of a novel oral arginase 1/2 inhibitor, that showed promising results in terms of enhancing the anti-tumour effect of PD-1 inhibition in murine experimental gliomas.<sup>55</sup> Blocking T-cell cholesterol esterification by genetic ablation or pharmacological inhibition of the key cholesterol esterification enzyme acetyl-CoA acetyltransferase 1 (by an inhibitor avasimibe) potentiated the anti-tumour activity of CD8<sup>+</sup> T-cells in mice.<sup>15</sup> Inhibiting/silencing PCSK9, a serine protease that modulates cholesterol metabolism, has been found to promote intra-tumoral infiltration of cytotoxic T-cells.<sup>56</sup> These suggest that inhibiting cholesterol esterification, PCSK9, and arginase could potentially be exploited to improve the immune checkpoint inhibitor therapy outcome for cancer.

Improving T-cell mitochondrial fitness by supplementation with nicotinamide riboside (NR), a member of the vitamin B<sub>3</sub> family and a potent modifier of energy metabolism, is a feasible approach to improve T-cell anti-tumour response. Mice treated with oral or intra-tumoral administration of NR exhibited decreased ROS levels, improved mitochondrial function, and enhanced T-cell responsiveness to anti-PD-1.<sup>30</sup> Other approach could apply T-cell-specific costimulatory molecule 4-1BB, that increases mitochondrial biogenesis.<sup>57</sup> Reprogramming or forced expression of PGC1 $\alpha$  in T-cells has been found to promote mitochondrial biogenesis, mass, and function,<sup>28,58</sup> and thus could be harnessed to improve an anti-tumour immunity. Targeting the ribonuclease REGNASE-1 in mouse models of melanoma and leukemia improved T-cell mitochondrial metabolism and reprogrammed CD8<sup>+</sup> T-cells to long-lived effector cells with extensive accumulation, better persistence, and robust effector function in tumours.<sup>59</sup> Since mitochondria also define T-cell stemness,<sup>60</sup> mitochondrial fitness is an important consideration for therapies involving hematopoietic stem cell or lymphocyte transfer for cancer treatment. These data open an important

Target	Drug/Agent	Development stage	Cancer type/Experimental model	
Targeting TME purinergic signalling				
CD73	Oleclumab (anti-CD73) + Durvalumab	Phase 2 (NCT04668300)	Recurrent, refractory, or metastatic sarcoma	
	BMS-986179 (anti-CD73) + Nivolumab	Phase 1/2a (NCT02754141)	Advanced/metastatic solid cancer	
	CPI-006 (anti-CD73) + Ciforadenant or Pembrolizumab	Phase 1/1b (NCT03454451)	Advanced cancer	
	NZV930 (anti-CD73) + PDR001 ((anti-PD-1) ± NIR178 (A2AR antagonist)	Phase 1/1b (NCT03549000)	Advanced cancer	
	LY3475070 (orally bioavailable small molecule inhibitor of CD73) ± Pembrolizumab	Phase 1 (NCT04148937)	Advanced cancer	
	TY/23 (mouse anti-CD73)	Experimental, <i>in vivo</i>	Mouse models of lung metastasis and breast cancer	
	Antibody-directed co-blockade of CD73 and A2AR	Experimental, <i>in vivo</i>	Mouse models of melanoma and mammary adenocarcinoma	
	CD73-04 (mouse anti-CD73)	Experimental, <i>in vivo</i>	Mouse models of colon, breast carcinoma	
	AD2 (human anti-CD73)	Experimental, <i>in vivo</i>	Human breast tumour xenografted in mice	
	APCP ( $\alpha\beta$ -methylene adenosine-5'-diphosphate)	Experimental, <i>in vivo</i>	Human breast tumour xenografted in mice	
	AB-680 (small molecular inhibitor of CD73) + Zimberelimab + Nab-Paclitaxel + Gemcitabine	Phase 1 (NCT04104672)	Gastrointestinal malignancies	
	OP-5244 (small molecule CD73 inhibitor)	Experimental, <i>in vitro</i>	Human cancer cell lines	
	CD39	TTX-030 (anti-CD39) + Budigalimab / Pembrolizumab ± Docetaxel / Gemcitabine	Phase 1/1b (NCT04306900)	Solid tumour
		ES002023 (anti-CD39)	Phase 1 (NCT05075564)	Advanced solid tumour
SRF617 (anti-CD39)		Phase 1 (NCT04336098)	Advanced solid tumour	
9-8B (anti-CD 39)		Experimental, <i>in vivo</i>	Fibrosarcoma patient-derived xenograft mice	
POM (small molecule NTPDase inhibitor)		Experimental, <i>in vivo</i>	Mouse model of hepatic metastatic cancer	
A1R	DPCPX (A1R antagonist)	Experimental, <i>in vivo</i>	Human renal cell carcinoma patient-derived xenograft mice	
A2AR	ZM241385 (A2AR antagonist ligand)	Experimental, <i>in vivo</i>	PC9 xenograft model	
	ZM241365 (A2AR antagonist ligand) + anti-CTLA4	Experimental, <i>in vivo</i>	B16-F10 melanoma model	
	SCH58261 (A2AR antagonist)	Experimental, <i>in vivo</i>	B16F10 CD73 <sup>+</sup> and 4T1.2 tumour model	
	FSPTP (A2AR blocker)	Experimental, <i>in vivo</i>	B16F10 and MB49 bladder carcinoma mouse models	
	Ciforadenant (A2AR antagonist) + Daratumuab (anti-CD38)	Phase 1 (NCT04280328)	Multiple myeloma	
	Ciforadenant (small molecule inhibitor of T-cell A2AR) ± Atezolizumab (anti-PD-L1)	Phase 1/1b (NCT02655822)	Advanced cancer	

Table 1 (Continued)



Target	Drug/Agent	Development stage	Cancer type/Experimental model
A2BR	EOS-448 (anti-TIGIT) + Pembrolizumab (anti-PD-1) ± Inupadenant (A2AR antagonist)	Phase 1/2 basket study (NCT05060432)	Advanced solid tumours
	PBF-509 (A2AR antagonist) ± PDR001 (anti-PD1)	Phase 1/1b (NCT02403193)	Advanced non-small cell lung cancer
	AZD4635 (small molecule inhibitor of A2AR) + Durvalumab ± Cabazitaxel	Phase 2 (NCT04495179)	Metastatic castrate-resistant prostate cancer
	AZD4635 + Durvalumab or Oleclumab (anti-CD73)	Phase 2 (NCT04089553)	Prostate cancer
	PSB1115 (A2BR antagonist)	Experimental, <i>in vivo</i>	4T1.2 mouse tumour model
	ATL-801 (A2BR antagonist)	Experimental, <i>in vivo</i>	MB49 bladder and 4T1 breast tumours in syngeneic mice
A3R	Etrumadenant (A2AR/A2BR dual antagonist) / Zimberelimab (anti-PD1)	Phase 1b/2 (NCT04381832)	Metastatic castrate resistant prostate cancer
	CF102 (A3R agonist)	Phase 2 (NCT02128958)	Hepatocellular carcinoma
	CF101 (small molecule agonist of A3R)	Experimental, <i>in vivo</i>	Colon, prostate carcinoma and melanoma mouse models
Targeting lactic acid build up in the TME			
MCT1	AZD3965 (MCT1 inhibitor)	Phase 1 (NCT01791595)	Advanced cancer
LDH	Gossypol (natural non-selective inhibitor of LDH)	Phase 2 (NCT00540722)	Glioblastoma multiforme
	FX11 (small-molecule inhibitor of LDHA)	Experimental, <i>in vivo</i>	Human B-lymphoma and pancreatic cancer xenograft models
	Oxamate (competitive inhibitor of LDH)	Experimental, <i>in vitro</i>	Patient samples of gastric cancer; SGC7901, BGC823 and GES-1 cell lines
	1-(Phenylseleno)-4-(Trifluoromethyl) Benzene (small molecule inhibitor of LDH)	Experimental, <i>in vitro</i>	NCI-H460, MCF-7, Hep3B, A375, HT29, LLC cell lines
	Quinoline 3-sulfonamides (small molecule inhibitor of LDHA)	Experimental, <i>in vivo</i>	Hepatocellular carcinoma models
	N-hydroxyindole (small molecule inhibitor of LDH)	Experimental, <i>in vitro</i>	Pancreatic ductal adenocarcinoma; cervical cancer
	Galloflavin (small molecule inhibitor of LDH)	Experimental, <i>in vitro</i>	PLC/PRF/5 human cell line
	HIF-1 $\alpha$	EZN-2968 (antisense oligonucleotide against HIF-1 $\alpha$ )	Phase 1 (NCT01120288)
Restoration of mitochondrial functions in TILs			
Mitochondrial glycerol-3-phosphate dehydrogenase (G3P)	Metformin (G3P non-competitive inhibitor) ± Pembrolizumab (anti-PD1)	Phase 1 (NCT03311308)	Advanced melanoma
Second mitochondria-derived activator of caspase (SMAC)	LCL161 (SMAC mimetic) ± Cyclophosphamide	Phase 2 (NCT01955434)	Recurrent & refractory multiple myeloma
Targeting "ionic-checkpoint"			
K <sub>Ca</sub> 3.1	SKA-346 (K <sub>Ca</sub> 3.1 activator)	Experimental, <i>in vitro</i>	K562 cell line

**Table 1: List of TME-targeting agents beyond the immune-checkpoint blockers and their status.**

avenue for improving T-cell mitochondrial function as an approach to augment the efficacy of cancer immunotherapies and adoptive cell therapies.

Targeting specific enzymes (such as IDO), ubiquitination and proteasome in the TME is also an important approach. For example, bortezomib, a proteasome inhibitor, augments lymphocyte stimulatory signalling in the TME to sustain CD8<sup>+</sup> T-cell anti-tumour function.<sup>61</sup> Bortezomib has been approved by the FDA for the treatment of multiple myeloma and mantle cell lymphoma. Since E3 ligases regulate PD-L1 stability, modulators of these enzymes can enhance T-cell anti-tumour activity by reducing PD-L1/PD-1 binding. As an example, resveratrol activates the  $\beta$ -TrCP ligase which catalyzes PD-L1 degradation thereby increases T-cell anti-tumour activity in triple-negative and HER2-positive breast cancer.<sup>62</sup>

Our somewhat improved understanding of the K<sup>+</sup> channels in T-cells has led to the development of specific K<sup>+</sup> channel modulators. However, studies investigating the K<sup>+</sup> checkpoint in the TME are very limited. Overexpressing genes encoding for K<sup>+</sup> channels in human tumour-specific T-cells *via* retroviral delivery before adoptive transfer into patients has been suggested.<sup>48</sup> Alternatively, pharmacological activation of K<sup>+</sup> channels is an attractive therapeutic space for exploration. Riluzole, a non-specific activator of the K<sub>Ca3.1</sub> channel, was found to enhance cisplatin uptake into cancerous cells in colorectal cancer patients with cisplatin resistance.<sup>63</sup> Experimental data show that both approaches could rescue T-cells from high-[K<sup>+</sup>]<sub>e</sub>-mediated suppression and enhance tumour-clearance by effector T-cells.<sup>48,49</sup> To this end, K<sub>Ca3.1</sub> presents itself as a better potential therapeutic target due to its unique expression profile on naïve T-cells and T<sub>CM</sub>. Since less differentiated T-cells are more potent effector T-cells, targeting K<sub>Ca3.1</sub> has an advantage over K<sub>v1.3</sub>. It would be imperative to test a combinatorial therapeutic approach that overrides the K<sup>+</sup> checkpoint and engages immune checkpoint blockade strategy.

### Challenges associated with empowering T-cells in the TME

Given the convergent metabolic programs between T-cells and tumour cells, shutting down metabolic pathways entirely would likely lead to immunosuppression, and unfavourable clinical outcomes. Selectively potentiating T-cells within the TME would be more rational, but also more challenging.

Therapeutic approaches aimed at blocking/inhibiting CD73, CD39, and PRs to counter high levels of [ATP]<sub>e</sub> and adenosine in the TME have been developed, but each of them has some drawbacks. For example, low selectivity towards CD39 is a major problem with the CD39/NADase 1 chemical inhibitor, POM-1.<sup>64</sup>

Schiff bases of tryptamine (*e.g.*, compound SBT-C6) strongly inhibit CD39, but with a low degree of selectivity.<sup>37</sup> While another Schiff base of tryptamine, named SBT-C1, demonstrated selectivity towards CD39, its ability to competitively inhibit CD39 in an [ATP]<sub>e</sub>-rich TME is limited.<sup>37</sup> Two new quinolone derivatives, compounds QD-3F and QD-3T, showed selective inhibition of the CD39 and are promising agents for future studies.<sup>65</sup> A humanized monoclonal antibody targeting CD39, IPH52, is awaiting clinical trial testing.<sup>36</sup>

Although arginine deprivation therapy has shown promising results in preclinical studies and clinical trials, the clinical application of this approach remains challenging due to technical issues.<sup>55,66,67</sup> Moreover, this approach is largely confined to ASS(-) tumours. While targeting protein degradation the TME is another attractive therapeutic strategy, proteasome inhibitors lack a defined ligand-binding site and selectivity, leading to serious side effects. However, despite these challenges, using small molecules to target the E3 ubiquitin-protein ligase Mdm2 has been partially successful.<sup>68</sup> Targeting lactate dehydrogenase A by small molecule inhibitors is also an attractive option to overcome the lactate barrier in the TME, but challenges remain. These underscore the importance of employing protein engineering to improve drugs and their formulations.

Activating K<sup>+</sup> channels in TILs has been proposed as an attractive strategy for improving immunotherapeutic outcomes. Riluzole showed benefit in a phase 0 trial in patients with advanced melanomas,<sup>69</sup> but was unsuccessful in a phase II trial.<sup>70</sup> There is no existing specific activator for K<sub>Ca3.1</sub>. Serious complications may be encountered in applying engineered T-cells to upregulate K<sub>Ca3.1</sub>. Post-infusion expression of inhibitory receptors could contribute to undesired suppression of effector T-cells.<sup>71</sup> More effort therefore should be invested to overcome the above-mentioned challenges.

### Outstanding questions

Ion channels K<sub>v1.3</sub> and K<sub>Ca3.1</sub> are expressed in mitochondria besides the cell membrane. It remains to clearly understand the expression kinetics of these mitochondrial channels in TILs to better inform future targeting strategies for improving anti-tumour immunity. K<sub>Ca3.1</sub> is also present in myofibroblasts, microglia, red blood cells, B-cells, and macrophages. It would therefore be important to understand how a K<sub>Ca3.1</sub> activator may affect various cell types. Since cells of hematolymphoid malignancies also express K<sub>v1.3</sub> and K<sub>Ca3.1</sub> channels, it would be interesting to know the implication of activating K<sub>Ca3.1</sub> with immunotherapies. T-cells in patients with head and neck cancer exhibit reduced K<sub>Ca3.1</sub> expression;<sup>72</sup> but the mechanism behind this defect remains unclear. Are these T-cells with reduced K<sub>Ca3.1</sub> more susceptible to high-[K<sup>+</sup>]<sub>e</sub>-induced suppression than normal T-cells? It remains unclear whether

immune checkpoint inhibitors alter the expression of these channels on the cell membrane or in mitochondria. Potential roles of other cellular ions, such as manganese, zinc, selenium, magnesium, and iron in anti-tumour immunity are yet to be explored.

The natural anti-inflammatory compound curcumin increases the ubiquitination of PD-L1 in breast and lung cancer and in melanoma and improves the susceptibility of tumour cells to anti-CTLA4 therapy. However, curcumin was not effective in other cancer models and thus requires further investigation. It also blocks the Kv1.3 channel and inhibits the activities of T<sub>EM</sub> cells. It would therefore be interesting to explore how this might impact curcumin drug therapy. The voltage-sensors of Kv1.3 are rich in arginine possessing the motif RXXRXXRXXRXXRXX. It would be fascinating to know if arginine depletion affects K<sup>+</sup> channels, which are an important consideration for the safety and efficacy of the arginine deprivation strategy.

Acidification within the TME causing pH to drop from 7.2-7.4 to 6.4-6.8, reduces c-type inactivation of Kv1.3, which would allow the channels to remain open for longer. It remains unexplored how that might impact the intracellular K<sup>+</sup> concentration and Ca<sup>2+</sup> signalling. Other drugs, like galloflavin, have the potential to neutralize the acidic microenvironment and thereby reduce tumour invasiveness and metastasis, but they are in very early stages of development. Further studies are required to fully delineate the above mechanisms and possible side-effects.

## Conclusions and perspectives

The success of immune checkpoint inhibitor-based immunotherapies has invigorated research in immune surveillance and dramatically changed the therapeutic landscape for cancers. Although several immune checkpoint blockers were approved, there have been increasing concerns on the application of these treatment modalities as a one-size-fit-all cure to cancer because they work more effectively in some cancer types over others. Moreover, intrinsic and acquired resistance often prevent therapeutic efficacy of immune checkpoint inhibitors and patients do not respond. A remaining challenge therefore is to unleash the full power of the anti-tumour immune response by enabling T-cells to overcome the multiple immunosuppressive effects of the TME. These issues call for attention to explore other therapeutic facets of empowering T-cells to fight against cancer. We argue that modulating T-cell function within the TME using an arsenal of combinatorial approaches may achieve robust and durable anti-tumour response. In this regard, immunotherapies as logic gates for guiding cancer treatment have opened-up unprecedented opportunities for precision medicine. The pursuit of this strategy necessitates a better understanding of mutual determinism and potential crosstalk of these

checkpoints as it is possible that they function in synergy. Improved understanding of TMEs would certainly be beneficial for guiding personalized cancer immunotherapy and advance our odds to develop a cure to cancer.

## Search strategy and selection criteria

The literature search for this review was carried out electronically. References were identified by searches of PubMed between 2004-2022 (latest search date: 8<sup>th</sup> of June 2022) and cross-references from relevant articles. The search terms used were - “tumour micro-environment”, “immune suppression and tumour”, “immune checkpoint”, “immune checkpoint inhibitors”, “immunotherapy”, “cancer”. After screening the abstracts, the final reference list was generated and reviewed in-depth based on relevance to the topics covered in this Review. Most articles published within the last 5 years are used.

## Contributors

N.K.V.: conceptualization, design, writing - original draft, writing - review & editing, figures, visualization, supervision, funding acquisition; B.H.S.W.: literature review on ionic checkpoint and interpretation; Z.S.P.: literature review on enzymes and interpretation; A.U.: literature review on metabolic aspects and interpretation, table and figures; R.V.: literature review on mitochondrial dysfunction and interpretation; G.K.J.R.: literature review on proteosomes and interpretation; S. D.: writing - review & editing; V.G.S.: writing - review & editing; G.K.C.: writing - review & editing; N.F.G.: design, writing - review & editing. All authors approved the final version of this manuscript.

## Declaration of interests

The authors declare no competing interest.

## Acknowledgements

This research was supported, in part, by the Singapore Ministry of Education (MOE) under its MOE Academic Research Fund (AcRF) Tier 2 (MOE2017-T2-2-004) and Tier 1 (2020-T1-001-062) grants, and the National Research Foundation Singapore under its Open Fund - Large Collaborative Grant (OFLCG18May-0028) and administered by the Singapore Ministry of Health's National Medical Research Council (NMRC). N.F.G. acknowledges funding support from the NMRC Transition Award (NMRC/TA/0051/2016) and the NMRC Centre Grant (TETRAD 2). B.H.S.W. and Z.S.P. were provided with PhD fellowship by HealthTech NTU and Lee Kong Chian School of Medicine, Nanyang Technological University Singapore. Funders had no direct role in paper design, data collection, data analysis, interpretation, and writing of the paper.

## References

- 1 Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoeediting. *Annu Rev Immunol.* 2004;22:329–360.
- 2 Hanahan D. Hallmarks of cancer: new dimensions. *Cancer Discov.* 2022;12:31–46.
- 3 Twomey JD, Zhang B. Cancer immunotherapy update: FDA-approved checkpoint inhibitors and companion diagnostics. *AAPS J.* 2021;23:39.
- 4 Morad G, Helmink BA, Sharma P, Wargo JA. Hallmarks of response, resistance, and toxicity to immune checkpoint blockade. *Cell.* 2021;184:5309–5337.
- 5 Dall'Olio FG, Marabelle A, Caramella C, et al. Tumour burden and efficacy of immune-checkpoint inhibitors. *Nat Rev Clin Oncol.* 2022;19:75–90.
- 6 Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quiescence. *Immunity.* 2013;38:633–643.
- 7 Niu Y, Mayr T, Muders MH. Competition for nutrients or cell intrinsic programming? - Metabolic mechanisms behind the tumor promoting immune microenvironment in cancer. *Signal Transduct Target Ther.* 2021;6(1):279.
- 8 Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science.* 2009;324:1029–1033.
- 9 Blagih J, Coulombe F, Vincent EE, et al. The energy sensor AMPK regulates T cell metabolic adaptation and effector responses in vivo. *Immunity.* 2015;42:41–54.
- 10 Han C, Ge M, Ho PC, Zhang L. Fueling T-cell antitumor immunity: amino acid metabolism revisited. *Cancer Immunol Res.* 2021;9:1373–1382.
- 11 Ma X, Bi E, Lu Y, Su P, Huang C, Liu L, et al. Cholesterol induces CD8<sup>+</sup> T cell exhaustion in the tumour microenvironment. *Cell Metab.* 2019;30:143–156e5.
- 12 Rodriguez PC, Quiceno DG, Ochoa AC. L-arginine availability regulates T-lymphocyte cell-cycle progression. *Blood.* 2007;109:1568–1573.
- 13 Martí I, Líndez AA, Reith W. Arginine-dependent immune responses. *Cell Mol Life Sci.* 2021;78:5303–5324.
- 14 Katz JB, Muller AJ, Prendergast GC. Indoleamine 2,3-dioxygenase in T-cell tolerance and tumoral immune escape. *Immunol Rev.* 2008;222:206–221.
- 15 Yang W, Bai Y, Xiong Y, et al. Potentiating the antitumor response of CD8(+) T cells by modulating cholesterol metabolism. *Nature.* 2016;531:651–655.
- 16 Baek AE, Yu YA, He S, et al. The cholesterol metabolite 27 hydroxy-cholesterol facilitates breast cancer metastasis through its actions on immune cells. *Nat Commun.* 2017;8:864.
- 17 Wang B, Zhao Q, Zhang Y, et al. Targeting hypoxia in the tumour microenvironment: a potential strategy to improve cancer immunotherapy. *J Exp Clin Cancer Res.* 2021;40:24.
- 18 Wang JX, Choi SYC, Niu X, et al. Lactic acid and an acidic tumour microenvironment suppress anticancer immunity. *Int J Mol Sci.* 2020;21:8363.
- 19 Wang ZH, Peng WB, Zhang P, Yang XP, Zhou Q. Lactate in the tumour microenvironment: From immune modulation to therapy. *EBioMedicine.* 2021;73:103627.
- 20 Parks SK, Mueller-Klieser W, Pouyssegur J. Lactate and acidity in the cancer microenvironment. *Annu Rev Cancer Biol.* 2020;4:141–158.
- 21 Watson MJ, Vignali PDA, Mullett SJ, et al. Metabolic support of tumour-infiltrating regulatory T cells by lactic acid. *Nature.* 2021;591:645–651.
- 22 Fischbeck AJ, Ruehlend S, Ettinger A, et al. Tumour lactic acidosis: Protecting tumour by inhibiting cytotoxic activity through motility arrest and bioenergetic silencing. *Front Oncol.* 2020;10:589434.
- 23 Kumagai S, Koyama S, Itahashi K, et al. Lactic acid promotes PD-1 expression in regulatory T cells in highly glycolytic tumour microenvironments. *Cancer Cell.* 2022;40:201–218.
- 24 Colegio OR, Chu NQ, Szabo AL, et al. Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature.* 2014;513:559–563.
- 25 Raychaudhuri D, Bhattacharya R, Sinha BP, et al. Lactate induces pro-tumour reprogramming in intratumoural plasmacytoid dendritic cells. *Front Immunol.* 2019;10:1878.
- 26 Fu Y, Lan T, Cai H, Lu A, Yu W. Meta-analysis of serum lactate dehydrogenase and prognosis for osteosarcoma. *Medicine.* 2018;97:e0741.
- 27 Gan J, Wang W, Yang Z, Pan J, Zheng L, Yin L. Prognostic value of pretreatment serum lactate dehydrogenase level in pancreatic cancer patients: a meta-analysis of 18 observational studies. *Medicine (Baltimore).* 2018;97:e13151.
- 28 Scharping NE, Rivadeneira DB, Menk AV, et al. Mitochondrial stress induced by continuous stimulation under hypoxia rapidly drives T cell exhaustion. *Nat Immunol.* 2021;22:205–215.
- 29 Liu X, Peng G. Mitochondria orchestrate T cell fate and function. *Nat Immunol.* 2021;22:276–278.
- 30 Yu YR, Imrichova H, Wang H, et al. Disturbed mitochondrial dynamics in CD8<sup>+</sup> TILs reinforce T cell exhaustion. *Nat Immunol.* 2020;21:1540–1551.
- 31 Fu SH, Yeh LT, Chu CC, Yen BL, Sytwu HK. New insights into Blimp-1 in T lymphocytes: a divergent regulator of cell destiny and effector function. *J Biomed Sci.* 2017;24:49.
- 32 Crompton JG, Sukumar M, Roychoudhuri R, et al. Akt inhibition enhances expansion of potent tumour-specific lymphocytes with memory cell characteristics. *Cancer Res.* 2015;75:296–305.
- 33 Vardhana SA, Hwee MA, Berisa M, et al. Impaired mitochondrial oxidative phosphorylation limits the self-renewal of T cells exposed to persistent antigen. *Nat Immunol.* 2020;21:1022–1033.
- 34 Chamoto K, Chowdhury PS, Kumar A, et al. Mitochondrial activation chemicals synergize with surface receptor PD-1 blockade for T cell-dependent antitumour activity. *Proc Natl Acad Sci USA.* 2017;114:E761–E770.
- 35 Falzoni S, Donvito G, Di Virgilio F. Detecting adenosine triphosphate in the pericellular space. *Interface Focus.* 2013;3:20120101.
- 36 Viganò S, Alatzoglou D, Irving M, et al. Targeting adenosine in cancer immunotherapy to enhance T-cell function. *Front Immunol.* 2019;10:925.
- 37 Battastini AMO, Figueiró F, Leal DBR, Doleski PH, Schetinger MRC. CD39 and CD73 as promising therapeutic targets: what could be the limitations? *Front Pharmacol.* 2021;12:633603.
- 38 Kuras Z, Kucher V, Gordon SM, et al. Modulation of Kv1.3 channels by protein kinase A I in T lymphocytes is mediated by the disc large 1-tyrosine kinase Lck complex. *Am J Physiol Cell Physiol.* 2012;302:C1504–C1512.
- 39 Li WH, Qiu Y, Zhang HQ, et al. P2Y2 receptor promotes cell invasion and metastasis in prostate cancer cells. *Br J Cancer.* 2013;109:1666–1675.
- 40 De Marchi E, Orioli E, Pegoraro A, et al. The P2×7 receptor modulates immune cells infiltration, ectonucleotidases expression and extracellular ATP levels in the tumour microenvironment. *Oncogene.* 2019;38:3636–3650.
- 41 Yang M, Ma C, Liu S, et al. HIF-dependent induction of adenosine receptor A2b skews human dendritic cells to a Th2-stimulating phenotype under hypoxia. *Immunol Cell Biol.* 2010;88:165–171.
- 42 Challier J, Bruniquel D, Sewell AK, Laugel B. Adenosine and cAMP signalling skew human dendritic cell differentiation towards a tolerogenic phenotype with defective CD8(+) T-cell priming capacity. *Immunology.* 2013;138:402–410.
- 43 Yu J, Sun J, Wang SE, et al. Upregulated expression of indoleamine 2,3-dioxygenase in primary breast cancer correlates with increase of infiltrated regulatory T cells in situ and lymph node metastasis. *Clin Dev Immunol.* 2011;2011:469135.
- 44 Finley D. Recognition and processing of ubiquitin-protein conjugates by the proteasome. *Annu Rev Biochem.* 2009;78:477–513.
- 45 Wang YT, Chen J, Chang CW, et al. Ubiquitination of tumour suppressor PML regulates prometastatic and immunosuppressive tumour microenvironment. *J Clin Invest.* 2017;127:2982–2997.
- 46 Liao L, Song M, Li X, et al. E3 Ubiquitin Ligase UBR5 drives the growth and metastasis of triple-negative breast cancer. *Cancer Res.* 2017;77:2090–2101.
- 47 Chandy KG, Norton RS. Immunology: channelling potassium to fight cancer. *Nature.* 2016;37:497–499.
- 48 Eil R, Vodnala SK, Clever D, et al. Ionic immune suppression within the tumour microenvironment limits T cell effector function. *Nature.* 2016;537:539–543.
- 49 Ong ST, Ng AS, Ng XR, et al. Extracellular K<sup>+</sup> dampens T cell functions: Implications for immune suppression in the tumour microenvironment. *Bioelectricity.* 2019;1:169–179.
- 50 Cahalan MD, Chandy KG. The functional network of ion channels in T lymphocytes. *Immunol Rev.* 2009;231:59–87.
- 51 Goggi JL, Khanapur S, Ramasamy B, et al. Imaging Kv1.3 expressing memory T cells as a marker of immunotherapy response. *Cancers.* 2022;14:1217.

- 52 Gurusamy D, Clever D, Eil R, Restifo NP. Novel “elements” of immune suppression within the tumour microenvironment. *Cancer Immunol Res*. 2017;5:426–433.
- 53 Kim S, Lee M, Song Y, et al. Argininosuccinate synthase 1 suppresses tumor progression through activation of PERK/eIF2 $\alpha$ /ATF4/CHOP axis in hepatocellular carcinoma. *J Exp Clin Cancer Res*. 2021;40(1):127.
- 54 Fernandes HS, Silva Teixeira CS, Fernandes PA, Ramos MJ, Cerqueira NM. Amino acid deprivation using enzymes as a targeted therapy for cancer and viral infections. *Expert Opin Ther Pat*. 2017;27:283–297.
- 55 Pilanc P, Wojnicki K, Roura AJ, et al. A novel oral arginase 1/2 inhibitor enhances the antitumour effect of PD-1 inhibition in murine experimental gliomas by altering the immunosuppressive environment. *Front Oncol*. 2021;11:703465.
- 56 Liu X, Bao X, Hu M, et al. Inhibition of PCSK9 potentiates immune checkpoint therapy for cancer. *Nature*. 2020;588:693–698.
- 57 Kawalekar OU, O'Connor RS, Fraietta JA, et al. Distinct signaling of coreceptors regulates specific metabolism pathways and impacts memory development in CAR T cells. *Immunity*. 2016;44:380–390.
- 58 Dumauthioz N, Tschumi B, Wenes M, et al. Enforced PGC-1 $\alpha$  expression promotes CD8 T cell fitness, memory formation and antitumour immunity. *Cell Mol Immunol*. 2021;18:1761–1771.
- 59 Wei J, Long L, Zheng W, et al. Targeting REGNASE-1 programs long-lived effector T cells for cancer therapy. *Nature*. 2019;576:471–476.
- 60 Sukumar M, Liu J, Mehta GU, et al. Mitochondrial membrane potential identifies cells with enhanced stemness for cellular therapy. *Cell Metab*. 2016;23:63–76.
- 61 Pellom S, Dudimah D, Thounaojam M, et al. Bortezomib augments lymphocyte stimulatory cytokine signaling in the tumour microenvironment to sustain CD8<sup>+</sup> T cell antitumour function. *Oncotarget*. 2016;8:8604–8621.
- 62 Verdura S, Cuyàs E, Cortada E, et al. Resveratrol targets PD-L1 glycosylation and dimerization to enhance antitumour T-cell immunity. *Aging*. 2020;12:8–34.
- 63 Pillozzi S, D'Amico M, Bartoli G, et al. The combined activation of K<sub>Ca</sub>3.1 and inhibition of Kv11.1/hERG1 currents contribute to overcome Cisplatin resistance in colorectal cancer cells. *Br J Cancer*. 2018;118:200–212.
- 64 Wall MJ, Wigmore G, Lopatár J, Frenguelli BG, Dale N. The novel NTPDase inhibitor sodium polyoxotungstate (POM-1) inhibits ATP breakdown but also blocks central synaptic transmission, an action independent of NTPDase inhibition. *Neuropharmacology*. 2008;55:1251–1258.
- 65 Hayat K, Afzal S, Saeed A, et al. Investigation of new quinoline derivatives as promising inhibitors of NTPDases: synthesis, SAR analysis and molecular docking studies. *Bioorg Chem*. 2019;87:218–226.
- 66 Zhang Y, Chung SF, Tam SY, Leung YC, Guan X. Arginine deprivation as a strategy for cancer therapy: An insight into drug design and drug combination. *Cancer Lett*. 2021;502:58–70.
- 67 Hall PE, Ready N, Johnston A, et al. Phase II study of arginine deprivation therapy with pegarginase in patients with relapsed sensitive or refractory small-cell lung cancer. *Clin Lung Cancer*. 2020;21:527–533.
- 68 Vassilev LT, Vu BT, Graves B, et al. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science*. 2004;303:844–848.
- 69 Yip D, Le MN, Chan JL, et al. A phase 0 trial of riluzole in patients with resectable stage III and IV melanoma. *Clin Cancer Res*. 2009;15:3896–3902.
- 70 Mehnert JM, Silk AW, Lee JH, et al. A phase II trial of riluzole, an antagonist of metabotropic glutamate receptor 1 (GRM1) signaling, in patients with advanced melanoma. *Pigment Cell Melanoma Res*. 2018;31:534–540.
- 71 Abate-Daga D, Hanada K, Davis JL, Yang JC, Rosenberg SA, Morgan RA. Expression profiling of TCR-engineered T cells demonstrates overexpression of multiple inhibitory receptors in persisting lymphocytes. *Blood*. 2013;122:1399–1410.
- 72 Chimote AA, Balajthy A, Arnold MJ, et al. A defect in K<sub>Ca</sub>3.1 channel activity limits the ability of CD8<sup>+</sup> T cells from cancer patients to infiltrate an adenosine-rich microenvironment. *Sci Signal*. 2018;11(527):eaq1616.