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Targeting the tumour microenvironment and T cell metabolism for effective cancer immunotherapy

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Abbreviations:

A2AR – adenosine A2A receptor; ACT – adoptive cell transfer; BH4 – tetrahydrobiopterin; CAR – chimeric antigen receptor; FAO – fatty acid oxidation; Kyn – kynurenine; OXPHOS – oxidative phosphorylation; PEP – phosphoenolpyruvate; TIL – tumour-infiltrating lymphocytes; TME – tumour microenvironment,

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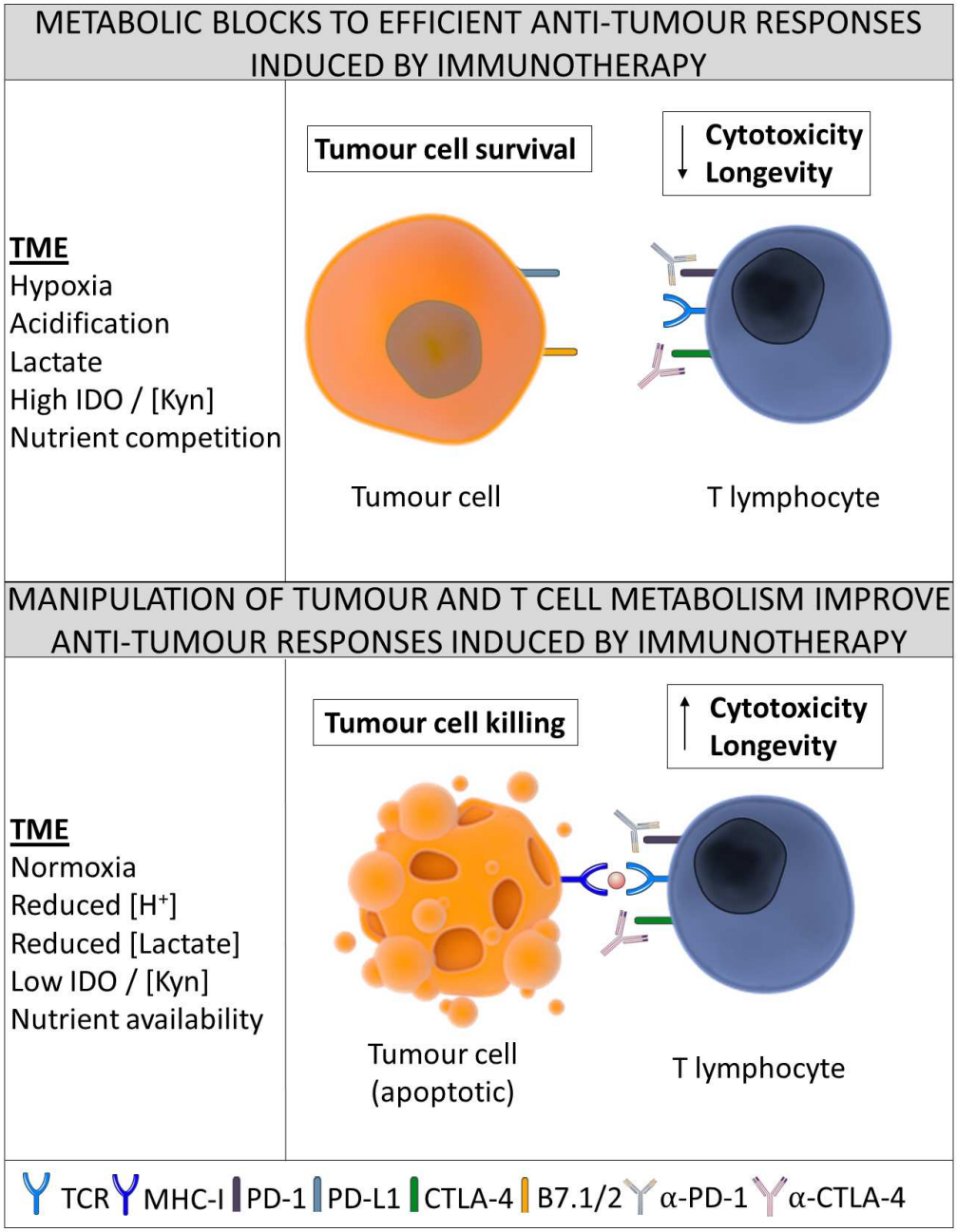
ABSTRACT

The successful implementation of immunotherapies has provided new impetus in the fight against cancer. Antibody-mediated blockade of immune checkpoint molecules PD-1 / PD-L1 and CTLA-4 has had a dramatic impact upon the treatment of previously intractable cancers such as malignant melanoma, whilst adoptive cell therapies using chimeric antigen receptor-bearing T cells have proven highly efficacious in B cell leukaemia. Furthermore, significant progress has been made in understanding the mechanisms by which tumours evade or become resistant to these immunotherapies. In this regard, approaches to broaden the applicability and enhance the efficacy of immunotherapies increasingly include modulation of tumuor and immune cell metabolism. In this mini-review we highlight the most recent studies describing novel approaches and targets for the manipulation of the tumour microenvironment and T cell metabolism and describe how these approaches are being combined with current immunotherapies in preclinical studies.

The efficacy of cancer immunotherapies, such as checkpoint inhibitors and adoptive T cell transfer, can be influenced by the presence of a nutrient-depleted and challenging tumour microenvironment, resulting in immune cell dysfunction. We discuss pre-clinical studies showing how targeting cancer and immune cell metabolism can improve anti-tumour immunotherapeutic responses.



INTRODUCTION

During the past decade, the development and increased use of cancer immunotherapies has resulted in impressive clinical benefits. Function blocking antibodies targeted to the immune checkpoint molecules PD-1/PD-L1 and CTLA-4 have been approved by the US Food and Drug Administration (FDA) for the treatment of advanced melanoma, non-small cell lung cancer, renal cell carcinoma and Hodgkin's disease, among others [1]. Furthermore, adoptive cell transfer (ACT) of *ex vivo*-expanded autologous tumour-infiltrating lymphocytes (TILs) or genetically engineered chimeric antigen receptor T (CAR-T) cells has been the other pillar of cancer immunotherapy [2, 3]. Despite these successes, responses to cancer immunotherapy are variable and only a subset of patients exhibit durable responses [4, 5].

It is likely that variation in tumour immunogenicity and the presence of immunosuppressive signals within the tumour microenvironment determine the efficiency of patient anti-tumour immune responses as well as immunotherapies [1, 4, 6]. Weakly immunogenic tumours are typically characterised by a lack of immune infiltration, and in particular a lack of $CD8^+$ CTLs [6]. Poor immune reactivity and infiltration to tumours is prognostic for resistance to checkpoint inhibitors whilst immunotherapeutic responses are enhanced in so-called immune 'hot' tumours [4]. Moreover, there is increasing appreciation of the interplay between tumour cell and immune cell metabolism in the outcome of anti-tumour immune responses. In this regard, cancer cells and anti-tumour lymphocytes, such as effector $CD8^+$ T cells, have similar metabolic requirements and compete for nutrient resources, including glucose and amino acids, within the tumour microenvironment (TME) [7]. Furthermore, in addition to depriving anti-tumour immune cells of key nutrients, tumour cells create a challenging environment characterised by hypoxia, acidic pH and high levels of immunosuppressive metabolites. Thus, new approaches that combine existing immunotherapies with strategies that overcome the metabolically challenging TME and enhance infiltration, effector responses and longevity of anti-tumour T cells are being investigated [6]. In this mini-review, we focus on the most recent studies describing mechanisms for manipulating the immunosuppressive TME and enhancing anti-tumour T cell responses through modulating metabolic pathways, and how these approaches can be used to improve the efficacy of immune checkpoint blockade and T cell ACT for the treatment of cancer.

IMPACT OF THE TUMOUR MICROENVIRONMENT AND CHECKPOINT INHIBITORS ON T CELL METABOLISM

T cells undergo a metabolic switch upon engagement of the T cell receptor (TCR). Whereas naïve T cells uptake low levels of glucose and utilize mitochondrial oxidative phosphorylation (OXPHOS) to fuel their energy requirements, effector T cells increase nutrient uptake and rely on aerobic glycolysis, glutaminolysis and lipid synthesis to support metabolic and functional demands. The metabolic regulation of T cell activation has been reviewed extensively in recent times [7-9]. Briefly, aerobic glycolysis is relatively inefficient in the production of ATP but enables T cells, and other proliferating and inflammatory immune cell types, to use glucose to fuel the pentose phosphate pathway (PPP). The PPP generates nicotinamide adenine dinucleotide phosphate (NADPH) that in turn is rate-limiting for the production of amino acids, nucleic acids and fatty acids and the production of biomass. Furthermore, a number of recent studies have shown important roles for glycolytic enzymes in non-glycolytic processes that regulate T cell activation and effector function (reviewed in [8]). Tumour cells frequently share a similar glycolytic metabolic profile with activated T cells. Consequently, cancer cell nutrient uptake creates a TME characterized by the limited availability of glucose, lipids and amino acids and, therefore, lymphocytes are suppressed due to the inability of the cells to sustain energetic needs [10-12]. Furthermore, T cells and other anti-tumour immune cells are inhibited by tumour-derived waste products, such as lactate or kynurenine, and low oxygen levels [10].

The mechanisms by which immune checkpoint receptors block T cell responses also involves manipulation of T cell metabolism. Thus, PD-1 engagement results in a progressive state of metabolic exhaustion in T cells by inhibiting glucose and amino acid uptake and catabolism [13, 14]. Instead, PD-1 signals favour the engagement of fatty acid oxidation (FAO) which is required for the long-term survival and fitness of chronically activated T cells [13, 15]. In effector T cells, immune checkpoint blockade shifts T cell metabolism back towards glycolysis. Thus, T cell metabolic impairment plays a pivotal role in the suppression of anti-tumour responses.

APPROACHES TO MANIPULATE T CELL AND TUMOUR GLYCOLYTIC METABOLISM

Glycolytic metabolism is required for efficient T cell effector function and is dampened within the TME, in part, as a result of low levels of glucose availability [11, 12]. However, a recent study demonstrated that glucose deprivation is not the only limiting factor for engagement of the

glycolytic pathway by $CD8^+$ TILs. Gemta and colleagues demonstrated that TILs have reduced enolase-1 activity, a glycolytic enzyme that catalyses the conversion of 2-phosphoglycerate to phosphoenolpyruvate (PEP) [16]. Importantly this deficit could be reversed as provision of the downstream metabolite pyruvate improved the metabolic fitness and functional capacity of TILs [16]. Furthermore, previous studies showed that PEP is a key metabolite in the regulation of anti-tumour responses as, in addition to its function in glycolysis, PEP regulates the cytoplasmic Ca_{2+} concentration and NFAT1 activation in T cells [12]. Under conditions of reduced glucose availability and glycolytic flux, restoration of intracellular PEP levels by overexpression of the gluconeogenesis enzyme PEP carboxykinase 1 in either $CD4^+$ or $CD8^+$ T cells results in improved anti-tumour responses upon ACT [12, 17].

Constitutive activation of glycolysis is linked to terminal differentiation of effector T cells. Therefore, whilst glycolysis permits enhanced cytokine secretion and production of cytolytic proteins, it is also associated with decreased T cell persistence and non-durable tumour responses (Fig. 1). Furthermore, it is well established that ACT of memory phenotype T cells results in superior and sustained anti-tumour responses as compared to effector T cells [18]. Thus, several studies have focused on attenuating glycolysis to promote a memory-like phenotype. Treatment with the glycolysis inhibitor 2-deoxyglucose enabled the differentiation of long-lived memory T cells with enhanced anti-tumour function upon ACT [19]. Furthermore, recent work has demonstrated that T cells lacking the serine/threonine Pim kinases or control T cells treated with a Pim kinase inhibitor had substantially reduced glycolytic metabolism and adopted a memory-like phenotype [20]. Consequently, ACT using melanoma-specific pmel T cells pre-treated with the PIM kinase inhibitor AZD1208 improved tumor clearance and survival when compared to controls. Furthermore, combination of AZD1208, PD-1 blockade and T cell ACT had synergistic effects against B16 melanoma in mice [20]. Another potential approach to improve anti-tumour responses was revealed by the finding that tumour-associated Tregs are also dependent upon the glycolytic pathway for their suppressive function. A new study showed that TLR8 ligation inhibits glucose uptake and glycolytic metabolism specifically in Tregs [21]. Furthermore, TLR8 agonists promoted anti-tumour responses following T cell ACT in a xenograft model of human melanoma [21].

Increased tumour glycolysis correlates with poor therapeutic responses to ACT [22]. Nonetheless, dampening glycolytic metabolism *in vivo* is complex due to the difficulties of specifically targeting

tumour cells. A role for tumour-associated macrophages (TAM) in promoting tumour cell glycolytic metabolism was recently described. TAM production of inflammatory cytokine TNF enhanced tumour cell glycolysis, whilst depletion of TAMs using chlodronate rendered otherwise resistant Lewis lung cancers sensitive to anti-PD-L1 therapy [23]. A potential cheap and non-toxic approach to manipulating tumour cell metabolism was reported by Gonzalez and colleagues who demonstrated that *in vivo* administration of the monosaccharide mannose interferes with tumour cell glycolysis [24]. Mechanistically, mannose treatment regulates apoptotic proteins resulting in an enhanced sensitivity of cancer cells to chemotherapeutic drugs. Sensitivity to mannose varied between tumour cell lines and was associated with expression levels of phosphomannose isomerase [24]. A further consideration is the role of mannose in enhancing Treg differentiation and suppressing inflammatory T cell responses [25]. Therefore, the outcome of mannose treatment in cancer will likely depend on the balance between anti-tumour effects and immunosuppressive effects.

APPROACHES TO MANIPULATE T CELL AND TUMOUR MITOCHONDRIAL METABOLISM

Mitochondrial metabolism has a key role in the development and maintenance of memory T cells with long-lived anti-tumoral activity (Fig 1). Pearce and colleagues demonstrated that CD28 co-stimulation during T cell priming regulates memory cell formation by eliciting enhanced FAO and spare respiratory capacity (SRC), as well as morphological mitochondrial remodelling [26, 27]. In the absence of CD28 co-stimulation, tumour-specific T cells suppressed tumour growth within the first 15 days after ACT but failed to maintain durable responses, resulting in tumour relapse. Treatment of anti-CD3/CD28-primed T cells with etoxomir, an irreversible inhibitor of carnitine parmitoyltransferase (Cpt) 1, dampened durable anti-tumour responses, highlighting the importance of long-chain FAO for the efficacy of memory phenotype T cell ACT [27].

Within the TME, exhausted TILs frequently have diminished or absent CD28 expression. However, recent work determined that expression of the costimulatory TNF receptor family member 4-1BB was elevated on $CD8^+PD\text{-}1^+Tim3^+LAG3^+$ TILs from B16 melanomas [28]. 4-1BB co-stimulation enhanced mitochondrial function in $CD8^+$ T cells via peroxisome proliferator activated-receptor (PPAR) gamma coactivator 1α (PGC-1α), a transcription factor involved in mitochondrial biogenesis. Furthermore, 4-1BB agonists enhanced the efficacy of anti-PD-1 therapy and T cell ACT in B16 melanoma models *in vivo* [28]. Several additional lines of evidence have also determined the utility

of targeting PPAR signalling pathways to improve T cell immunotherapy. In glucose- and oxygen-deprived mouse melanoma models, TILs upregulate PPAR-alpha signalling and use FAs as an energy source [29]. Promotion of FA catabolic pathways *in vitro* improved the subsequent anti-tumour function of adoptively transferred $CD8^+$ T cells, and synergized with PD-1 blockade resulting in superior control of tumour growth [29]. Similarly, Chamoto and colleagues show that Luperox, a reactive oxygen species (ROS) generator, synergizes with PD-L1 mAb therapy against MC38 colon adenocarcinoma cells in mice. ROS accumulation did not directly affect tumour cell killing but elevated T cell responses through upregulation of PGC-1α [30]. Small molecule activators of PGC-1α (oltipraz and bezafibrate) potentiated PD-1 blockade therapy. More recently, the same group reported that bezafibrate in combination with anti-PD-L1 induces expression of genes involved in mitochondrial biogenesis and FAO, such as PGC-1α or Cpt1b, and anti-apoptotic genes including Bcl2, Birc3 and API5. Overall, PGC-1α activation results in an increased number of tumour-reactive T lymphocytes infiltrating the TME thereby boosting PD-1 blockade therapy [31]. By contrast to the effects of Luperox *in vivo*, Pilipow and colleagues demonstrated that ROS limitation, using the antioxidant N-acetylcysteine (NAC), favours the generation of long-lived stem cell memory T (Tscm) cells [32]. ACT using NAC-generated Tscm CD19 CAR-T lymphocytes demonstrated enhanced clearance of lymphoblastic leukaemia cells in immunodeficient mice [32]. These studies demonstrate that targeting ROS in anti-tumour therapy has complex context-dependent effects.

A study from the Delgoffe laboratory indicated that targeting tumour cell mitochondrial metabolism might also represent a viable approach to enhancing immunotherapies. Their results demonstrated that different human melanoma cell lines varied substantially in their reliance upon mitochondrial or glycolytic metabolic pathways [33]. Cell lines with dysregulated oxidative metabolism induced higher levels of intra-tumoral hypoxia in immunodeficient mice whilst knockdown of mitochondrial complex 1 subunit *Ndufs4* in melanoma cells reversed this effect [33]. Importantly, anti-PD-1 induced $CD8^+$ T cell infiltration and function was enhanced in *Ndufs4*, but not GLUT1, knockdown tumours, as compared to control. These data suggest that, in this model, tumour cell-intrinsic oxidative but not glycolytic metabolism dampens anti-PD-1 efficacy.

TARGETING THE TUMOUR MICROENVIRONMENT TO IMPROVE IMMUNOTHERAPIES

The uncontrolled growth of tumour cells leads to the formation of hypoxic non-vascularized areas whilst high degrees of tumour hypoxia are associated with poor clinical outcomes. Hypoxic areas

within tumours typically lack infiltrating T cells [34] and several recent studies have addressed the question of whether reversing tumour hypoxia can enhance T cell infiltration and responses to cancer immunotherapy. One such study investigated the impact of evofosfamide (TH-302), an alkylating chemotherapeutic pro-drug that is activated under conditions of hypoxia. Jayaprakash and colleagues reported that treatment of mice with TH-302 restored normoxia and enhanced T cell infiltration into TRAMP-C2 prostate tumors [34]. Importantly, TH-302 treatment markedly enhanced the therapeutic efficiency of PD-1/CTLA-4 checkpoint blockade [34]. Similarly, respiratory hyperoxia (breathing 60% O_2) enhanced infiltration of anti-tumour $CD8^+$ T cells and reduced tumour burden within the lung when combined with dual CTLA-4/PD-1 blockade in a MCA205 fibrosarcoma pulmonary mouse model [35]. In this study, supplemental oxygenation repressed Treg numbers and levels of the inhibitory cytokine transforming growth factor (TGF)β [35]. Furthermore, metformin treatment also reduces tumour hypoxia and potentiates the effects of PD-1 blockade against B16 melanoma and MC38 colorectal carcinomas in mice [36].

Alternative approaches to counteracting the effects of tumour hypoxia have included targeting the immunoinhibitory effects of adenosine signalling. Within hypoxic tumours, stabilisation of hypoxia-inducible factor (HIF) transcription factors increases expression of CD73, a cell surface nucleotidase that promotes synthesis of extracellular adenosine. Binding of the A2A adenosine receptor (A2AR) suppresses inflammatory immune responses by anti-tumour cells, including T lymphocytes, NK cells, DCs and macrophages. Antagonising A2AR or inhibiting CD73 has synergistic effects against tumours when combined with anti-PD-1 [37]. Furthermore, a recent study showed that resistance to PD-L1 blockade in mouse models of non-small cell lung cancer was associated with enhanced expression of CD38 by tumour cells and A2AR-induced immune suppression [38]. Consistent with an important role for CD38 expression in mediating tumour escape, CD38 or A2AR antagonists enhanced the therapeutic effects of anti-PD-L1.

Glycolytic solid tumours excrete high levels of lactate whilst tumor expression of high levels of lactate dehydrogenase A (LDHA) is associated with poor prognosis in human melanoma [39]. LDHA function is particularly important for tumor cell growth under hypoxic conditions [40] whilst the acidic TME generated by lactate secretion blocks T cell cytokine production and cytolytic capacity [39] and induces NK cell apoptosis [41]. In addition, a recent study demonstrated that an acidic microenvironment within melanoma induces expression of the transcriptional repressor protein ICER

in macrophages and promotes their polarization to an anti-inflammatory, pro-tumorigenic phenotype [42]. Pre-clinical studies to determine the impact of neutralization of tumour acidity on anti-tumor immunity have yielded promising results. Thus, sodium bicarbonate therapy slowed the growth of Yumm1.1 melanomas in mice [43]. Bicarbonate therapy alone did not impede the growth of aggressive B16 melanomas in mice but enhanced the therapeutic efficacy of anti-PD-1 or anti-CTLA-4 [43]. Furthermore, long-term survival rate after ACT of melanoma-specific pmel TCR transgenic T cells were improved when combined with bicarbonate (40% v 10% with ACT alone) [43].

Indoleamine 2,3-dioxygenase (IDO), produced within the TME by malignant cells, Tregs, stromal cells and tolerogenic DCs, converts the essential amino acid tryptophan (Trp) to kynurenine (Kyn) [44]. The lack of Trp results in decreased mTORC1 signalling, activation of the stress kinase GCN2 and triggers cell cycle arrest and apoptosis in effector T cells [44]. Recent studies have shown that Kyn further contributes to immunosuppression in the TME by promoting PD-1 expression by TILs through upregulation and activation of the aryl hydrocarbon receptor (AHR) transcription factor [45]. The discovery of a novel role for the enzyme cofactor tetrahydrobiopterin (BH4) in T cells has revealed potential new approaches to counteract the inhibitory effects of Kyn in the TME. Penninger and colleagues demonstrated that the synthesis of BH4 was important for TCR-induced T cell proliferation and influenced mitochondrial metabolism [46]. Furthermore, *in vitro* BH4 supplementation could reverse the inhibitory effects of Kyn on T cells whilst delivery of BH4 *in vivo* enhanced T cell infiltration and reduced growth of E0771 and TC1 orthotopic tumours [46]. High levels of IDO expression in the TME is correlated with poor prognosis and promotes resistance to CTLA-4 blockade [47] and CD19-CAR-T cells [48]. A recent study evidenced the beneficial role of IDO1 inhibitors in long-term survival in combination with radiotherapy and PD-1 blockade in a mouse model of glioblastoma [49]. Similarly, studies performed in the murine B16.SIY melanoma model showed improved tumour control when combining anti-PD-L1 or anti-CTLA-4 with the IDO inhibitor INCB23843 [50].

CONCLUDING REMARKS

The TME has long been recognized as hostile to effective immune responses. The development of checkpoint inhibitors demonstrated the capacity of endogenous immune responses to be reinvigorated and combat tumour growth, whilst advances in ACT approaches have also resulted in dramatic improvements in patient outcomes. Complimentary approaches to reduce the impact of

the nutrient-deprived and immunosuppressive TME on anti-tumour responses may help to optimise immunotherapies. Furthermore, identification of the pathways and targets that determine T cell metabolic fitness are likely to result in new approaches to enhance the durability and efficacy of anti-tumour responses following T cell ACT. The discovery of such novel approaches to target tumour cell and immune metabolism will likely broaden the applicability and enhance the efficacy of immune-based anti-cancer therapies in the future.

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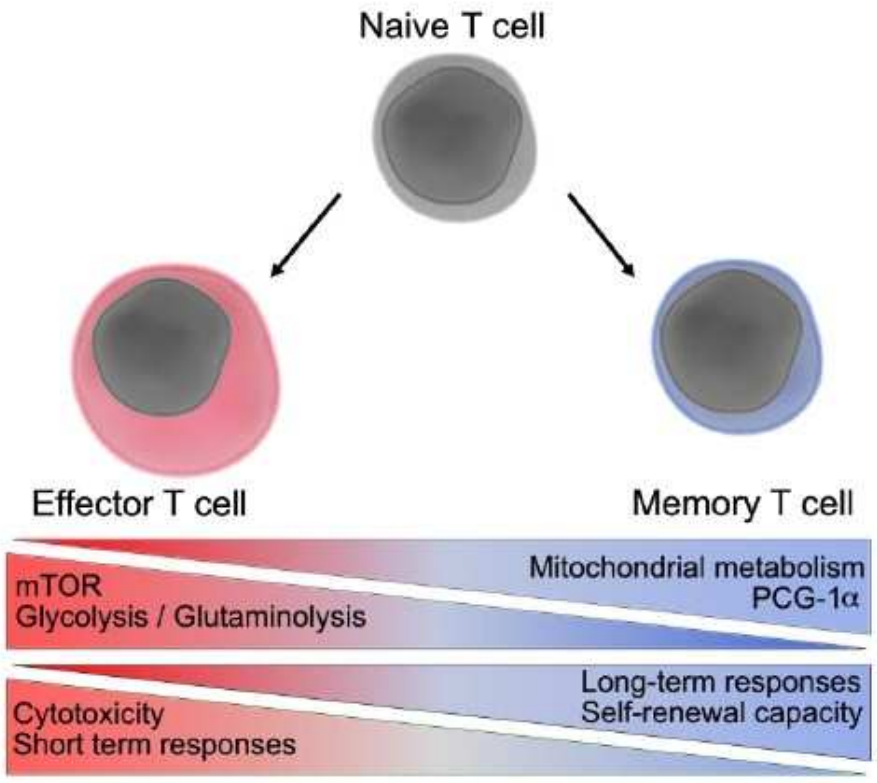
CONFLICT OF INTEREST The authors declare no financial or commercial conflict of interest

Figure Legend

Figure 1. Distinct metabolic and functional features of memory and effector T cells in cancer.

Activation of the mTOR signalling pathway favours engagement of aerobic glycolysis and glutamine catabolic pathways, and preferentially results in differentiation of naïve $CD8^+$ T cells to an effector phenotype. These rapidly growing and highly proliferative T cell populations are characterised by potent cytotoxic function, but are short-lived and prone to functional and metabolic exhaustion within the TME. By contrast, memory phenotype T cells rely on mitochondrial metabolism and FAO, frequently under the control of the PGC1α transcriptional programme. Memory phenotype T cells have the capacity to self-renew and provide long-lived responses to malignancies.



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