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Metabolic reprograming of anti-tumor immunity

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Abstract

Immunotherapy techniques designed to engage T cells against tumor cells can generate sustained and complete responses in patients whose cancers were resistant to more traditional treatment options. Powering the T cell response – how a T cell gets its energy – plays an important role in the effectiveness of T cell destruction of cancer. Recent work in the field indicates that the modulation of T cell metabolism may allow for improved anti-cancer activity by generating T cells with optimal characteristics for the elimination of tumor cells. In this review, we discuss the key metabolic properties of anti-cancer T cells, along with potential methods to improve T cell immunotherapy through metabolic modulation.

Introduction

The progression of cancer from localized tumor to metastatic disease is associated with reduced patient survival and poor efficacy of further therapeutic interventions [1]. There is currently a lack of therapeutic options to successfully treat metastatic solid tumors with surgery, chemotherapy and radiation strategies often failing to fully arrest disease progression [2, 3]. Increasingly, immunotherapy can be used to treat patients with cancer. This includes the adoptive transfer of naturally-occurring tumor infiltrating lymphocytes (TIL) or genetically-engineered T cells and the use of immune checkpoint inhibitors to boost the function of T cells [4–6•]. Cancer immunotherapy has been successfully utilized to mediate complete and durable clinical responses in patients with several types of cancer including melanoma and acute lymphoblastic leukemia (ALL) [7–9], and is currently being explored as a potential therapeutic strategy in numerous other types of cancer [10]. Recent research has begun to elucidate some of the mechanisms by which T cell mediated cancer immunotherapy works to eliminate disseminated tumor cells and indicates that T cell

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differentiation status and the metabolic properties of T cells may play an important role in regulating their anti-tumor functionality [11••].

The contextual basis for much of our current understanding of the role of metabolism in regulating tumor immunity is derived from a series of studies on $CD8^+$ T cell differentiation. $CD8^+$ T cells can be divided into subsets such as naïve (T_N), stem cell memory (T_{SCM}), central memory (T_{CM}), effector memory (T_{EM}) and terminally differentiated effector cells (T_{EFF}) [12]. Importantly, it has been clearly established that various subsets of T cells have distinct metabolic profiles that regulate function [13, 14]. Interestingly, there is a negative correlation between the degree of differentiation of T cells and their capacity for anti-tumor function [15]. In human patients that have undergone TIL therapy, increased telomere length and CD27 expression in infused T cells have been correlated with improved tumor clearance [16]. Consistent with these findings, the adoptive transfer of fully-differentiated terminal effectors (T_{EFF}) was found to be less effective in controlling tumor growth than utilizing less-differentiated T_{SCM} or T_{CM} subsets in mouse models of large vascularized melanoma [17, 18]. These studies suggest that the acquisition of a fully-differentiated terminal effector phenotype limits the *in vivo* expansion and survival capacity of T cells following adoptive transfer, which likely limits the effectiveness of their anti-tumor response. Conversely, cells with increased self-renewal potential appear to possess increased therapeutic activity [17, 19].

Cellular metabolic processes regulate self-renewal capacity, as evidenced by studies in the settings of hematopoietic stem cells (HSC) and memory [20–22••]. In the HSC setting, increased metabolic activity may directly contribute to the loss of quiescence through the generation of high reactive oxygen species (ROS) levels that can impair long-term self-renewal properties [23–25]. Similarly, the increased mitochondrial metabolism and ROS generation driven by T cell activation is necessary for effector function and proliferation [26, 27] but also may compromise the long-term self-renewal capacity of memory T cell subsets (T_{SCM} and T_{CM}). (Figure 1).

In this review, we discuss the metabolic requirements for T cells used in immunotherapy, nutrient competition between T cells and tumor, a metabolic checkpoint in the tumor microenvironment, and the metabolic properties of T cells that successfully eliminate tumors. We summarize and appraise recent reports that have highlighted the importance of metabolic maintenance in T cells by the mTOR signaling pathway in self-renewal [28–30] and in T-cell quiescence [31], memory versus effector T-cell differentiation [32, 33], ROS levels [20, 34] and anti-tumor activity [35]. Because T cell differentiation and functional activity are metabolically linked [36•], it is possible for the first time to characterize the metabolic properties of T cells that associated with enhanced anti-tumor efficacy and to program metabolic pathways to optimize the clinical efficacy of T cell-based therapies.

Metabolic requirements of immunotherapy

The lifespan of T cells employed in immunotherapy encompasses several phases, each with different functional and metabolic demands. Tumor-specific T cells must be successfully isolated from a patient's tumor and then cultured *in vitro* to generate large numbers of cells

[16]. Upon transfer, tumor-specific T cells must be able to engraft, localize to the tumor, survive in the harsh microenvironment it is likely to encounter there, finally, mount a sustained attack against tumor cells by proliferating and producing inflammatory cytokines and molecules that trigger tumor cell lysis. At each stage of this process, the T cells must balance the metabolic demands of energy maintenance, cell survival and persistence with those of rapid proliferation and inflammatory function. This balancing act requires the utilization of diverse metabolic programs [36].

Studies have shown aerobic glycolysis is required for T cells to achieve full inflammatory functionality [37–39], while T cells with a memory phenotype of increased persistence typically utilize an oxidative metabolic program characterized by increased mitochondrial fatty acid oxidation and spare respiratory capacity (SRC) [13, 40]. These metabolic programs can determine not only function but also fate. T cells with enforced glycolysis may become terminally differentiated [41] and the imposition of an oxidative metabolic program may drive T cell quiescence and loss of effector function [42•].

Thus, the metabolic requirements for successful T cell tumor immunotherapy are dynamic and likely to require the metabolic machinery for both long-term survival (characterized by quiescence and fatty acid-based metabolism) and a vastly more energetically demanding effector function that is appears to involve high levels of glycolysis as well as oxidative phosphorylation. Recent work has begun to shed light on how the metabolic programs of T cells can be harnessed to promote improved anti-tumor response.

Metabolic programs that promote successful therapy

Effector T cells require a metabolic program of aerobic glycolysis to proliferate and generate effector cytokines such as IFNγ [43, 44], however the utilization of glycolytic metabolism during *in vitro* priming and expansion of T cells can result in reduced functional activity *in* vivo. Blocking glucose metabolism using the hexokinase inhibitor 2-deoxyglucose (2-DG) [41] or by restricting the activity of Akt [45] during *in vitro* priming limits T-cell differentiation. These metabolic manipulations resulted in more potent anti-tumor activity and increased metabolic fitness in mouse models of melanoma. While causative proof has not been shown, increased mitochondrial spare respiratory capacity was also observed as a result of these metabolic interventions. Inhibition of cholesterol metabolism can also promote anti-tumor activity [46]. Recent work in the chimeric antigen receptor (CAR) T cell setting has similarly shown that oxidative metabolism is associated with cell persistence and longevity [47].

T cells with elevated mitochondrial membrane potential ($\Delta\psi m$) and ROS are associated with reduced anti-tumor efficacy [20]. Interestingly, T cells with low-$\Delta\psi m$ during *in vitro* priming had phenotypes consistent with memory formation such as high spare respiratory capacity (SRC) and expression of memory genes, and also exhibited superior persistence compared with high $\Delta\psi m$ cells. In contrast, cells with high $\Delta\psi m$ were more glycolytic, expressed effector genes and had increased *in vitro* functionality (Figure 2). It seems plausible that increased ROS abundance, observed in T cells with high $\Delta\psi m$, is detrimental to the long-term survival of T cells [20]. The degree of metabolic activity of T cells may be

regulated by the mitochondrial dynamics of the cell, with mitochondrial fusion promoting cell longevity and improved anti-tumor response, while mitochondrial fission drives terminal effector differentiation [48••]. Elevated metabolic activity during *ex vivo* expansion may result in T cells that have low ability to persist *in vivo* and consequently generate a poor anti-tumor response. A recent study indicated that arginine supplementation in the culture media during *ex vivo* expansion can promote oxidative metabolism and inhibit glycolytic metabolism to yield more effective anti-tumor T cells [49]. Thus, culture conditions in which the metabolic activity of anti-tumor T cells is limited during *ex vivo* expansion may result in the production of cells with beneficial characteristics for immunotherapy.

Once T cells are transferred *in vivo* and have made their way to the tumor site, the utilization of a much more active metabolic phenotype is likely required for anti-tumor efficacy [41, 50]. Enforced glycolysis through genetic or pharmacological enhancement of hypoxia inducible factor (HIF) such as mutation or knockout to the Von Hippel Lindau (VHL) gene [51] or the prolyl hydroxylases (PHD) [52••] can result in elevated anti-tumor activity. Additionally, glycolytic metabolism may directly support the generation of inflammatory cytokines that are necessary for anti-tumor activity through epigenetic regulation [43]. The association of high $\Delta\psi m$ with cytokine production by T cells [20] may indicate that sustained mitochondrial activity by T cells at the tumor site would be required for tumor control. Conversely, engagement of PD1 at the tumor site [42] or an excess of tumor-derived potassium in the tumor microenvironment can act to inhibit the functionality of anti-tumor T cells [53]. Two recent studies have shown that the loss of metabolic activity through Akt-mediated inhibition of PPAR-gamma coactivator 1α (PGC-1α) can reduce effector function and result in poor anti-tumor activity in T cells [54, 55••]. Furthermore, overexpression of PGC-1α in T cells has been shown to improve anti-tumor activity, suggesting that reprogramming mitochondrial biogenesis of T cells might represent an alternative strategy to improve TIL function for cancer treatment. In contrast, a recent report has demonstrated that mitochondrial FAO is not necessary for memory T cell formation and function. Sustained glycolysis through conditional knockout of von Hippel Lindau tumor suppressor protein (Vhl), that increases HIF activity, supports increased T cell persistence [56••]. This finding seems to contradict the view that memory cells have low glycolytic rates and may point to the complexities of HIF signaling [57, 58].

Nutrient Competition of T cells and tumor, a metabolic checkpoint in the Tumor Microenvironment

It is increasingly appreciated that tumors are not merely a homogeneous mass of malignant cells, but rather a complex structure containing vascular and stromal cells that support the tumor as well as diverse array of infiltrating immune cells including lymphocytes and myeloid-derived cells [16, 59]. Thus, success in T cell-mediated cancer therapy is not only achieved by adequate trafficking of T cells within the tumor deposit, but may also be largely determined by whether T cells successfully compete for nutrients in an immunosuppressive environment. Effector T cells appear to compete with tumor cells for glucose that enables them secrete IFN-γ and eradicate established tumor. Glucose deprivation suppresses TCR-dependent activation of $Ca2^{+}$ and NFAT signaling and this leads to T cell hypo-

responsiveness. PD-1 ligand (PD-L1) expression by tumor cells activates the AKT/mTOR pathway to promote tumor cell glycolysis [50, 59••]. Antibodies that block the PD-1/PD-L1 checkpoint may restore glucose in tumor microenvironment, permitting T cell glycolysis and IFN-g production. A new study also reported that ovarian cancers imposed glucose restriction on T cells and dampened their effector function [60]. Taken together, it has become increasingly clear that glucose availability within the tumor microenvironment regulates T cell effector function.

Improve metabolic fitness of human T cells for cancer immunotherapy

Immunotherapy using adoptive transfer of naturally-occurring, tumor-infiltrating T cells (TIL) can mediate the complete regression in some patients with metastatic melanoma [16]. TIL are heterogeneous with respect to their state of differentiation (as illustrated in Figure 3) and the generation of large numbers of autologous TIL for adoptive transfer requires *in vitro* expansion. Current methods of expansion of TIL can trigger Akt and mTOR activity, driving the terminal differentiation of T cells. We and others have demonstrated that restraining Akt [45] or mTOR [30] activity during T cell priming or enhancing STAT3 activity [61] and Wnt-β-catenin signaling in human T cells [18], can arrest T cell development, maintaining stem cell-like memory T cells. This arrested development of effector T cells is associated with enhanced metabolic properties including reduced glycolysis and increased utilization of fatty acid oxidation, improving long-term survival and anti-tumor activity of human T cells.

Model of T cell differentiation based on metabolic activity

We therefore propose a model for anti-tumor T cell immunometabolism, extending our previous work with T cell differentiation, in which cells with high metabolic activity during *in vitro* expansion and priming results in the acquisition of a terminal effector phenotype. High metabolic activity, with concomitant high $\Delta\psi m$ and ROS levels leads to short-lived cells with poor anti-tumor efficacy **(**Figure 4**)**. In contrast, cells with restrained metabolic activity *in vitro* are preserved in a functional state where increased self-renewal and persistence are favored, allowing for long-lived and improved anti-tumor function. A critical aspect of this model is that restraints on the metabolic activity of T cells at the tumor site must be released to enable effector function and tumor destruction.

Conclusions

The emerging interface between immune response and metabolism ‘immunometabolism’ helps us understand the bio-energetic requirements of T cell differentiation, cell-fate decision and function of T cells. Current methods for generating T cell products for adoptive immunotherapy have the pitfall of driving cells toward terminal differentiation and senescence. Approaches that are informed by a knowledge of the metabolic requirements for the optimal function of anti-tumor T cells are likely to be the subject of intense work in the field of immunometabolism.

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Highlights

- Memory T cells have quiescent metabolism.
- Activation of T cells triggers both glycolysis and oxidative phosphorylation.
- Elevated T cell metabolic activity is necessary at the tumor site to promote tumor killing
- High mitochondrial membrane potential ($\Delta\Psi m$) is associated with cytokine production and capacity for cytotoxic function.
- Metabolic reprogramming of T cells may improve TCR and chimeric antigen receptor (CAR) based immunotherapy.

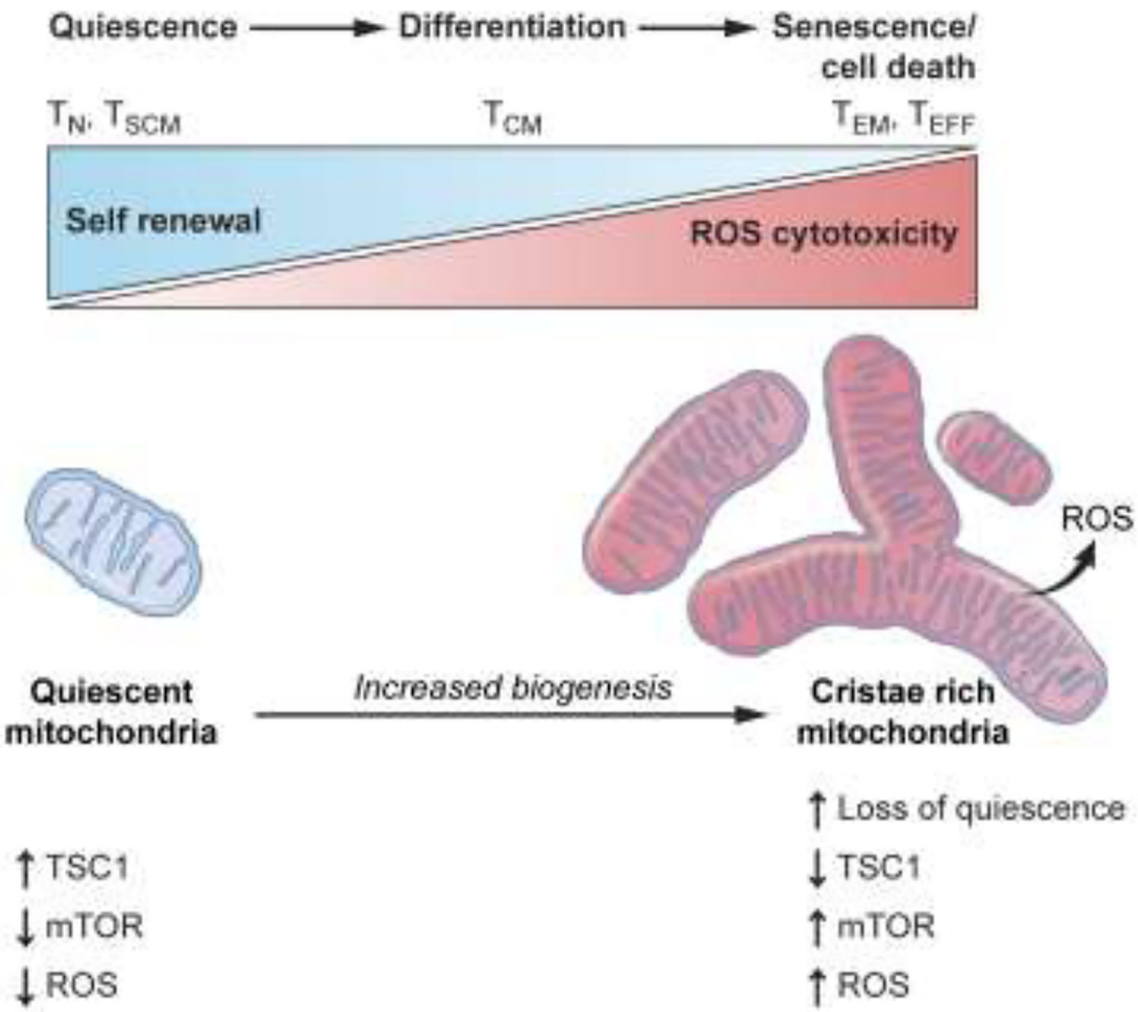

Figure 1. Mitochondrial ROS levels may impair long-term self-renewal program in CD8+ T cells.

T cell activation and differentiation are accompanied by an increase in mTOR activity that results in loss of quiescence and gain of metabolic activity such as increased mitochondrial biogenesis, mitochondrial ROS and oxidative stress. This model includes observations that cellular differentiation, i.e. the acquisition of effector functions are associated with increase in ROS production within mitochondria. Cellular differentiation is accompanied by loss of 'stemness' – the capacity of cells to be multipotent and self-renewing. In this model, less-differentiated T cell subsets such as stem cell memory (T_{SCM}) and central memory (T_{CM}) have reduced levels of ROS whereas terminally differentiated effector memory and effector T cells (T_{EM} and T_{EFF}) display increased ROS levels that are required for their effector function such as cytotoxicity. Increased oxidative stress and DNA damage as a result of ROS accumulation may directly drive $CD8^+$ T cells towards terminal differentiation with characteristics of loss of T cell proliferation, T cell effector function and impaired self-renewal.

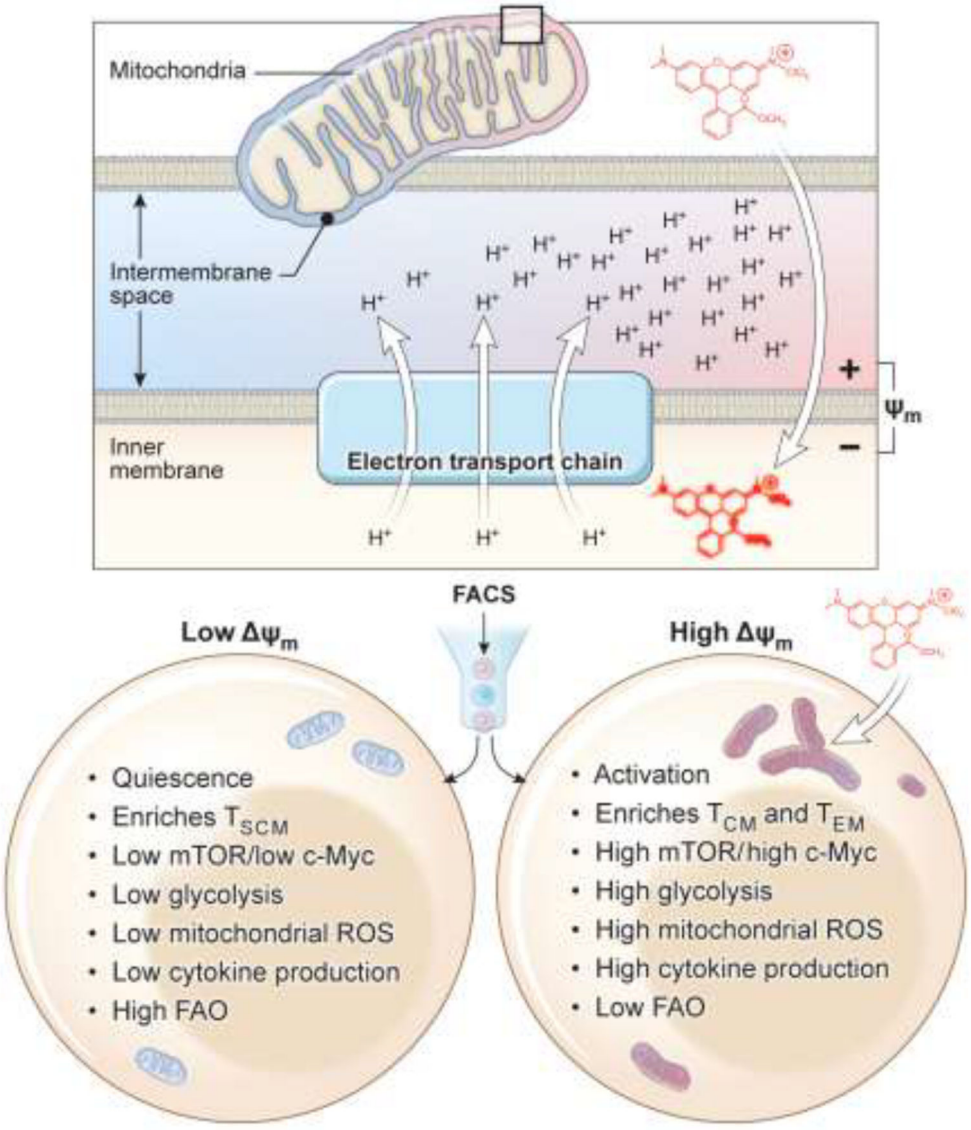

Figure 2. Metabolic sorting of therapeutic T cells based on mitochondrial activity for T-cell based immunotherapy.

Similar to cell surface markers that identify superior T cells for clinical applications, sorting T cells based on mitochondrial ROS and membrane potential (Δψm) can be explored for cell-based therapy. After T cell priming, sorting activated T cells based on mitochondrial activity into low-Δψm or high-Δψm would allow us to enrich for metabolic fit T cells for sustained T cell effector function, long-term persistence and anti-tumor activity.

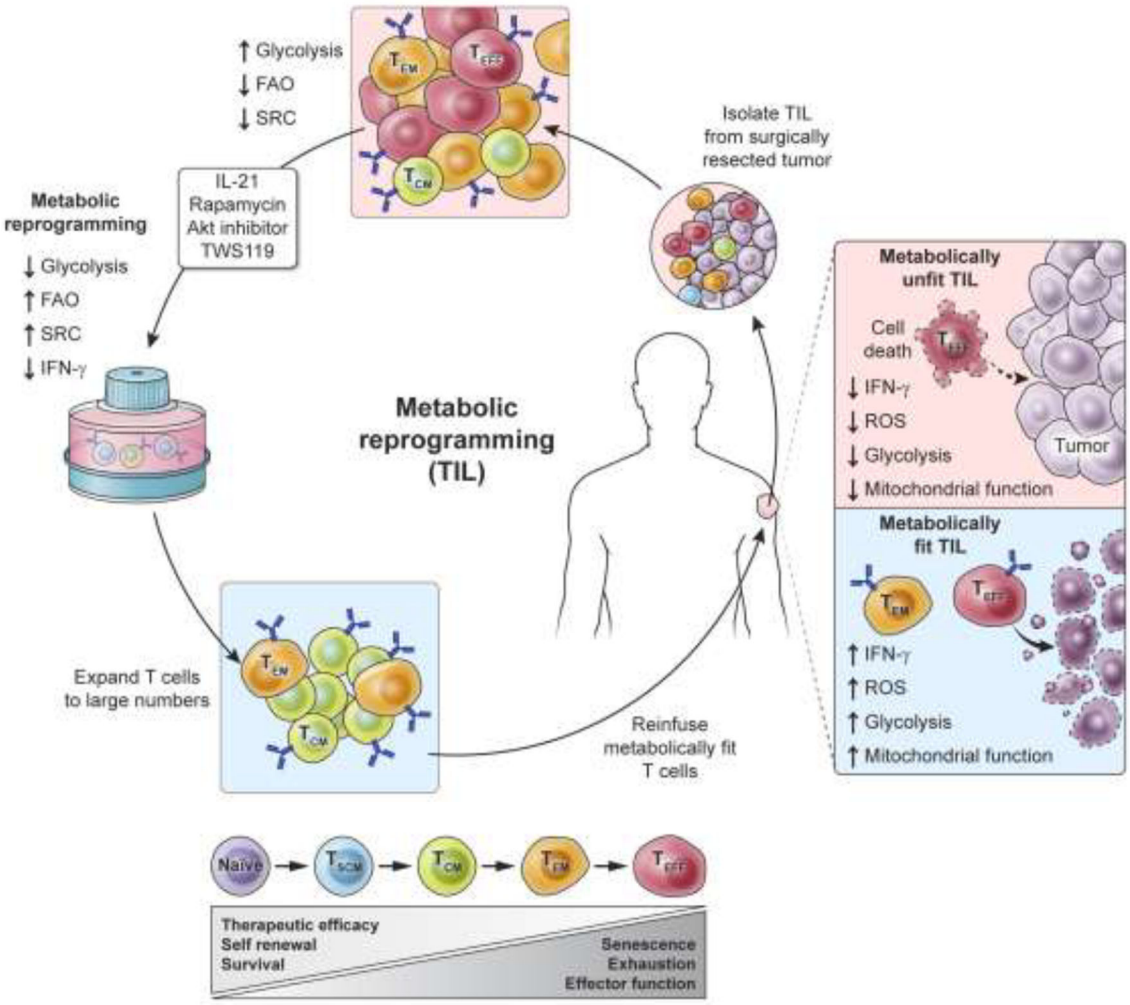

Figure 3. Clinical approaches to enhance "metabolic fitness" of anti-tumor T cells for advanced cancer.

Immunotherapy using adoptive transfer of tumor-specific T cells can mediate dramatic tumor regression in patients with advanced cancer. Animal models have revealed that "metabolically fit" anti-tumor T cells—those with enhanced fatty acid oxidation (FAO) and spare-respiratory capacity (SRC) and lower rates of glycolytic activity—have improved efficacy in eradicating established tumor. Because these metabolic features in mouse models are associated with improved long-term persistence and tumor regression, we propose enhancing "metabolic fitness" of T cells with pharmacologic agents targeting key metabolic pathways. This novel immunometabolic approach may improve the clinical efficacy of T cell-based therapies for patients with advanced cancer.

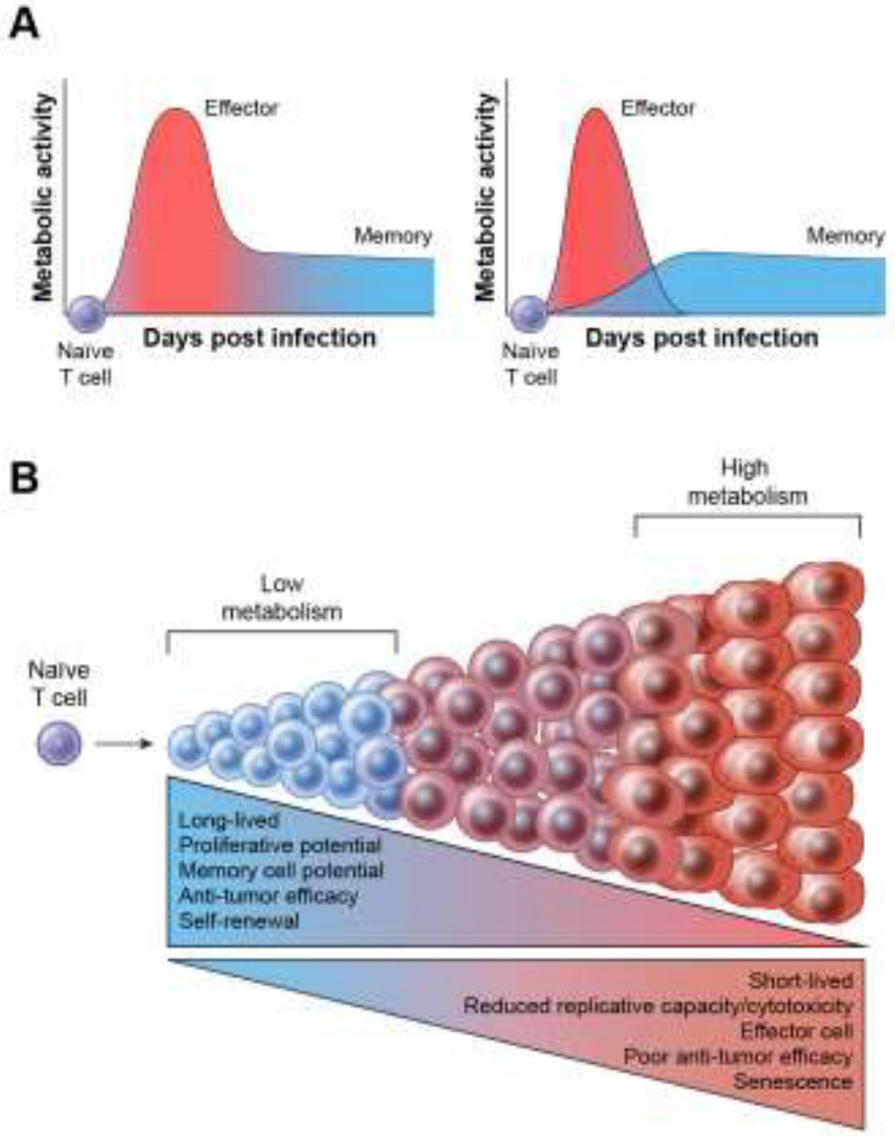

Figure 4. Model of T cell differentiation based on metabolic activity of lymphocytes.

Upon encountering cognate antigen, naïve $CD8^+$ T cells (T_N) undergo massive clonal expansion and differentiate into short-lived effector and long-lived memory $CD8^+$ cells. This cell fate commitment is accompanied by changes in metabolic activity (increase in glycolysis, mitochondrial membrane potential and mitochondrial ROS) after T cell activation. Two dominant competing metabolic models of T cell differentiation are shown (Panel A). The left side of the panel depicts the off-on-off model in which quiescent cells become effector cells with a high metabolic rate which gradually de-differentiate into memory cells with more quiescent metabolism. The right side of Panel A depicts a model of progressive differentiation in which T cells with a highly active metabolic state die, in large part because of their production of ROS and granzymes. In this 'developmental model,' long-lived memory T cells can only originate from cells that retain relative metabolic quiescence. Panel B. We propose that high levels of metabolic activity may drive $CD8^+$ T

cells toward a terminally differentiated effector state that is associated with limited lifespan and replicative potential, impaired antitumor efficacy and ultimately cell senescence. In contrast, low levels of metabolic activity during T cell priming favors the formation of long-lived memory $CD8^+$ T cells that have enhanced long-term survival and anti-tumor immunity.