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# Metabolic regulation of T cell longevity and function in tumor immunotherapy

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## Summary

Cancer immunotherapy is an increasingly successful strategy for the treatment of patients who have advanced or conventional therapy-resistant cancers. T cells are key mediators of tumor destruction and their specificity for tumor-expressed antigens is of paramount importance, but other T cell-intrinsic qualities such as durability, longevity and functionality also play important roles in determining the efficacy of immunotherapy. The cellular energetic pathways that are utilized by T cells play a key role in regulating each of these qualities. Metabolic activity, which both regulates and is regulated by cellular signaling pathways and epigenetics, also profoundly influences the trajectories of T cell differentiation and fate. In this review, we discuss how cell metabolism influences T cell anti-tumor activity, the metabolic qualities of highly-functional T cells and strategies to modulate metabolism for improving the immune response to tumors.

## Introduction

The multi-generational effort to provide better treatments for patients with cancer has resulted in clear improvements in therapeutic options and clinical outcomes. The adoption of defined chemotherapy, radiation and surgical strategies in the post-World War II years has resulted in improved patient outcomes for many types of cancer (DeVita et al., 1975; DeVita et al., 1972; Devita et al., 1970) and the advent of targeted therapies that directly inhibit oncogenic signaling pathways has also provided benefits for patients with certain cancers (Kantarjian et al., 2012). However, to date, there are very few examples of curative therapies for patients with metastatic cancer, in which cancer has disseminated throughout the body (Rosenberg, 2012; Sridhara et al., 2010). Further, treatment options are limited and often only yield short-lived responses in patients for whom frontline chemotherapeutic, radiation

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and surgical treatment strategies have failed to control disease (Armstrong, 2002; Oriol et al., 2010). Consequently, therapies that can successfully treat metastatic cancers or conventional treatment-resistant tumors would be of great benefit to many cancer patients.

Efforts to harness the body's immune system to treat cancer, collectively known as cancer immunotherapy, have emerged as a powerful set of approaches to address these key needs. Many immunotherapy techniques focus on modifying the activity of T lymphocytes (T cells) to drive an anti-tumor response (Rosenberg, 2012). Encouraging results demonstrate that a number of T cell-based cancer therapies have achieved success in mediating complete, durable responses in patients with several types of metastatic cancer (Robbins et al., 2011; Rosenberg et al., 1988; Zhao et al., 2005). Further, these approaches can achieve these same responses in patients with tumors that were previously resistant to conventional treatment strategies (Maude et al., 2014; Rosenberg et al., 2011; Topalian et al., 2012).

While these clinical successes with immunotherapy approaches are promising, there are several limitations that must be considered and overcome to broaden the number of patients for whom immunotherapy can be successfully utilized. First, immunotherapy strategies are not successful in all patients. While immunotherapy can sometimes mediate complete, long-lasting responses, many patients experience partial or non-durable responses or may have no response to immunotherapy (Maude et al., 2014; Rosenberg et al., 2011; Topalian et al., 2012). Additionally, although immunotherapy can provide clinical benefit in a number of cancer types such as melanoma (Rosenberg et al., 2011; Topalian et al., 2012), acute lymphoblastic leukemia (Maude et al., 2014), synovial sarcoma (Robbins et al., 2011) and lung cancer (Brahmer et al., 2012; Topalian et al., 2012), it is not yet clear to what degree immunotherapy will benefit patients with other types of cancer. Therefore, approaches to improve and broaden the efficacy and utility of this therapy are needed.

While many factors play roles in mediating effective immune responses to cancer, including tumor-intrinsic and microenvironmental considerations, the characteristics of the T cells that are used to mount an anti-cancer immune response are likely a critical factor in determining clinical outcome (Klebanoff et al., 2012). There is mounting evidence in both human patients and in animal models suggesting that T cell replicative potential and differentiation status are important regulators of anti-tumor activity (Gattinoni et al., 2009; Klebanoff et al., 2005; Louis et al., 2011; Rosenberg et al., 2011; Zhou et al., 2005). In both settings, treatment of cancer using T cells with characteristics of heightened cellular longevity, such as increased self-renewal and replicative capacity, is associated with improved anti-tumor responses (Sukumar et al., 2017). Therefore, factors that promote these characteristics in T cells may be potential avenues through which the efficacy of immunotherapy could be improved.

The study of organismal aging and longevity has highlighted the key link between metabolic activity and longevity (Lopez-Otin et al., 2016). Cellular metabolic pathways have been shown to play key roles in regulating the fate, function (O'Neill et al., 2016) and longevity of T cells (Chang and Pearce, 2016). Additionally, the modulation of T cell metabolism has been explored as a potential therapeutic target to enhance or suppress immune responses in numerous settings, including anti-tumor immunity (O'Sullivan and Pearce, 2015). Therefore,

modulating the metabolic properties of the T cells utilized in cancer immunotherapy is a promising strategy towards improving anti-tumor function and therapeutic benefit.

In this review, we discuss the key role of cellular metabolic pathways in governing the T cell response to tumor. We describe advances in the understanding of the role that T cell metabolic properties play in regulating the efficacy of the anti-tumor response. We highlight the interface between cellular metabolic pathways and anti-tumor T cell longevity and functionality against cancer, focusing on the metabolic characteristics of effective T cell responses against tumor and discussing metabolic considerations unique to the tumor microenvironment. Finally, we evaluate potential metabolic avenues through which T cell-mediated immunotherapy can be optimized for heightened clinical efficacy.

## Principles of immunotherapy to treat human cancer

Cancer immunotherapy is predicated on the idea that, as a result of unique mutations or protein expression patterns, tumor cells may be recognized and eliminated by the immune system with a high degree of specificity (Lim and June, 2017; Rosenberg and Restifo, 2015). Although there are several forms of cancer immunotherapy strategies currently under study (Guillerey et al., 2016), we will limit our discussion in this review to therapies that are considered as being mediated through the action of T cells. The therapeutic strategies that seek to utilize T cells to destroy tumors can largely be divided into three categories: 1) genetic modification of T cells to confer the ability to specifically recognize cancer cells. 2) isolation of tumor-specific T cells from existing tumor masses, followed by *ex vivo* expansion and reinfusion into the patient. 3) treatment of patients with agents designed to activate tumor-specific T cells *in situ* in the tumor.

The genetic modification of T cells to impart the ability to specifically recognize and eliminate tumor cells has been achieved through transducing T cells with chimeric antigen receptors (CARs) or tumor-antigen specific T cell receptors (TCRs). A CAR is a synthetic cell surface receptor composed of an extracellular targeting element to mediate recognition of tumor-expressed surface proteins, along with signaling domains that mediate T cell function (Lim and June, 2017). Conversely, TCRs are naturally occurring T cell surface receptors that can recognize peptide fragments (or antigens) that are presented to them by the major histocompatibility complex (MHC)/human leukocyte antigen (HLA) system. TCRs within a single person are incredibly diverse and collectively have the capacity to recognize many different antigens (Nikolich-Zugich et al., 2004). In the immunotherapy setting, TCRs can recognize peptide fragments that are generated from mutated tumor proteins and may also recognize germline antigens that are sometimes aberrantly expressed in tumors (Rosenberg and Restifo, 2015). The transduction of peripheral, non-tumor specific T cells with tumor-targeting CARs or TCRs can transform these cells into potent cancer killers (Figure 1a).

CAR and TCR-transduced T cells have each been utilized clinically to achieve durable, complete regressions of cancer. T cells transduced with CARs recognizing CD19, a cell surface marker expressed in many B cell leukemias and lymphomas, have been shown to mediate specific destruction of cancer cells in human patients (Kochenderfer et al., 2010;

Locke et al., 2017; Maude et al., 2014; Porter et al., 2011), and can yield durable responses in patients for whom frontline chemotherapy was unsuccessful (Park et al., 2016). CD19-CAR therapy is generally well tolerated by patients, but can lead to the destruction of normal cell types that express CD19, such as healthy B cells (Maude et al., 2015). Consequently, in principle, CAR therapy is restricted to targeting cell surface molecules uniquely expressed by tumor cells or those that are only expressed by dispensable normal tissues (Kakarla and Gottschalk, 2014). Due to a lack of such targets (Klebanoff et al., 2016), and perhaps a number of other factors that are incompletely elucidated (Yong et al., 2017), CAR therapy has shown extremely limited efficacy against solid tumors.

In contrast, TCR-mediated immunotherapy does not require the cell surface expression of tumor antigens and instead relies on tumor-specific mutations generating proteins that are processed and presented to T cells through the MHC/HLA system on tumor cells (Rosenberg and Restifo, 2015). A population of T cells that is capable of recognizing and mounting immune responses against cancer cells can sometimes be identified within patient tumors (Muul et al., 1987; Rosenberg et al., 1988; Rosenberg et al., 1986). These T cells likely possess TCRs that are able to recognize unique mutations expressed in tumor cells, allowing them to specifically attack malignant tissue (Robbins et al., 2013; Tran et al., 2017; van Rooij et al., 2013). In some cases, T cells have been identified that can recognize cancer-germline antigens such as MAGE-A3 and NY-ESO that can be aberrantly expressed by tumor cells and are not highly expressed in normal tissue (Scanlan et al., 2002). The identification and sequencing of TCRs that recognize these types of proteins has allowed for their transduction into patient peripheral T cells followed by expansion into large numbers and infusion into the patient. There have been clinical successes in limited trials of this therapy (Robbins et al., 2011), but the identification of TCRs that recognize epitopes generated by commonly held driver mutations in cancer such as KrasG12D may allow for future widespread applications (Tran et al., 2016; Wang et al., 2016).

A second strategy to utilize T cells in immunotherapy against tumors utilizes naturally occurring tumor-reactive T cells. This approach, known as adoptive transfer (ACT) of tumor infiltrating lymphocytes (TIL), reviewed in (Rosenberg and Restifo, 2015), involves the surgical extraction of tumor masses and the *in vitro* expansion of resident tumor-specific T cells. Patients are infused with expanded populations of tumor reactive T cells and dosed with high levels of interleukin 2 (IL-2) to promote anti-cancer activity (Figure 1B). This therapeutic approach has been found to mediate complete and durable responses in some patients with advanced melanoma (Rosenberg et al., 2011) and can yield durable partial responses in other solid epithelial cancer settings (Tran et al., 2016; Tran et al., 2014). Efforts are currently underway to expand the types of cancer that can be treated with adoptive transfer of TIL. In principle, any patient with a resectable tumor that contains tumor-reactive T cells may benefit from this approach.

The final type of T cell mediated immunotherapy is the restoration of immune function against cancer cells *in situ*. Strategies to accomplish this include the administration of cytokines such as IL-2 to promote T cell growth, survival and function (Klapper et al., 2008) and the use of immune checkpoint inhibitors that target cell surface proteins such as PD-1/PD-L1 or CTLA4 to counteract immune suppressive signals in the tumor

microenvironment (Pardoll, 2012). There is also a high level of interest in utilizing vaccines against tumor antigens to drive T cell anticancer function (Carreno et al., 2015), but these strategies are yet to demonstrate clinical efficacy. Immune checkpoint inhibitor therapy currently has by far the biggest impact in the immuno-oncology sphere, and has been used to mediate complete and durable patient responses against a wide range of tumors including melanoma (Topalian et al., 2012), lung cancer (Reck et al., 2016), head and neck cancer (Ferris et al., 2016), bladder cancer (Powles et al., 2014) and even gastrointestinal tumors expressing high mutational load (such as those seen in microsatellite instability) (Le et al., 2016).

The nature of the protocols used for CAR T cell, tumor antigen specific-TCR transduced T cell and ACT therapies, in which cells are expanded or modified in an *in vitro* setting prior to infusion into patients, provides an opportunity for the genetic or pharmacological modulation of T cell properties (Rosenberg and Restifo, 2015) such as metabolism (Sukumar et al., 2017) to improve the functional characteristics of the cells.

## Differentiation status regulates T cell lifespan

A key characteristic that determines immune functionality and longevity in T cells is the degree of differentiation of the cell. Mature T cells are categorized into two broad functional groups based on their exclusive expression of the cell surface markers CD4 or CD8 (Germain, 2002). $CD4^+$ T cells are known as helper T cells ($T_H$ cells) and act to modulate immune responses by producing cytokines to enhance or suppress inflammation (Ziegler, 2016). $CD8^+$ T cells are known as cytotoxic T cells ($T_C$ cells) and act to kill pathogen-infected or malignant cells by secreting inflammatory cytokines along with cell lytic molecules such as perforin and granzyme (Zhang and Bevan, 2011). Both $CD4^+$ and $CD8^+$ T cells can mount anti-tumor immune responses (Quezada et al., 2010).

$CD4^+$ and $CD8^+$ cells circulate through the body searching for antigens that stimulate their TCR, indicating the presence of infection or malignant growth (Gowans and Knight, 1964). Prior to an initial antigen encounter, T cells are referred to as naïve ($T_N$) (Cossarizza et al., 1996). After antigen encounter, T cells are activated and undergo rapid growth and proliferation and begin to produce inflammatory cytokines and cytotoxic granules to support a sustained and robust immune response (Smith-Garvin et al., 2009). This process, known as differentiation, involves a progressive acquisition of inflammatory character and involves a host of changes to cellular functions (Gattinoni et al., 2012). Additionally, depending on the cytokine environment upon antigen encounter, T cells may further specify their lineage into a number of discrete subsets (Masopust and Schenkel, 2013) that are characterized by the expression of functionally-defining transcription factors(Zhu et al., 2010). T cells can specify their lineages into inflammatory subsets such as Th1 or Th17 for $CD4^+$ cells (Zhu et al., 2010) or Tc1 (Sad et al., 1995) and Tc17 for $CD8^+$ cells (Huber et al., 2009). T cells may also acquire immune-suppressive characteristics as regulatory T cells (Treg) (Smith and Kumar, 2008; Zhu et al., 2010). The differentiation and lineage subset specification of T cells drives and is driven by dramatic shifts in cellular signaling and metabolic pathways (Sukumar et al., 2017).

The differentiation state and subset specification of T cells can influence their relative longevity. $CD8^+$ T cell differentiation states include $T_N$, stem central memory T cells ($T_{SCM}$), central memory T cells ($T_{CM}$), effector memory T cells ($T_{EM}$) and effector T cells ($T_{EFF}$) and finally terminally differentiated $T_{EMRA}$ (Restifo and Gattinoni, 2013), with $T_N$, $T_{SCM}$ and $T_{CM}$ cells exhibiting increased longevity and replicative capacity compared with $T_{EM}$, $T_{EFF}$ and $T_{EMRA}$. The transition from $T_N$ to $T_{EMRA}$ status is marked by a progressive change in the expression of transcription factors (Gattinoni et al., 2012), along with epigenetic and metabolic changes (Figure 2) that promote the acquisition of effector character (Crompton et al., 2016). Concurrently, increased T cell differentiation also leads to reduced telomere length (Weng et al., 1995), and the repression of transcription factors that promote self-renewal and cell longevity (Gattinoni et al., 2012). While increased differentiation status is characterized by reduced self-renewal capacity and longevity, these states are also correlated with increased immune functionality, including the increased production of inflammatory cytokines and cell lytic molecules such as granzyme B and interferon γ (Gattinoni et al., 2006). Subset specification also likely regulates T cell longevity, with Th17 and Tc17 cells exhibiting considerable self-renewal capacity, while Th1 and Tc1 cells tend to be short-lived terminal effectors (Muranski et al., 2011). Taken together, the process of cell differentiation and lineage specification has profound impacts on the self-renewal capacity and longevity of T cells.

## T cell differentiation: Persistence is key for anti-tumor responses

T cells exhibiting characteristics of minimal differentiation and increased self-renewal capacity have proven superior to terminally differentiated effector cells in mounting immune responses against established tumors (Gattinoni et al., 2009). In human patients in the adoptive transfer of TIL setting, T cells that mount effective anti-tumor responses are characterized by increased persistence and survival *in vivo* (Gattinoni et al., 2011; Robbins et al., 2004). Cells with early differentiation states, including $T_N$ cells, $T_{SCM}$ and $T_{CM}$ cells are more efficacious in treating established vascularized melanoma than terminally differentiated $T_{EM}$ or $T_{EFF}$ cell subsets (Gattinoni et al., 2005). Subsets characterized by increased longevity such as Th17 cells provide superior anti-tumor activity against melanoma compared with short-lived Th1 (Muranski et al., 2008). While detailed clinical evaluations of human T cell differentiation status and anti-tumor efficacy have not been conducted in clinical trial settings, positive correlations have been found between the expression of memory cell markers along with telomere length in T cells and the degree of response against tumors (Huang et al., 2006; Huang et al., 2005; Rosenberg et al., 2011; Zhou et al., 2005). These studies indicate that T cell longevity may be an important factor in determining the efficacy of the immune response against tumor.

## T cell metabolism is a dynamic aspect of immune responses

The metabolic activity of T cells is regulated, with each differentiation state and lineage subset of T cells exhibiting a unique metabolic profile (MacIver et al., 2013). The metabolic demands for T cells are not static and shift over the course of an immune response, requiring a dynamic regulation of cellular metabolic processes (Buck et al., 2015). The metabolic needs for circulating $T_N$ are low, with these cells generally utilizing low levels of

mitochondrial oxidation of glucose-derived pyruvate or fatty acids to maintain cellular ATP levels (MacIver et al., 2013). However, upon encounter with antigen, T cells rapidly begin to alter their cellular signaling and metabolic pathways to prioritize proliferation and immune functionality, including the production of inflammatory cytokines and lytic molecules (O'Neill et al., 2016). This entails dramatically increased activity through anabolic signaling pathways including the PI3K/Akt/mTOR and Ras/MEK/ERK pathways to promote cell growth and replication (Buck et al., 2015). Metabolically, these activated T cells undergo a similar shift towards anabolic growth, with dramatically increased metabolic activity focused on the generation of biosynthetic intermediates such as nucleotides, proteins and membranes that are necessary for cellular growth and proliferation (MacIver et al., 2013).

In a process similar to the aerobic glycolysis (often termed the Warburg Effect) that is sometimes observed in cancer cells (Macintyre and Rathmell, 2013), activated T cells exhibit elevated rates of glycolytic activity. Also similar to what is observed in tumor cells, activated T cells also increase amino acid metabolism (Wang et al., 2011) and fatty acid synthesis (O'Neill et al., 2016). Differentiated $CD8^+$ T cells are generally thought to become more reliant on glycolytic metabolism as they progress towards terminal differentiated effector status (Sukumar et al., 2013). Anabolic metabolic activity likely directly contributes to the inflammatory immune response, with T cell aerobic glycolysis promoting the production of inflammatory cytokines by maintaining acetyl-CoA pools substrates that are necessary for epigenetic promotion of *Ifng* gene expression (Peng et al., 2016). Glycolytic flux may also promote inflammation through enhancing *Ifng* mRNA translation into protein (Chang et al., 2013). After the antigen driving an immune response has been cleared from the body, memory T cells remain in circulation, establishing immunological memory to allow for rapid recall responses to any future encounters with the same antigen or disease (Farber et al., 2014). These memory T cells are characterized by a metabolic profile that is similar to that of naïve T cells, with a reliance on mitochondrial metabolism and fatty acid oxidation (FAO) (O'Sullivan et al., 2014).

In addition to the general metabolic program of naïve, antigen-activated and memory T cells, each lineage subset of T cells has a unique metabolic profile. In the $CD4^+$ compartment, inflammatory effector cells such as Th1, Th2 and Th17 have been found to be glycolytic and reliant on anabolic signaling and metabolic pathways, while Treg have been characterized as utilizing an oxidative metabolism in which pyruvate and fatty acids are burned in the mitochondria (Beier et al., 2015; Gerriets et al., 2015; Liu et al., 2015; Michalek et al., 2011). Detailed studies on the metabolic programs utilized by each lineage subset of $CD8^+$ T cells have not been conducted to date. However, there appears to be some degree of plasticity in the metabolic programs that each T cell subset utilizes. For instance, Th17 cells can extensively utilize oxidative metabolism *in vivo* (Franchi et al., 2017) and Treg cells can be glycolytic (Gerriets et al., 2016; Procaccini et al., 2016). It is possible that these results reflect differences between *in vitro* and *in vivo* modeling systems, indicating that further work to characterize the *in vivo* metabolic phenotype of T cell subsets will likely be necessary and informative.

Metabolic dysfunction in T cells can lead to the loss of proper immune function and homeostasis. For instance, imposing increased anabolic metabolism in quiescent, antigen-

naïve T cells through transgenic expression of the glucose transporter Glut1 (Jacobs et al., 2008) or by genetically increasing the activity of the mTOR pathway (Pollizzi et al., 2015) results in the loss of immune quiescence and elevated production of inflammatory cytokines. Conversely, reducing the ability of activated T cells to engage anabolic metabolic programs with the genetic deletion of Glut1 (Macintyre et al., 2014), or by impairing amino acid uptake (Nakaya et al., 2014), or reducing fatty acid synthesis (Berod et al., 2014) results in compromised immune responses to antigens that are reflected by reduced T cell numbers and inflammatory cytokine production. Similarly, inhibiting pyruvate dehydrogenase kinase (PDHK), a positive regulator of aerobic glycolysis, can reduce the inflammatory capacity of Th17 cells and promote the generation and suppressive function of Treg cells (Gerriets et al., 2015). Memory and regulatory T cell functionality is also influenced by cell metabolism, as the inhibition of mitochondrial metabolism and FAO may prevent memory cell formation (van der Windt et al., 2012) and reduce Treg induction (Michalek et al., 2011) and functionality (Beier et al., 2015; Gerriets et al., 2015). Taken together, it appears that the coupling of the metabolic activity of T cells with the functional needs of each T cell lineage is an important aspect of maintaining proper immune homeostasis and functionality.

## Metabolism impacts cellular signaling and epigenetics to govern longevity

In the setting of organismal longevity, it has been conclusively demonstrated that metabolic interventions can provide a therapeutic strategy to enhance lifespan (Lopez-Otin et al., 2016). Caloric restriction (CR) along with pharmacological mimetics such as metformin, are linked to increased longevity (Lin et al., 2002; Martin-Montalvo et al., 2013; Weindruch and Walford, 1982). At a cellular level, CR (and pharmacological or genetic mimetics) acts through the regulation of cellular signaling pathways that are key players in modulating the activities of cell intrinsic metabolic pathways (Figure 3) (Johnson et al., 2013). Mechanistically, CR and mimetics inhibit the activity of anabolic signaling pathways including the PI3K-Akt-mTORC1 pathway, while promoting the activity of signaling pathways such as the AMPK signaling cascade and autophagy, which serve to promote catabolic metabolism (Galluzzi et al., 2014; Marino et al., 2014). Showing the key link between the regulation of anabolic growth and longevity, the pharmacological inhibition of mTORC1 with rapamycin has been demonstrated to extend lifespan in aged mice (Harrison et al., 2009). The inhibition of anabolic cellular signaling pathways results in the suppression of pro-growth metabolic pathways including glycolysis, amino acid metabolism and fatty acid synthesis, while simultaneously promoting catabolic metabolic pathways such as FAO (Buck et al., 2015). Intriguingly, there is some evidence that metabolic pathways can directly influence organismal longevity, with glycolysis having been demonstrated to negatively regulate lifespan (Priebe et al., 2013; Schulz et al., 2007). In elegant studies, Ristow and colleagues found that increased glucose availability decreased *C. elegans* lifespan, while the genetic and pharmacological inhibition of glycolytic metabolism extended lifespan in an AMPK-dependent fashion (Schulz et al., 2007). Conversely, the whole-body genetic overexpression of genes involved in FAO (Paik et al., 2012) extended lifespan (Lee et al., 2012) in *D. melanogaster*. Organismal and cellular aging is associated with profound alterations in epigenetic modifications and consequently, changes in gene expression (Lopez-Otin et al., 2016; Sun et al., 2014). Cellular metabolic pathways may also

regulate longevity by controlling the availability of substrates for epigenetic modifications such as acetyl-CoA for acetylation and methionine for methylation (Sharma and Rando, 2017). Overall, it is clear that the activities of cellular signaling and metabolic pathways play key roles in determining the biological aging of individual cells and organisms. The principles that have been elucidated in the field of aging research may hold value in considering methods to improve the T cell response to cancer.

## Metabolic activity is coupled to T cell longevity

Many of the metabolic principles that regulate cell biology are conserved across species and various cell types (Caspi et al., 2006). There is now mounting evidence that metabolic pathways, as in other cell settings, play a key role in the regulation of T cell longevity (Chang and Pearce, 2016). Increased activity in anabolic cell signaling pathways such as the PI3K-Akt-mTOR pathway, through genetic loss of negative regulators of the pathway such as PTEN (Huynh et al., 2015; Shrestha et al., 2015), tuberous sclerosis complex (TSC) proteins (Shrestha et al., 2014), AMPK (MacIver et al., 2011), or key regulators of autophagy (Wei et al., 2016) imposes increased activity in anabolic metabolic pathways in T cells. T cells with heightened mTORC1 activity are characterized by increased glycolytic metabolism, along with high levels of pentose phosphate pathway (PPP) and amino acid metabolism pathway activities (Pollizzi et al., 2016; Verbist et al., 2016). These alterations drive rapid proliferation, but can also result in decreased cell persistence (Pollizzi et al., 2016; Verbist et al., 2016) and an increased susceptibility to cell death (MacIver et al., 2011). Similarly, the catabolic autophagy process is essential for T cell memory and persistence, as genetic deletion of *Atg5* or *Atg7* results in impaired memory cell formation and long-term survival of $CD8^+$ T cells (Xu et al., 2014). Conversely, the inhibition of the mTORC1 signaling pathway using the pharmacological inhibitor rapamycin has been demonstrated to increase T cell persistence in the context of infection (Araki et al., 2009; Pearce et al., 2009). Signaling pathways that drive catabolic metabolic programs, such as IL-15, which promotes mitochondrial metabolism, have been found to promote T cell longevity *in vivo* (van der Windt et al., 2012). Likewise, AMPK pathway activity can promote the persistence and survival of normal and malignantly-transformed T cells through regulating the activity of metabolic pathways (Blagih et al., 2015; Kishton et al., 2016). Beyond survival, AMPK signaling has been demonstrated to be required for antigen recall responses (Rolf et al., 2013). The pharmacological activation of AMPK signaling via metformin treatment has also been demonstrated to promote T cell persistence *in vivo* (Pearce et al., 2009).

Although cellular signaling pathways can influence many cell phenotypes beyond metabolism, direct alterations in the activities of metabolic pathways alone are sufficient to modulate the longevity of T cells. T cells that take up low levels of glucose are metabolically fit for long term survival and memory cell formation (Sukumar et al., 2013; Verbist et al., 2016). Further, inhibiting T cell glycolysis by pharmacological inhibition with 2-deoxyglucose results in increased cell lifespan and memory cell induction (Sukumar et al., 2013). Conversely, the imposition of glycolytic metabolism through genetic overexpression of Pgam1 decreases the long term survival of T cells (Sukumar et al., 2013). These results

suggest that glycolysis itself, while crucial for enabling T cell inflammatory character, imposes a penalty of reduced longevity on the cell.

Altering the metabolic phenotype of long-lived memory cells also results in reduced functionality. The reduction of memory T cell FAO through pharmacological or genetic inhibition of Cpt1a or lysosomal acid lipase (LAL) results in significant reductions in T cell persistence (O'Sullivan et al., 2014), indicating that usage of mitochondrial oxidation is an important aspect of T cell longevity. Another common feature of T cells that exhibit increased longevity and persistence is increased mitochondrial spare respiratory capacity (SRC), as measured upon treatment of cells with the uncoupling agent FCCP (van der Windt et al., 2012). SRC may be reflective of the capacity of the cell to utilize FAO, but uncertainty remains in the biological meaning of this measurement (Pfleger et al., 2015). However, it is unclear whether SRC is causative of increased T cell persistence, or perhaps is a feature that is simply correlated with increased longevity. The stabilization of hypoxia inducible factor 1α (HIF1α) through the loss of the tumor suppressor Von Hippel-Lindau (VHL) results in T cells with increased *in vivo* persistence, along with elevated glycolysis and no increase in SRC (Phan et al., 2016). Consequently, further work is likely necessary to definitively understand the role of fatty acid oxidation and mitochondrial metabolism in the promotion of T cell longevity.

Additional layers of nuance are indicated by studies suggesting that the overall rate of cellular metabolic activity, rather than that of specific pathways, may be important in determining cell longevity. Indeed, T cells and hematopoietic stem cells (HSCs) with increased mitochondrial membrane potential, indicative of high rates of glycolysis and basal mitochondrial oxidative metabolism, have reduced persistence and self-renewal capacity when compared with cells exhibiting low mitochondrial membrane potential (Sukumar et al., 2016). The reduction of mitochondrial mass through the pharmacological promotion of mitophagy was similarly found to promote HSC self-renewal and longevity (Vannini et al., 2016). High $\Delta\psi m$ in T cells is characterized by reduced expression of memory-associated genes that are associated with cell longevity and self-renewal potential, indicating that increased mitochondrial activity may drive T cells towards a terminally differentiated state (Sukumar et al., 2016). Interestingly, high $\Delta\psi m$ in T cells is associated with increased reactive oxygen species (ROS) production and DNA damage (Sukumar et al., 2016). While a clear role has been established for ROS in promoting T cell response to antigens (Sena et al., 2013), it is possible that elevated mitochondrial activity also causes lifespan-reducing DNA damage. Taken together, there appears to increasing evidence that T cells (and HSCs) with high rates of metabolic activity may be characterized by reduced self-renewal capacity and longevity, while moderated metabolic activity can promote increased longevity and memory cell generation (Figure 4A).

## Two-stage metabolism for T cell longevity in immunotherapy

There has been considerable research effort to understand the metabolic properties of highly effective anti-tumor T cells, along with how metabolism can be targeted as a possible approach to improve the anti-tumor efficacy of immunotherapy (Chang and Pearce, 2016). An emerging narrative that can be found in many studies is that optimal anti-tumor T cell

metabolism is bi-phasic. During the *in vitro* growth and priming phase of immunotherapy, in which T cells are transduced with a CAR or tumor-specific TCR or TIL are grown up to large numbers prior to infusion into the patient, the maintenance of a program of moderated metabolic activity yields improved anti-cancer activity (Sukumar et al., 2017). Conversely, at the site of the tumor *in vivo*, an effective anti-tumor T cell response requires robust metabolic activity to support the production of biosynthetic intermediates for cell proliferation along with the expression of key inflammatory proteins such as interferon γ and granzyme B (Figure 4B).

## Moderated in vitro metabolism promotes increased anti-tumor activity in vivo

The notion that moderating metabolic activity in the *in vitro* setting is useful to promote cell longevity and proliferative capacity is supported by the results of several studies. Repressing the activity of the glycolytic pathway during *in vitro* priming and expansion of anti-tumor T cells by treating them with 2-deoxyglucose resulted in increased and sustained T cell infiltration of melanoma after infusion in a murine model, yielding improved tumor clearance (Sukumar et al., 2013). T cells that were treated with this protocol exhibited increased expression of genes encoding for glycolytic proteins at the tumor site, and were also able to produce increased levels of inflammatory cytokines *in vivo* (Sukumar et al., 2013). Similarly, the pharmacological inhibition of Akt, a key positive regulator of glycolytic metabolism, during *in vitro* T cell expansion also resulted in decreased *in vitro* glycolytic activity that corresponded with improved subsequent *in vivo* anti-tumor activity (Crompton et al., 2015). In each of these studies, the observed increase in anti-tumor activity as a consequence of inhibiting glycolysis was also correlated with increased T cell longevity and persistence in antigen-recognition models. Further supporting the principle that glycolytic metabolism may be a negative regulator of T cell longevity in the anti-tumor setting, CD19-CAR T cells expressing a CD28 co-stimulatory domain that promoted glycolysis were found to exhibit reduced *in vivo* persistence when compared with cells transduced with a 4-1BB co-stimulatory domain that drove oxidative metabolism (Kawalekar et al., 2016). While the anti-tumor efficacy of each construct was not evaluated in the study, anti-tumor T cell persistence has previously been shown to correlate with anti-tumor efficacy in human patients (Robbins et al., 2004). It has also been observed that the supplementation of *in vitro* culture medium with exogenous arginine inhibited T cell glycolytic metabolism while promoting oxidative pathways, leading to increased cell persistence and anti-tumor activity *in vivo* (Geiger et al., 2016). These results suggest that detailed studies on the effects of various concentrations of *in vitro* culture media components such as amino acids may yield novel strategies to modify anti-tumor T cell function. The modulation of T cell metabolism to promote anti-tumor activity has also been accomplished through altering the mitochondrial dynamics of the cell. Pearce and colleagues observed that the process of mitochondrial fission promotes the usage of glycolytic metabolism, while mitochondrial fusion drives mitochondrial metabolism and FAO (Buck et al., 2016). The pharmacological promotion of mitochondrial fusion in tumor-specific T cells during *in vitro* culturing limited glycolytic metabolism and promoted anti-tumor activity *in* vivo. Taken together, these studies indicate that limiting the glycolytic activity of T cells during *in vitro*

culturing can preserve their subsequent replicative capacity and longevity to mediate a successful immune response against tumors *in vivo*.

Moderation of the activity of additional metabolic pathways in anti-tumor T cells can also be considered to improve *in vivo* efficacy. T cells that exhibit high mitochondrial activity as measured by $\Delta\psi m$ and ROS production during *in vitro* expansion were found to have reduced longevity and anti-tumor functionality upon *in vivo* transfer (Sukumar et al., 2016). Reducing the activity of fatty acid and cholesterol biosynthesis pathways during *in vitro* culturing may also improve the *in vivo* anti-tumor activity of T cells (Yang et al., 2016). Future studies examining the role of additional metabolic pathways, such as the pentose phosphate pathway and the metabolism of additional amino acids will result in a more complete understanding of how various metabolic pathways interface with T cell longevity and anti-tumor function.

## Elevated metabolic activity at the tumor site promotes T cell function

In contrast to the metabolic moderation of *in vitro* T cell metabolism that improves subsequent *in vivo* activity, efficacious T cell responses to cancer at the tumor site are characterized by high rates of metabolic activity (Sukumar et al., 2017). Glycolytic metabolism can directly contribute to the production of inflammatory cytokines such as interferon gamma through epigenetic promotion of gene transcription (Peng et al., 2016), and the elevated expression of genes that encode for proteins that promote glycolytic metabolism such as *Slc2a1* (Glut1) or *Pkm2* in T cells that have localized to a tumor are associated with increased cytokine production and anti-tumor response (Sukumar et al., 2013). The imposition of glycolytic metabolism in anti-tumor T cells *in vivo* through the genetic loss of negative regulators of HIF signaling also results in improved tumor clearance (Clever et al., 2016). Similarly, transgenic expression of Glut1 or constitutively active Akt mediated increased effector function in T cells in a murine model of ALL (Siska et al., 2016). There are also indications that elevated mitochondrial metabolic activity in T cells *in vivo* may promote anti-tumor activity. Consistent with the idea that ROS activity plays a key role in mediating T cell signaling after antigen recognition (Sena et al., 2013), cells that exhibit high $\Delta\psi m$ have elevated ROS levels and increased production of inflammatory cytokines *in vitro* (Sukumar et al., 2016). A recent report demonstrated that the pharmacological enhancement of mitochondrial metabolic activity to increase ROS production in T cells can mediate increased T cell activity against tumors. In this study, treatment of melanoma bearing mice with mitochondrial activating chemicals synergized with checkpoint inhibitor blockade to improve T cell anti-tumor response in a ROS mediated process (Chamoto et al., 2017). Supporting the idea that elevated T cell metabolism at the tumor site is necessary for a robust immune response to cancer, tumor mediated inhibition of T cell metabolic activity via repression of the PI3K pathway by PD-L1 ligation (Chang et al., 2015; Patsoukis et al., 2015) or increased potassium in the tumor microenvironment (Eil et al., 2016) have been shown to suppress anti-tumor immunity. Likewise, reduced mitochondrial biogenesis mediated through the loss of PGC1α expression in T cells in the tumor microenvironment has been implicated as a mechanism of reduced anti-tumor activity (Scharping et al., 2016). Conversely, a recent report indicated that neo-antigen reactive T cells that are capable of mediating tumor regression in mice upon vaccination against tumor

antigens or checkpoint blockade are characterized by increased metabolic activity (Gubin et al., 2014).

Taken together, these studies indicate that after infusion into the body and localization to the tumor site, an effective T cell response to cancer is characterized by robust metabolic activity, with high rates of glycolysis and mitochondrial activity to support the proliferative and immunological demands of a sustained attack on the tumor.

## T cells face a hostile tumor environment with fierce competition for nutrients

However, there are several factors in the tumor microenvironment that inhibit the ability of anti-tumor T cells to fully engage in robust metabolic activity. Indeed, tumor metabolic properties along with inhibitory factors in the tumor microenvironment play an active role in repressing T cell metabolism and functionality (Siska and Rathmell, 2015). The idea of metabolic competition between T cells and tumor cells has been appreciated as a possible mechanism that may govern how effectively T cells eliminate tumor. Two studies found that tumors that are highly glycolytic can deplete glucose levels in the tumor microenvironment, dampening the ability of T cells to maintain anabolic growth signaling and produce inflammatory cytokines (Chang et al., 2015; Ho et al., 2015). Increasing T cell competitiveness in acquiring nutrients through overexpression of PCK1 (Ho et al., 2015) or by checkpoint blockade therapy targeting CTLA-4 and PD-1 (Chang et al., 2015) results in increased T cell metabolic activity, anabolic signaling and response to tumor. Interestingly, PD-L1 expression in tumor cells may promote tumor cell glycolysis in an autocrine signaling loop (Chang et al., 2015). Additional studies highlighting the importance of amino acids in promoting T cell growth and biosynthesis (Hosios et al., 2016), along with proliferation and effector character (Sinclair et al., 2013) suggest that the high rates of amino acid uptake commonly exhibited by tumor cells (Wang and Holst, 2015) could potentially also act to inhibit the anti-tumor T cell response. There is also emerging evidence that some tumors may engage in high rates of fatty acid uptake (Beloribi-Djefaflia et al., 2016). Given that effector T cells have been found to take up fatty acids at high rates (O'Sullivan et al., 2014), it is possible that this may be another environmental nutrient that T cells must compete for in the tumor microenvironment. There are also indications that metabolic alterations found in some tumors as a consequence of oncogenic mutations may also directly influence T cell function. Treatment of T cells with the "oncometabolite" 2-hydroxyglutarate (2-HG), generated at high levels by tumors expressing mutant forms of IDH (Dang et al., 2009), results in reduced effector character (Tyrakis et al., 2016). Mutant IDH tumors generate an immunosuppressive environment through high concentrations of 2-HG at the tumor site that can be overcome by the pharmacological inhibition of 2-HG production by tumor cells (Kohanbash et al., 2017). Taken together, these results suggest that the inhibition of T cell metabolism by the metabolic activity of tumor cells is an important mechanism of immunosuppression in the tumor.

Immunosuppressive, non-tumor cells in the tumor microenvironment also regulate T cell metabolism to inhibit anti-tumor function. Treg may have a metabolic advantage to survive

and function in the tumor microenvironment (Angelin et al., 2017), and can act to promote the expression of indoleamine 2,3-dioxygenase (IDO) in dendritic cells, resulting in tryptophan depletion (Fallarino et al., 2003) and inhibited immune activity in anti-tumor T cells (Munn et al., 1999). Additionally, Treg cells may express CD39, allowing them to hydrolyze extracellular ATP in a process that is also known to reduce the functionality of an immune response (Borsellino et al., 2007). Additional subsets of suppressive cell types exist in the tumor microenvironment, including myeloid-derived suppressor cells (MDSCs), which can also act to reduce the availability of key amino acids including arginine (Rodriguez et al., 2004) and tryptophan (Yu et al., 2013), resulting in the suppression of anti-tumor T cell responses. Further suppressive elements can be generated by tumor-derived lactate, which can act to promote the polarization of immunosuppressive macrophages that act to promote tumor growth (Colegio et al., 2014) and can increase Treg induction under *in vitro* polarizing conditions (Angelin et al., 2017), indicating that tumor production of lactate is one mechanism through which the tumor microenvironment acquires immunosuppressive characteristics.

These studies lead to a model in which anti-tumor T cells are in competition for essential metabolic substrates with not only tumor cells, but also with additional suppressive immune cells subsets (Figure 5). To mount a successful and sustained immune response against tumor cells, anti-tumor T cells must be able to overcome the difficulties of a metabolically hostile environment.

## Strategies to optimize T cell metabolism for immunotherapy

How, then, can researchers leverage cellular metabolic pathways to improve the efficacy of tumor immunotherapy? Numerous approaches have been taken towards modulating T cell metabolism with this goal in mind. As previously discussed, the clinical protocols that are used to produce anti-tumor T cells, in which there is extensive culturing and *in vitro* manipulation of cells prior to infusion into the patient, provide an excellent opportunity for metabolic intervention to promote increased *in vivo* anti-tumor activity (Rosenberg and Restifo, 2015). Currently utilized protocols for anti-tumor T cell expansion rely on high dose IL-2 and can drive T cells towards a terminal effector differentiation status characterized by high rates of metabolic activity, particularly glycolysis. Modifications to culturing techniques may allow for an improved T cell product for infusion into patients (Figure 6). A number of studies in model organisms have indicated that the modulation of T cell metabolism during *in vitro* culture and expansion can improve subsequent anti-tumor efficacy *in* vivo. Limiting the ability of T cells to engage glycolysis during *in vitro* culture through direct inhibition of hexokinase by 2-DG results in improved anti-tumor efficacy against established and vascularized melanoma (Sukumar et al., 2013). Elevated $\Delta\psi m$ is indicative of highly active mitochondria and is associated with increased mTORC1 activity and poor anti-tumor efficacy (Sukumar et al., 2016). Therefore, targeting mTORC1 with rapamycin may be one method to control $\Delta\psi m$ to promote a more favorable mitochondrial energetic profile for improved anti-tumor response. Similarly, pharmacological modulation of mitochondrial dynamics with the small molecule Mdivi has been utilized in T cells to promote fused mitochondrial structures and inhibit mitochondrial fission during *in vitro* culture, resulting in improved anti-tumor activity *in vivo* (Buck et al., 2016). Targeting Akt

with pharmacological inhibitors has also been utilized *in vitro* to reduce T cell glycolysis and promote mitochondrial metabolism, yielding cells that had heightened anti-tumor properties *in vivo* (Crompton et al., 2015). c-Myc signaling activity has previously been observed to drive anabolic growth and metabolism in activated T cells (Wang et al., 2011), and the pharmacological inhibition of c-Myc with the BRD inhibitor JQ1 during *in vitro* culture of T cells has been found to improve *in vivo* persistence and anti-cancer function (Kagoya et al., 2016). Additional approaches that have been evaluated include the activation of the Wnt signaling cascade (Gattinoni et al., 2009) and the inclusion of additional cytokines such as IL-15 (Klebanoff et al., 2004) and IL-21 (Hinrichs et al., 2008) in culturing media. These signals are known to promote catabolic metabolism (Scholz et al., 2016; van der Windt et al., 2012), and each resulted in reduced cell differentiation during *ex vivo* expansion and a consequent improvement in anti-tumor activity *in vivo*.

An important open question is whether metabolism can be modulated *in vivo* to boost anti-tumor T cell functionality. Attempts to improve anti-tumor T cell function through the modulation of metabolism *in vivo* have also shown some promise in pre-clinical models. For instance, genetically enhancing T cell metabolic activity *in vivo* through the overexpression of PCK1 (Ho et al., 2015) or Glut1 (Siska et al., 2016) may allow for improved functionality and metabolic competitiveness in the tumor microenvironment. The use of sophisticated techniques for genetic modification of primary human T cells through CRISPR technology (Su et al., 2016) may allow for genetically modifying patient T cell metabolism in the clinical setting. Additionally, strategies to target tumor PD-L1 mediated inhibition of T cell functionality may act in part through changing the metabolic balance in the tumor. Anti-PD-L1 therapy has been shown to directly inhibit tumor cell glycolytic activity, resulting in a boost to T cell metabolic competitiveness (Chang et al., 2015). Immune checkpoint inhibitor therapy may act to promote T cell anabolic metabolism directly (Patsoukis et al., 2015). The targeting of immunosuppressive macrophages and Treg in the tumor microenvironment may also allow for more robust T cell responses against tumors. Suppressive macrophages (O'Neill and Pearce, 2016) and Treg (Liu et al., 2015) each have distinguishing cellular signaling and metabolic characteristics that may potentially allow for specific inhibition of their suppressive capacity while sparing the effector function of anti-tumor T cells.

While it is important to keep in mind that the metabolic similarities between tumor cells and immune cells may result in difficulties in achieving tumor-specific metabolic inhibition (Kishton and Rathmell, 2015; Macintyre and Rathmell, 2013), it may be possible to achieve a therapeutic window where T cell function is not compromised by metabolic inhibition. Interestingly, recent work in the tumor metabolism field has indicated that, in contrast to highly glycolytic cancer cell lines, primary tumor cells may be reliant on oxidative metabolic pathways *in vivo* (Davidson et al., 2016; Kishton et al., 2016), highlighting that further understanding of the *in vivo* metabolic characteristics of both tumor and T cells is critical. There are indications that the targeting of tumor oncogenic signaling pathways may allow for the inhibition of tumor metabolic pathways while sparing or even improving T cell immune function (Baudy et al., 2012; Ho et al., 2014). However, the *in vivo* targeting of certain oncogenic signaling pathways that are also critical to T cell function, such as the Myc pathway, could exert profound inhibitory effects on T cells (Wang et al., 2011).

Additional insight may be gained from the field of nutritional immunology. Compelling evidence indicates that systemic nutritional states of malnutrition (Lord et al., 1998; Saucillo et al., 2014) or obesity (Yang et al., 2010) can have dramatic effects on the functional characteristics of the immune system, including T cells (Gerriets and MacIver, 2014). These findings indicate that the nutritional status of cancer patients, who can have greatly reduced nutrient intake and sometimes experience a wasting syndrome known as cachexia (Nitenberg and Raynard, 2000), may play a role in determining the efficacy of anti-tumor T cell therapies. Studies to parse out the role of systemic nutritional status in regulating the anti-tumor immune response may allow for nutritional interventions to improve T cell function.

Key challenges remain in devising appropriate strategies to use metabolic interventions to promote T cell anti-tumor function *in vivo*. In addition to the further work required to characterize the *in vivo* metabolic dependencies of tumor cells and T cells, the role of metabolic pathways in the function of key players in the tumor microenvironment such as tumor stromal and vascular cells remain to be elucidated. Studies making use of cell-specific genetic modifications, which have previously demonstrated that a competition for nutrients in the tumor environment between tumor and T cells likely plays a role in influencing immune responses to cancer(Chang et al., 2015; Ho et al., 2015), are likely to play an important role in further understanding this complex biological issue. Further, the role of many key metabolic pathways such as mitochondrial oxidation, fatty acid synthesis and oxidation, along with amino acid metabolic pathways in tumor and T cells remains largely unknown in the context of tumor immunology. Studies that broaden the understanding of these complex interactions will likely generate novel metabolic targets to enhance the immune response against cancer cells.

## Summary and Conclusions

The field of tumor immunotherapy is currently rapidly growing in the clinical and research settings. While immunotherapy techniques such as CAR T cell therapy, the adoptive transfer of tumor infiltrating lymphocytes and immune checkpoint inhibitor therapies have been shown to be capable of mediating complete and durable responses in patients with a number of cancer types, further advances may increase the number of patients that can benefit from these techniques. Pre-clinical and clinical studies indicate that the longevity and durability of the T cells utilized in immunotherapy are likely to be important factors in determining the efficacy of therapy. The balance of T cell metabolic activity is a key regulator of these characteristics, and genetic and pharmacological interventions to modulate anti-tumor T cell metabolic properties have the potential to generate improved cell products for patient treatment.

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Does metabolism determine the fate of T cells? Kishton *et al.* reviews the relationships between metabolism, aging and epigenetics in T cells, which are at the heart of new cancer immunotherapies. By manipulating key metabolic pathways, the efficacy of T cells can be improved in the setting of cancer immunotherapy.

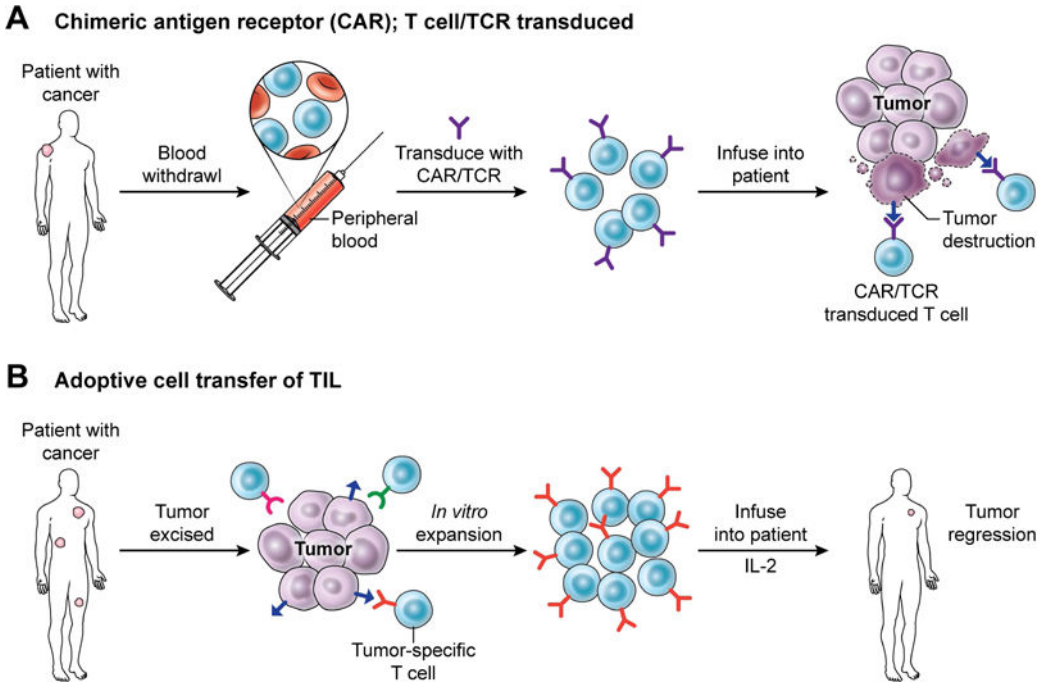


**Figure 1. T cell anti-tumor therapies**

There are several clinical techniques that have been utilized to harness the immune system to treat cancer. **(A)** In some instances, T cells are isolated from the peripheral blood of the patient and genetically transduced to express a chimeric antigen receptor (CAR) or T cell receptor (TCR) that confers the ability to specifically recognize and destroy tumor cells when re-infused into the patient. **(B)** Another technique is the adoptive transfer of anti-tumor T cells that were isolated from within a patient's tumor. Tumor-specific T cells are extracted from resected tumor samples, then expanded *in vitro*, followed by re-infusion into the patient and administration of the T cell growth factor IL-2.

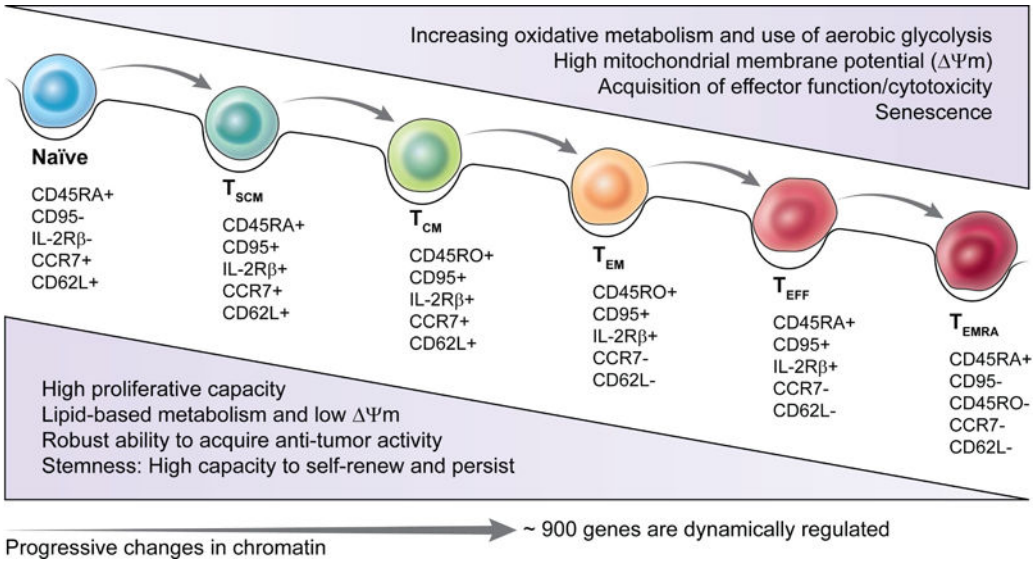


**Figure 2. Alterations in metabolic characteristics accompany T cell differentiation**

Naïve T cells, upon antigen encounter, undergo activation and differentiate into $T_{SCM}$, $T_{CM}$ and effector T cells. This differentiation is marked by the expression of various cell surface markers and transcription factors along with profound alterations in cellular metabolic pathways. In general, T cell activation and differentiation is characterized by increased reliance on glycolysis and mitochondrial membrane potential. These metabolic pathways are critical to mediate effector function of T cells responding to infection and cancer.

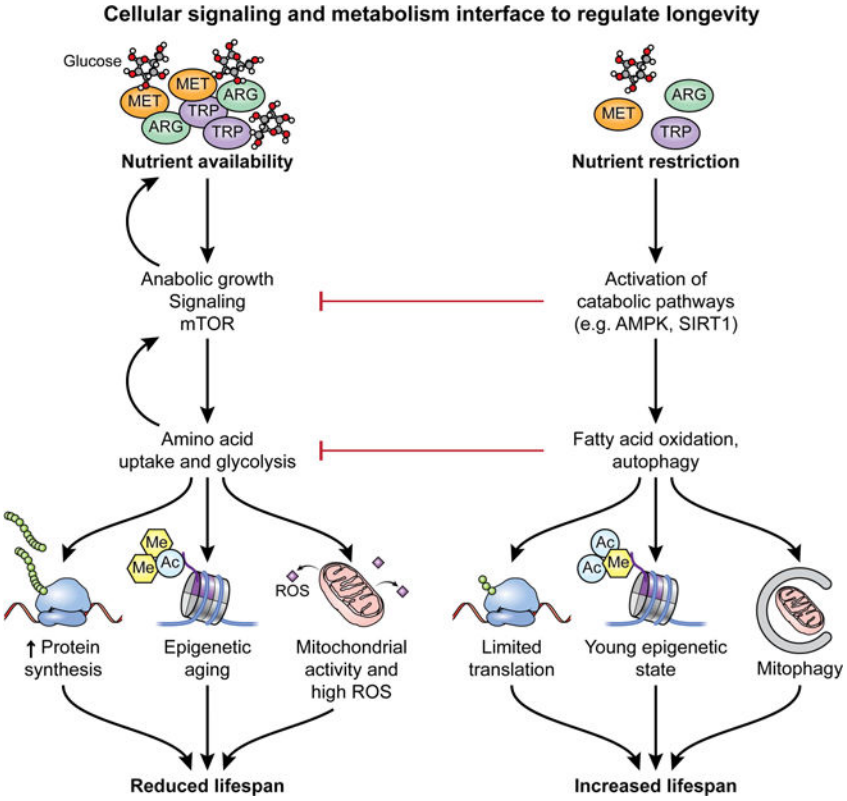


**Figure 3. Cellular signaling and metabolism interface to regulate longevity**

At an organismal and cellular level, the availability of key nutrients such as glucose and amino acids strongly influences longevity. Under conditions of nutrient abundance, anabolic growth signaling pathways including the PI3K-Akt-mTORC1 axis are active, resulting in a feed forward loop promoting greater uptake and usage of nutrients. This ultimately results in increased cell growth through elevated synthesis of proteins and other cellular building blocks. However, increased anabolic signaling is also associated with epigenetic aging and elevated mitochondrial activity and increased ROS production. Ultimately, these factors result in reduced lifespan. On the other hand, nutrient restriction activates catabolic signaling pathways such as AMPK or SIRT1, which in addition to actively inhibiting anabolic growth pathways, also act to promote catabolic metabolism including FAO and autophagy. Catabolic signaling and metabolism limits protein translation and biosynthesis, while promoting a young epigenetic state and increased mitochondrial turnover. Ultimately, nutrient restriction is a key promoter of increased longevity.

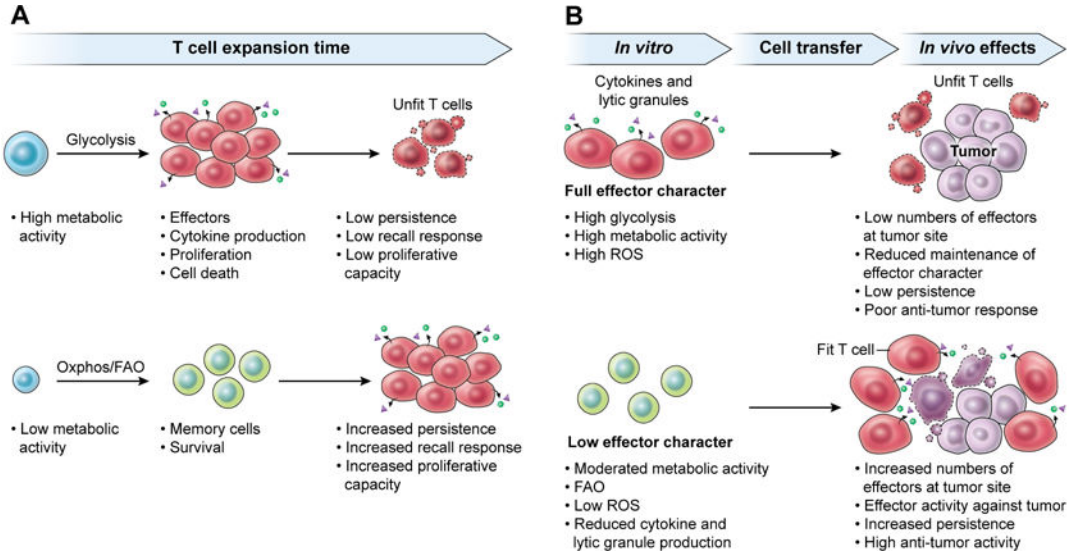


**Figure 4. Metabolism regulates T cell functionality and longevity**

The activity of metabolic pathways in T cells exerts a profound influence on immune function and lifespan. **(A)** In general, T cells that exhibit high metabolic activity, including elevated glycolysis, mitochondrial activity, and anabolic growth activity are characterized by inflammatory effector character. While these cells are capable of high levels of cytokine production and rapid proliferation, they exhibit poor long-term persistence. Conversely, cells that maintain low levels of metabolic activity are favored for long lifespans and memory formation, with the ability to expand and enact robust immune responses at later times. **(B)** The metabolic activity of T cells that are used for adoptive transfer to mediate anti-tumor responses also plays a key role in governing the efficacy of the response. Cells that achieve full effector character, with heightened metabolic activity, during *in vitro* expansion exhibit poor persistence and anti-tumor efficacy upon *in vivo* transfer. However, cells that exhibit moderated metabolic activity during *in vitro* expansion mediate efficacious anti-tumor responses, characterized by increased proliferation, production of inflammatory cytokines and lytic granules and ultimately improved tumor clearance.

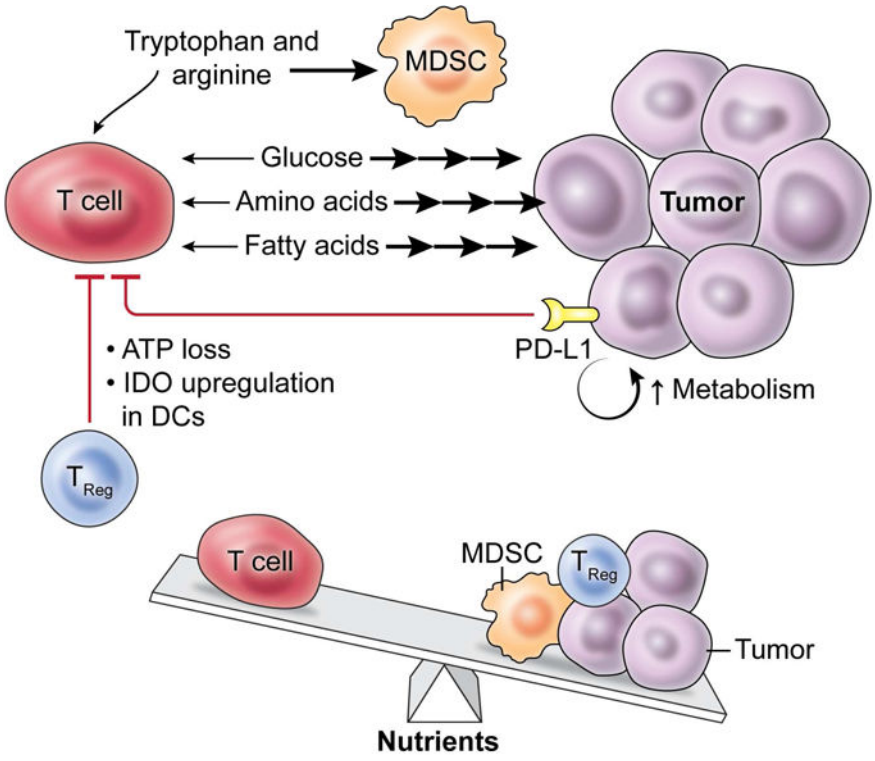


**Figure 5. The tumor microenvironment fosters a struggle for T cells to acquire key nutrients**

At the tumor site, several factors act in opposition to anti-tumor T cells acquiring sufficient nutrients to support immune function. Tumor cells are known to take up large amounts of key nutrients that are required for optimal T cell activity, including glucose, amino acids and fatty acids. Tumor expression of the inhibitory PD-L1 molecule acts to both directly inhibit T cell metabolism and to further promote the metabolic activity of the tumor. Anti-tumor T cells must also manage the impact of tumor-localized suppressive cell subsets such as MDSCs and Treg, which can act to deplete key nutrients such as arginine and tryptophan. Taken together, anti-tumor T cells must face and overcome a challenging metabolic microenvironment in order to successfully mount an immune response against tumors.

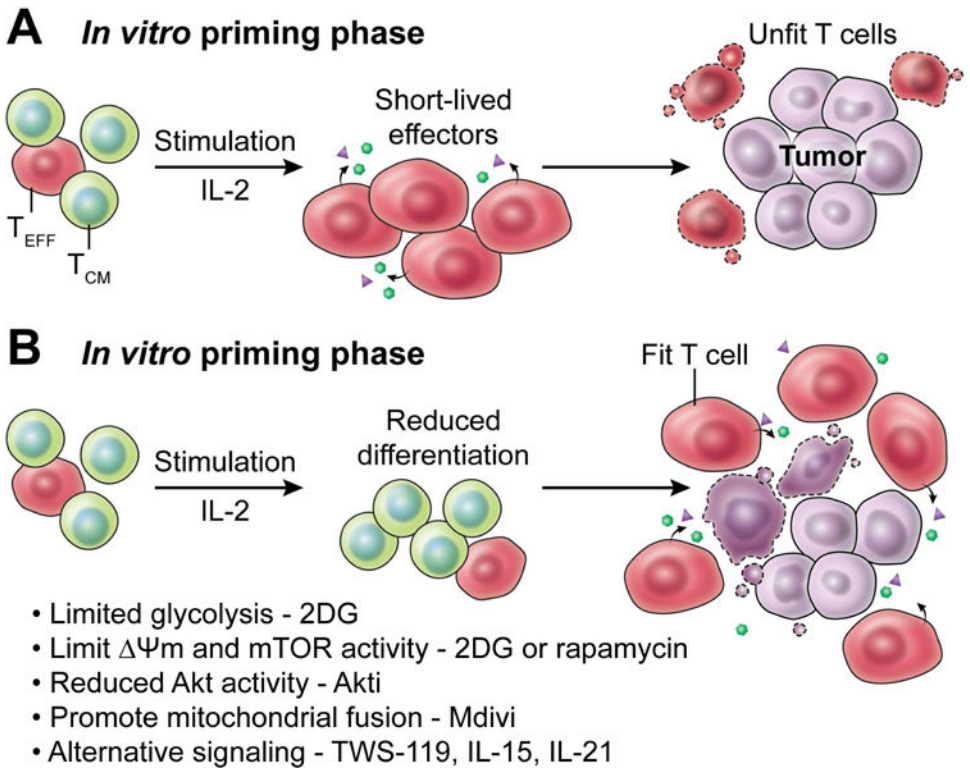


**Figure 6. Modifications to cell metabolism can boost T cell anti-tumor function**

**(A)** The protocols currently used to prime and expand anti-tumor T cells *in vitro* can lead to the generation of terminally differentiated effector cells that are unable to mount a robust response *in vivo* at the tumor site. However, **(B)** alterations to *in vitro* expansion protocols that maintain anti-tumor T cells in a state of reduced differentiation may allow for the generation of T cells that have increased fitness to engage in a sustained immune response against tumors *in vivo*. Adjustments to culturing conditions that have been shown to achieve this in pre-clinical settings include the limitation of anabolic growth pathways including glycolysis, mitochondrial activity and mTORC1 signaling with the glycolytic inhibitor 2-deoxyglucose or the mTORC1 inhibitor rapamycin. Additional interventions include the pharmacological inhibition of Akt activity, the promotion of mitochondrial fusion, and the activation of alternative signaling cascades such as the Wnt pathway that act to maintain T cells in a state of reduced differentiation.