Antitumor T cell reconditioning: improving metabolic fitness for optimal cancer immunotherapy

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Running Title
Metabolism in antitumor Immunity

Abstract
With the rapid rise of immunotherapy for cancer treatment, attention has focused on gaining a better understanding of T cell biology in the tumor microenvironment. Elucidating the factors underlying changes in their function will allow for the development of new therapeutic strategies that could expand the patient population benefiting from immunotherapy, as well as circumvent therapy resistance. Cancers go beyond avoiding immune recognition and inducing T cell dysfunction through co-inhibitory molecules. Recent work has demonstrated that the tumor microenvironment elicits metabolic changes in T cells that dampen their ability to respond and manipulating these metabolic changes can strengthen an antitumor immune response. Here we review the metabolic status of various types of T cells, the energetic state of the tumor microenvironment, and proposed modalities for improvement of immunotherapy through metabolic remodeling.

Introduction
It is now generally appreciated that cancer development and progression has an immune component, and clinically apparent cancer represents a failure of the immune system to destroy developing neoplasia. Thus, a key hurdle cancer cells need to overcome immune surveillance and attack is accomplished through “immunoediting” as well as creating a directly immunosuppressive environment (1). Cancer cells achieve this immunosuppression through the recruitment of immunosuppressive cells (regulatory T cells, myeloid derived suppressor cells) and expression of ligands for co-inhibitor “checkpoint” molecules such as programmed death-1 (PD-1). These co-inhibitory checkpoint molecules bind to their ligands and reduce T cell effector function against cancer cells. The past decade has seen the development of immunotherapeutic modalities targeting this immunosuppression using monoclonal antibody-mediated blockade of these receptor-ligand interactions, allowing T cells to reduce tumor burden. The impressive clinical response initiated a new wave of therapeutic possibilities harnessing the immune system. Currently, there are several US FDA approved antibodies inhibiting CTLA-4 and PD-1, for a number of indications. These include therapies in both treatment refractory and, in some cases, first line patients with melanoma, bladder cancer, advanced NSCLC, advanced renal cell cancer, bladder cancer, Hodgkin’s Lymphoma, and squamous cell carcinoma of the head and neck (2-4). Despite the remarkable results seen in the clinic with immunotherapy, many patients do not have a complete response and most have no response at all. Therefore, a better understanding of T cell biology, specifically in the tumor microenvironment, is needed to expand the repertoire of therapeutic agents targeting T cell function and design better combination therapies. Identifying the mechanisms by which cancer cells escape immune surveillance is currently an expansive field of research in cancer immunology (5-7).

One of these mechanisms is the metabolic landscape cancer cells create, especially in the solid tumor microenvironment. The metabolic state of the tumor microenvironment, such as oxygen levels, acidity, and nutrient availability, plays a critical role in T cell biology, affecting their infiltration, survival, and effector function. Furthermore, these metabolic landscapes can vary between patients of the same tumor type providing a variable environment for immune cells to survive and function which may account for the differential response to immunotherapy. Understanding how the tumor microenvironment metabolic state affects T cell function could be used as a predictor of response, providing a possibility to tailor immunotherapy to each patient as well as develop novel approaches to bolster T cell metabolism to improve current immunotherapeutic modalities.

**Metabolic states during the life of an antitumor T cell**
As naïve T cells specific for tumor antigens first see their cognate peptides, and are primed in the lymph node, proliferate and migrate to the tumor site, detect their antigen in the tumor microenvironment, and experience chronic stimulation over the course of days or weeks, they progress through a number of transcriptionally and epigenetically controlled differentiation states. T cells exhibit distinct metabolic profiles dependent on their activation state, which has been extensively reviewed (8, 9). Briefly, naïve T cells, having a lower metabolic demand, preferentially generate ATP through oxidative phosphorylation (OXPHOS) and fatty acid oxidation (FAO) over glucose fermentation through glycolysis. When T cells get activated they swiftly switch their metabolic programming to support rapid expansion by generating more energy and biomass. TCR signaling activates glucose and amino acid transporters and increases the rate of aerobic glycolysis. Importantly, even though activated T cells predominantly utilize aerobic glycolysis, OXPHOS still occurs (10-12).

Although T cells rely heavily on aerobic glycolysis for proliferation and function, a common misconception is that glycolysis occurs at the expense of mitochondria. T cells still require functional mitochondria for several key metabolic processes. Mitochondrial metabolism goes beyond being the “powerhouse of the cell” and generating ATP. Glycolytic byproducts are shuttled into the mitochondria and used in the TCA cycle for biosynthesis and programmed cell death (13). Mitochondria generate a wide range of biosynthetic intermediates that serve as building blocks for macromolecules. One example is acetyl-CoA, which is generated in the TCA cycle and needed for lipid and fatty acid synthesis. Acetyl-CoA has a critical role in gene expression through histone acetylation and consequently has been shown to regulate IFNγ production (14). Mitochondria can further generate biosynthetic intermediates through glutamine metabolism generating pyruvate and citrate through glutaminolysis, Glutamine is critical for T cell survival and effector function upon activation (15). Furthermore, glutamine metabolism is able to feed the TCA cycle when nutrients are scarce in the case of low glucose levels (16). As previously mentioned, even though activated T cells rely on aerobic glycolysis for activation and survival, OXPHOS still occurs. An important product generated by Complex I and III of the electron transport change is reactive oxidative species (ROS). ROS generated by mitochondrial flux are needed for T cell activation and antigen-specific expansion(17). T cells that lack complex III fail to translocate NFAT into the nucleus resulting in decreased IL-2 transcription (18).

Mitochondrial dynamics can dictate metabolic reprogramming in T cells through changes in mitochondrial fusion and fission where cells are directed towards OXPHOS or aerobic glycolysis, respectively. A balance between fusion and fission controls the mitochondria
positioning within the cell, where mitochondria can be repositioned in areas where energy is needed as well as Ca\(^{2+}\) buffering. Similar to what is observed in neurons, mitochondria are recruited to the immune synapse for T cell activation (19). The positioning of mitochondria to the immune synapse is directed by dynamin-related protein 1 (DRP1), a protein that drives mitochondrial fission which directs the position of mitochondria towards the immune synapse and promotes central supramolecular activation cluster (cSMAC) clustering needed for T cell activation (20). In the context of the TME, tumor infiltrating T cells acquire mitochondrial defects, characterized by an overall loss of mitochondrial mass and consequently a defect in oxygen consumption (21). Importantly, promoting mitochondrial biogenesis improves T cell function and reduces tumor burden demonstrating that mitochondrial integrity is critical for T cell activation and function. Nonetheless, there is a gap of knowledge on the dynamics between mitochondria morphology and positioning within immune cells and how the balance between the two can affect T cell infiltration, migration, and effector function in the tumor microenvironment.

A small proportion of effector T cells after pathogen clearance will become memory T cells. In the case of memory T cells, much like naïve cells, they rely on OXPHOS and FAO for energy production. Importantly, memory T cells have increases in mitochondrial mass and complexity, and therefore harbor an increased spare respiratory capacity (22). This respiratory capacity presumably provides memory T cells with the capacity to quickly generate ATP in the event of a secondary antigen exposure in comparison to naïve T cells (23). Lack of costimulatory molecules during T cell activation causes T cell anergy, producing cells that fail to proliferate and produce cytokines upon antigen re-encounter (24). Co-stimulation, essential for the prevention of T cell anergy, is critical to activate various components of biosynthetic and metabolic machinery (25). Importantly these findings highlight the importance of nutrient availability showing that amino acid and glucose deprivation induces T cell anergy reinforcing the link between metabolism and T cell function.

In addition to “effector”, “memory”, or “anergic” differentiation states, T cells also can enter a dysfunctional state known as T cell exhaustion. First described in chronic viral infections, it is now generally appreciated that T cells in tumors experience chronic activation due to antigen persistence and also become exhausted. Exhausted T cells become dependent on TCR stimulation, fail to effectively lyse target cells or secrete cytokines, and express high and sustained levels of multiple co-inhibitory molecules like PD-1, LAG-3, and TIM-3 (26). A number of recent studies suggests that T cell ‘exhaustion’ is an apt term; these cells are characterized by major metabolic defects driven both through chronic activation as well as the signaling of the co-inhibitory molecules they overexpress. Exhausted T cells expressing co-inhibitory molecules
show a deregulated metabolic profile that may play a role in the degree of response to
checkpoint blockade (26). Co-inhibitory molecule signaling can have a direct effect on metabolic
pathways linked to T cell exhaustion. PD-1 and CTLA-4 signaling inhibit glycolysis and
 glutaminolysis. Distinctly, unlike CTLA-4 signaling, PD-1 signaling can promote fatty acid oxidation, commonly observed in memory T cells (27). Additionally, CTLA-4 interact with PP2A a key phosphatase that inhibits mTOR signaling, a crucial signaling cascade for T cell differentiation and function (28). T cell exhaustion correlates to additional metabolic defects. T cells in the chronic viral infection and in the TME show a decrease in mitochondrial mass and function observed by mitochondrial depolarization, elevated levels of reactive oxidative species (ROS) and low spare respiratory capacity that occurs concomitantly with the progression to an exhausted phenotype (29). Chronic AKT signaling in tumor infiltrating T cells represses PGC1 alpha, a transcriptional co-activator responsible for mitochondrial biogenesis. Consequently, T cells lose mitochondrial mass and the ability to perform oxidative metabolism (21). Taken together, the metabolic state of a T cell goes hand in hand with its activation capacity and differentiation state and consequently their function. This programmed metabolic changes for effective function will become challenged when encountering the tumor microenvironment. Most importantly, understanding how the tumor microenvironment reconditions T cells opens the opportunity to target these changes, invigorate T cell function and potentially improve the response to immunotherapy.

**Metabolic hallmarks of the tumor microenvironment**

While T cells in various functional and dysfunctional states may have altered cell-intrinsic
metabolic profiles, there are several extrinsic factors that can affect T cell anti-tumor response
through metabolic repression in addition to other mechanisms such as the expression of co-inhibitory molecules. T cells that reach the tumor encounter an inhospitable environment with a poor blood supply, nutrient availability, low pH and low oxygen levels. All these aspects of the tumor microenvironment can alter signaling pathways, gene expression, and ultimately modify T cells activation, clonal expansion, and effector function against tumor cells (Figure 1). Here we will briefly describe how the aberrant metabolic state of the tumor microenvironment can have a functional effect on T cells.

**Nutrient availability in the tumor microenvironment**

Both, tumor cells and activated T cells, are required to grow and rapidly proliferate, therefore
they switch to rapid biosynthesis through upregulation of glycolysis over oxidative metabolism.
even in the presence of oxygen (30). During glycolysis, glucose is broken down into pyruvate and subsequently metabolized into lactic acid in the cytosol instead of entering the TCA cycle. Aerobic glycolysis generates ATP more rapidly, as well as supplying metabolic intermediates for biosynthesis such as pyruvate which is converted in acetyl-CoA for the production of lipids, amino acids and fatty acids (rather than used to drive OXPHOS). Ultimately, this increased glucose consumption by tumor cells can cause a glucose deficiency in the tumor microenvironment, with both tumor cells and T cells competing for available glucose for survival and function (31). In an environment with low glucose, T cells have a decrease level of the glycolytic metabolite phosphoenolpyruvate (PEP) which in turn has a negative effect on Ca⁺ flux and NFAT signaling, ultimately diminishing T cell activation (32). Glucose deprivation has also been shown to have a direct epigenetic effect on T cells through the repression of methyltransferase EZH2 expression, which in turn controls cytokine production and provides protection against cell death through Bcl2 expression (33). In the context of immunotherapy, checkpoint blockade can restore glucose in the tumor microenvironment allowing T cells to use glucose for glycolysis and increase INF gamma production (31).

In addition to glucose, T cells need to compete for amino acids such as glutamine, arginine and fatty acids. L-arginine promotes human T cell proliferation and limits T cell differentiation maintaining a more T cell memory phenotype. These cells rely less on glycolysis and promote OXPHOS (34). In the tumor microenvironment, myeloid-derived suppressor cells produce arginase 1 deplete the TME from arginine inhibiting T cell proliferation (35). Glutamine can be broken down into precursors used for the synthesis of amino acids and nucleotides which in turn supports proliferation and T cell activation. Consequently, low levels of glutamine can inhibit T cell proliferation and cytokine production (36). Furthermore, competition for glutamine, in conjunction with HIF1α stabilization, has been shown to affect the synthesis of S-2-hydroxyglutarate, causing an overall change in histone methylation and promoting CD8+ T cell differentiation and effector function (37). Fatty acids and their subsequent oxidation provide intermediates for the TCA cycle which generates citrate for lipid production important for Cd8+ T cell survival and clonal expansion (38).

Unlike effector T cells that become ineffective in a nutrient poor environment, Foxp3-expressing regulatory T cells (Treg cells) an immunosuppressive subset of CD4+ T cells, seem to thrive in the tumor microenvironment. The exact metabolic requirements of Treg cells in the TME are not fully understood but data shows that Tregs rely less on glycolysis, express low levels of glucose transporter GLUT1 and instead rely on lipid oxidation (39, 40). Furthermore, it has been reported that lactate and kynurerine can support the immunosuppressive functions of Treg cells.
These differential metabolic requirements of Tregs allow them to thrive in the tumor microenvironment and suppresses the antitumor immune response (40, 41). Nonetheless, the exact mechanisms of how Tregs support their metabolic demands for immunosuppression in the TME needs further study.

Hypoxia in the tumor microenvironment

A critical metabolic component of the tumor microenvironment is oxygen tension (Figure 1). The uncontrolled proliferation of cancer cells causes tumors to be poorly vascularized, creating a low oxygen or hypoxic environment. Furthermore, these changes in oxygen levels are highly heterogeneous throughout the tumor and among tumors which potentially give rise to a differential response to immunotherapy (42). Oxygen levels can directly affect the metabolism in tumor infiltrating T cells. When oxygen levels are low, HIF1α, a transcription factor tightly regulated by oxygen levels, is stabilized and promotes the transcription of several genes needed for glycolysis. Cells use this mechanism to avoid using OXPHOS in hypoxic conditions. Hypoxia not only enhances glycolysis, it can also affect T cell function by dampening TCR signaling (43, 44) as well as enhancing the presence of immunosuppressive cells such as Treg cells (45). Work is ongoing to further elucidate the effects of hypoxia on T cell function; nonetheless, it is clear that oxygen levels affect signaling pathways involved in T cell metabolism and T cell function. One can conclude that re-oxygenation or improving tumor vascularization could prove a viable avenue for reducing the hypoxic environment in tumors (42, 46, 47).

Acidity in the tumor microenvironment

In addition to lacking fuel sources, metabolic changes such as hypoxia and high rates of glycolysis cause the accumulation of metabolic end-products detrimental for T cell function. Hypoxia and increased levels of glycolysis lead to interstitial accumulation of lactate, protons and CO₂. Cells remove these metabolites in order to prevent acidosis (48). However, some of these mechanisms of removal, including the expression of lactate transporters (MCTs) and the production of carbonic anhydrase IX (CA-IX) for lactate and CO₂ diffusion, cause the accumulation of damaging metabolites that alters the acidity of the tumor microenvironment and diminishes T cell effector function (49-51). One might suggest that stabilizing the nutrient availability in the tumor microenvironment could potentially prove beneficial for T cell function and consequently immunotherapy (52). A recent study demonstrates that reducing lactate
accumulation by inhibiting lactate dehydrogenase A in tumors improves T cell and NK infiltration accompanied by IFNγ production (53).

However, not all tumor cells or type are equivalently metabolically deranged. While deregulated energetics are a new hallmark of cancer, there are many paths by which tumor cells can achieve this metabolic transformation, thus the type and degree of energetic deregulation can vary widely. At the genetic level, tumors acquire mutations that activate oncogenes and inhibit tumor suppressors to support cell growth and proliferation. These genetic alterations can give rise to a wide range of metabolic changes needed to support cell growth (54). The concept of metabolic tumor heterogeneity, where the metabolic landscape of a tumor type can vary from patient to patient as well as within a tumor, is currently an attractive field of research and recent findings have been extensively reviewed (55, 56). Metabolic analysis show that tumors can have different metabolic profiles between patients and within the same tumor (57) (Figure 1). Tumor heterogeneity is an important concept to keep in mind when developing therapeutic strategies. Is the therapy targeting a specific area of the tumor while the rest does not see any response? Is the therapy targeting the tumor as a whole and how can this be monitored? Thus, a better understanding of the metabolic changes that occur in different tumors and their microenvironment is needed to better understand how the tumor immune response would be affected and ultimately be able to better tailor immunotherapeutic strategies. Furthermore, it may be attractive to profile tumor microenvironment metabolism in order to identify how a patient may respond to current immunotherapies.

**Tumor microenvironment metabolic state as a predictor for immunotherapeutic response**

As the field moves forward, it would be beneficial to determine markers and strategies to assess the metabolic state of the tumor microenvironment between patients and assess if these markers can predict response to immunotherapy. Here we will mention a few examples of recent technologies that can be used for tumor metabolic screening (Figure 2).

One of the most common in vivo techniques used for tumor metabolic assessment is positron emission tomography (FDG-PET) which visualizes the aerobic glycolysis being exploited by tumors. FDG-PET is frequently utilized for tumor detection, mainly for staging and restaging in order to guide therapy. It has also been utilized to identify patients that will respond to therapy even before reduction of tumor size. This technology comes with its limitations. In particular, it depends on highly metabolic tumors that will quickly incorporate the glucose analog FDG. Some tumors such as prostate cancer and hepatocellular carcinoma have low metabolic
rates (58). Nonetheless, we can envision the development of improved tracers that can help determine the metabolic state of tumors and guide tumor detection and cancer therapy. One example is the development of PET tracers for the detection of hypoxia in solid tumors. There are currently several PET hypoxia tracers under investigation such as $^{18}$F-FMISO, $^{18}$F-FAZA and $^{64}$Cu-ATSM (59). These hypoxic tracers are being investigated for the assessment of therapeutic response such as radiation therapy (30). This is a small example of a variety of tracers currently being tested which have the potential to assess the metabolic heterogeneity of tumors in vivo (60-62). In the clinic, the implementation of genomics to assess genetic mutations and tailor therapy was a huge step forward for personalized medicine. Similarly, high-resolution metabolomics has advancing rapidly in recent years (63). Metabolomics can diagnose up to 20 inborn metabolic diseases (64). In the context of the tumor microenvironment, metabolomics can be used to assess various metabolites, including glucose, lactate, lipoproteins, and amino acids, which in various ways can modulate T cell function. A wide screen of metabolites can also give information on the state of full metabolic pathways.

Newer methods are being developed exploiting molecules utilized in metabolic pathways. One such method uses an NAD$^+$/NADH sensor called SoNar (65). NAD and NADH are carriers of H+ and e- in major metabolic pathways like glycolysis, TCA, fatty acid synthesis and others. These sensors change their conformation when they bind NAD$^+$ and NADH, emitting a fluorescent signal and can be used to detect the NAD$^+$ and NADH redox state in vitro and in vivo. Interestingly, these sensors can be utilized for high-throughput screening for new compounds that can target tumor metabolism by assessing changes in the redox state. Real time metabolic profiling is a powerful tool used for ex vivo analysis of a variety of cells including lymphocytes using Seahorse technology (21, 66). Seahorse technology, measures two major metabolic pathways (glycolysis and oxidative metabolism) in real time and can be employed to profile small numbers of tumor cells and lymphocytes (67). Assessing the metabolic state of TIL directly from patients could be extremely useful in determining T cell metabolic fitness and the potential response to immunotherapy. Furthermore, Seahorse technology could potentially be used to simultaneously metabolically profile matching tumor samples and TIL, allowing us to correlate the metabolic state of the tumor and immune cells. These are just a few examples among many demonstrating ongoing efforts for developing strategies to assess the metabolic state of the tumor microenvironment, and how the metabolic state can be utilized to develop a more personalized approach to immunotherapy.

Bolstering T cell metabolism to improve function in the tumor microenvironment
As previously discussed, the metabolic requirements T cells need for an effective antitumor response are highly dynamic. T cells require specific metabolic signals for clonal expansion, activation and effector function that are energetically demanding mostly relying on high levels of glycolysis (12, 39, 68), while on the other hand survival and persistence for sustain antitumor response rely on a more oxidative machinery like fatty acid oxidation and OXPHOS resembling a memory like phenotype (22, 69). Therefore, when developing strategies targeting metabolic pathways to improve T cell function it’s important to keep in mind that enhancing a single metabolic pathway over the other might not result in the most optimal antitumor response. Nonetheless, a number of studies have demonstrated the benefit of enhancing the metabolic capabilities of T cells to improve anti-tumor immune response (Figure 3). Enhancing the production of the glycolytic metabolite phosphoenolpyruvate (PEP) through the expression of phosphoenolpyruvate carboxykinase 1 (PCK1) improves T cell effector function and inhibits tumor growth in mouse melanoma models (32). On the other hand, It has been proposed that promoting mitochondrial function increasing OXPHOS and FAO enhances memory T cell presence and function (70). Overexpression of PGC1alpha promoting mitochondrial biogenesis and OXPHOS has shown to improve T cell antitumor activity (21). As an alternative mechanism to induce OXPHOS and a memory like phenotype, the induction of mitochondrial fusion pharmacologically using the DRP1 inhibitor Mdivi-1. T cells treated with Mdivi1 and adoptively transfer into tumor bearing mice mediated a significant reduction in tumor burden (21, 71) (Figure 3A).

Additional metabolic pathways needed for T cell function can be further exploited to improve current immunotherapies. Targeting cholesterol metabolism in T cells using an ACAT-1 inhibitor can improve the response to immunotherapy by inhibiting cholesterol esterification. By inhibiting the enzyme needed for esterification, cholesterol can accumulate in the cell membrane where is needed for TCR clustering and therefore T cell signaling and function. Furthermore, anti-PD1 in combination with ACAT-1 inhibition shows higher efficacy compared to monotherapy (72).

Not only can these metabolic pathways be targeted directly to the tumor but there is a therapeutic window in adoptive cell immunotherapies where T cell metabolic programs can be enhanced ex vivo (Figure 3B). Once these cells are reintroduced in the patient, T cells can function efficiently and achieve clinical efficacy (73). Even though T cells require aerobic glycolysis for full effector function, evidence show that enhancing glycolysis during in vitro expansion results in short lived T cells with poor effector function in vivo (74). Conversely, limiting glycolysis with the use of 2-DG, a hexokinase 2 inhibitor, increased the presence of
memory CD8+ T cells and reduced tumor burden after adoptive transfer. Similarly, restraining AKT activity allowed the expansion of TIL with a memory phenotype and increased the anti-tumor immune response (75). This shows evidence that maintaining a lower metabolic activity, less terminally differentiated T cell in vitro, once they are adoptively transfer these T cells are primed to function better, increase persistence and improve anti-tumor function.

Similarly, chimeric antigen receptor (CAR) T cells base therapies will also present themselves with differential metabolic programs that will impact their efficacy in the clinic. A recent study showed that by promoting mitochondrial biogenesis, higher spare respiratory capacity and fatty acid oxidation resulted in an increase of CAR T cell memory population (76). These metabolic characteristics were predominantly observed in CAR T cells that included a 41BB costimulatory domain compared to CD28. CAR T cell therapy with 41BB showed a better clinical response in CLL in comparison with CAR T cells with a CD28 costimulatory domain (77). One can envision that metabolic programs can be inhibited or enhanced in the process of CAR T cell vector design that may provide antigen redirection as well as a supportive metabolic reprogram.

Nevertheless, T cells still have to endure the tumor microenvironment: there may be a limit on how much bolstering we can do without taking into consideration its nutrient dearth landscape.

**Metabolic reconditioning of the tumor microenvironment to level the playing field for tumor-specific T cells**

As previously discussed, the metabolic state of the tumor microenvironment can have a suppressive effect on T cell effector function and consequently dampen the effects of immunotherapy. Therefore, modifying the tumor microenvironment by making it more permissive for T cell metabolism to properly function could be a viable approach to improve the response to immunotherapy.

An attractive metabolic target that would have an impact on T cell function is modulating the levels of hypoxia in the tumor microenvironment (Figure 3C). As mentioned before, low oxygen levels can initiate a signaling cascade in T cells that causes poor T cell function as well as promoting the presence of immunosuppressive cells. Simply by inducing respiratory hyperoxia through direct oxygenation in a mouse model, reports show a decreased hypoxia in the TME, reduced levels of HIF1alpha and a reduction in tumor growth in response to immunotherapy (78, 79). Pharmacologically, metformin (a type II diabetes drug) reduces hypoxia levels in the tumor microenvironment by reducing oxygen consumption in tumor cells and improves the response to PD-1 blockade even in a tumor model resistant to checkpoint blockade (42). Reduced tumor oxygenation also occurs from poor vascularization. Hypoxic tumors increase the levels of
angiogenic molecules like VEGF in order to promote vascularization, but this results in a disorganized vascular architecture. There are currently several investigational drugs that target tumor vascularization such as VEGF inhibitors. Bevacizumab is currently the only FDA approved VEGF inhibitor for patients with metastatic breast cancer, metastatic colorectal cancer, non-small cell lung cancer, and recurrent glioblastoma, and it is used in combination with chemotherapy (80, 81). While these inhibitors were designed to inhibit angiogenesis and vascularization and promote cell death, a study demonstrated that metered doses of VEGF inhibitors can actually improve tumor blood flow and oxygenation (82). Furthermore, recent studies arising from results observed with immunotherapies have shown that VEGF is immunosuppressive and its levels can be predictive for immunotherapy response (83, 84). One can envision the possibility of utilizing VEGF inhibitors at the right dose to normalize oxygen levels in the tumor microenvironment, allowing T cells to properly function and potentially improve the response to immunotherapy. Further studies are needed for the development of novel therapeutic modalities that can target the hypoxia-HIF1alpha signaling pathway.

To overcome nutrient competition, targeting specific metabolites or supplying the TIL with necessary metabolites can potentially benefit to anti-tumor immune response (Figure 3D). Metabolome analysis identified the reduction of the amino acid arginine in activated TIL. Reintroduction of L-arginine shifted glycolysis to OXPHOS, improved T cell survival and most importantly enhanced anti-tumor effects in vivo (34). Most recently, tryptophan metabolism has received much interest. Indoleamine 2,3 dioxygenase-1(IDO-1) is an IFNγ-induced enzyme that catalyzes the breakdown of tryptophan into kynurenine, this byproduct is detrimental for efficient T cell effector function. IDO secretion by cancer cells can also stimulating regulatory T cells (Tregs) infiltration compromising the anti-tumor immunosuppressive function of T cells. (Figure 3B). Furthermore, Several IDO inhibitors have recently being developed showing promising anti-tumor preclinical results. Two phase I/II clinical trials phase (ECHO-204 and ECHO-202) are investigating Epacadostat in combination with nivolumab and pembrolizumab, respectively, in advanced solid tumors and have shown positive preliminary results in melanoma patients and metastatic head and neck patients, respectively. These results will allow these combination therapies to move into phase III trials in both cancer types. Devising new therapeutic strategies to improve response to immunotherapy is a major goal of the cancer immunology field. These are just a few examples of the wide range of possibilities demonstrating how T cell metabolism in the tumor microenvironment can be exploited and in order to expand the therapeutic options in immunotherapy.
Concluding Remarks:
The development of checkpoint blockade and adoptive cell immunotherapies was groundbreaking, not only because of the impressive therapeutic response but also because it demonstrated that harnessing the immune response can be an effective anti-tumor strategy in a wide range of tumors. Checkpoint blockade therapies, however, focused on only one aspect of T cell biology: their dysfunction induced by ligation of inhibitory receptors. But on the basic side, the field of immune tolerance has revealed a myriad of ways that T cells can be rendered dysfunctional. It is clear that T cells can be both intrinsically metabolically exhausted, but also subject to the dearth energetic conditions of the tumor microenvironment. Here we have highlighted exciting new avenues of basic, translational, and clinical investigation in cancer immunometabolism. These data suggest that the metabolic defects in tumor immunity are central (but, notably, not solely responsible) in maintaining a T cell’s dysfunctional state. Thus, this suggests that a wide array of immunotherapies can be improved by metabolic conditioning. While it can be harrowing to imagine all of the potential pathways dampened by pathologic T cell differentiation or microenvironmental factors, it is also exciting to realize that understanding these pathways may uncover novel methods to manipulate immune cell metabolism and thus enhance the response to these life-saving therapies.

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**Figure Legends**

**Figure 1:** *Metabolic hallmarks of the tumor microenvironment:* The same tumor type can differ from patient to patient. In the TME there is a metabolic heterogeneity in terms of acidity, hypoxia and nutrient availability. These metabolic changes have an impact on the function of tumor infiltrating lymphocytes.

**Figure 2:** *Metabolism markets as predictors for immunotherapy response:* Different technical approaches currently used and in development for the analysis of the metabolic state in the TME. Assessment of the metabolic state of the tumor could predict response to immunotherapy.

**Figure 3:** *Therapeutic strategies to enhance metabolic fitness in the TME:* Different mechanisms by which T cell function could be enhanced by targeting the TME. **A.** Direct T cell metabolic targeting methods by enhancing mitochondrial biogenesis. **B.** Mechanisms to enhance T cell priming during expansion of T cells used for Adoptive Transfer and CAR T cell therapies. **C.** Mechanisms targeting metabolites and nutrient availability in the TME. **D.** Mechanisms to inhibit tumor hypoxia through oxygenation and pharmacological agents. T cell effector (Teff), memory T cells (Tm).
Figure 1: Metabolically permissive vs. Metabolically restrictive conditions in blood vessels.

- **Metabolically permissive**
  - T-cell effector function
  - T-cell proliferation
  - Cytokine production
  - Mitochondrial function

- **Metabolically restrictive**
  - Immunosuppressive Tregs
  - T-cell effector function
  - T-cell proliferation
  - Cytokine production

Key elements:
- Oxygen level
- Acidosis
- Glucose, amino acids, fatty acids
- Blood vessel
- Lactate
- Tumor cells
- T cells
- B cells
- NK cells
- Dendritic cells
- Glucose
- Lactate
- Treg
Figure 2:

Glycolysis

Glucose → G-6-P → F-6-P → 3PG → Pyruvate

Metabolomics

FDG-PET

Metabolomics

Seahorse analyzer

Hypoxia

NAD+/NADH

cpYFP

SoNar

NADH

TCA cycle

ETC

O2

F-FMISO

F-FAZA

Cu-ATSM

Sonication

F-6-P

G-6-P

3PG

Lactate

Pyruvate

Glucose

FDG-PET
Figure 3:

A. Mitochondria biogenesis • PGC1α
Mitochondrial fusion • Mdivi
T-cell function

B. Tumor
TIL expansion
Peripheral blood
In vitro priming
CAR transduction
Mitochondria biogenesis
AKT inhibitors
Mitochondrial fusion Mdivi

C. VEGF inhibitors
Tumor
O2
O2
Hypoxia
Oxygenation
Metformin
PD1 blockade

D. Epacadostat
Treg cells
Tryptophan
IDO
Kynurenine
ACAT-1
L-Arginine
Mitochondrial fusion
T-cell survival
Tumor
Therapy response

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