Review Article

Metabolic Exhaustion of CAR-T Cells in Solid Tumors: Mechanisms, Biomarkers, and Engineering Strategies to Restore Function

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ABSTRACT

Chimeric antigen receptor T-cell (CAR-T) therapy has already achieved a significant clinical effect in hematologic malignancies, yet so far, has not been effective in solid tumors because of the presence of numerous barriers in the tumor microenvironment. Metabolic exhaustion has been one of these factors that has been identified as a critical and underestimated causes of therapeutic failure. This review explores metabolic exhaustion as a key pathway of CAR-T dysfunction in solid tumors and how chronic antigen stimulation, nutrient competition, hypoxia, lactic acidosis, amino-acid depletion, mitochondrial injury and oxidative stress all contribute to decreased cellular fitness. These metabolic stresses slow down proliferation, persistence, polyfunctionality of cytokines, serial cytotoxicity and memory formation, thus leading to ephemeral responses or non-response. The review also notes the bidirectional communication between the signals of metabolic constraint and exhaustion, demonstrating that metabolic inadequacy is incorporated in the overall exhaustion program, as opposed to being an independent malfunction. Moreover, it integrates the existing methods of biomarkers in detecting metabolically exhausted CAR-T cells, such as phenotypic, functional, metabolic, and compartment-specific. Lastly, it assesses the engineering approaches to restore metabolic fitness, including metabolic rewiring, cytokine support modules, transcriptional and gene-editing strategies, hypoxia-adapted CAR designs, and manufacturing strategies to maintain stemness. Altogether, metabolic exhaustion offers a consistent mechanistic framework of CAR-T failure in solid tumors and can be used to provide a rational framework of creating more potent and longer-lasting next-generation cellular immunotherapies.

Keywords: CAR-T cells; solid tumors; metabolic exhaustion; tumor microenvironment; mitochondrial dysfunction; biomarkers; metabolic engineering.

INTRODUCTION

Chimeric antigen receptor T-cell (CAR-T) therapy is one of the most significant innovations in cancer immunotherapy. CAR-T therapy has gained multiple regulatory approvals due to its success in treating hematologic malignancies. CAR-T therapy is now the standard treatment for relapsed and refractory leukemias, lymphomas, and myeloma. However, solid tumors do not have a comparable level of success. CAR-T therapy has inconsistent clinical response in solid tumors and insufficient persistence for durable control. Therefore, the difference in CAR-T therapy response between hematologic malignancies and solid tumors is one of the most significant issues in cellular therapy[1]. Clinically, this gap holds critical importance as solid tumour's comprise the vast majority of the cancer burden. However, patients with advanced solid malignancies currently have no equivalent cellular immunotherapy that can provide reproducibly long-term disease control. Emerging analyses show the gap is not simply the result of poor targeting. It relates to decreasing CAR-T potency after initial tumor contact, especially in tumor microenvironments that are metabolically challenged and limit CAR-T proliferation, persistence, and cytotoxicity[2]. In recent years, numerous overviews have thoroughly described the major issues with CAR-T therapy for solid tumors, such as antigen selection, trafficking, stromal limitations, immunosuppression, and safety. These issues have been important for identifying the hurdles that need to be addressed to successfully translate the therapy. However, reviews that are this broad can promote a mechanistic approach that is backed by an increasing number of experimental and translational studies. These studies suggest that metabolic exhaustion is a primary cause of CAR-T therapy failure[3]. A focused review assessing metabolic exhaustion is warranted as newer studies have provided insight into the relationship between chronic antigen stimulation and metabolic stress. These studies have shown how nutrient deprivation, hypoxia, lactate buildup, mitochondrial dysfunction, and changes in transporter usage result in states characterized by exhaustion, reduced functionality, and decreased persistence. In recent studies, the impact of the aforementioned factors on metabolic stress has demonstrated that, in some cases, the stress can be measured, stratified, and even therapeutically altered[4]. This focus on mechanism is especially appropriate at this point in time as the biomarker space is moving beyond traditional phenotyping. This includes the higher-dimensional evaluation of the fitness of CAR-T, beyond the conventional metrics. With multi-omic assessments, the transcriptomic, proteomic, and cytokine signatures that may provide insights into the functional competence and quality of CAR-T manufacture and the outcome of the therapy, as well as the metabolic state of the CAR-T, become pertinent. The fitness of CAR-T may provide more information regarding the therapeutic failure beyond a biological analogy. The

framework may provide direct implications for the selection of patients and CAR-T optimization[5]. In the framework of the aforementioned context, the focal point of this review is the concept that metabolic exhaustion plays a critical and under-recognized role in the dysfunction of CAR-T cells in solid tumors. Metabolic exhaustion serves as a broad integrative paradigm through which persistent signaling, the challenging landscape of nutrients, and the stress of mitochondria can be understood as converging factors of therapeutic failure in solid tumors, rather than dealt with as separate issues[6]. Therefore, in an orderly manner, this review will analyze the causes of metabolic exhaustion in solid tumor CAR-T cells, the consequences that exhaustion entails for persistence, cytotoxicity, and the capacity to remember and subsequently act, the newly developed biomarkers for the identification of metabolically compromised and/or resilient cellular states, and the engineered solutions that restore lost function, such as metabolic armoring, hypoxia-adapted circuits, and other methods that address the altered mitochondrial and bioenergetics fitness[7]. By maintaining this narrowed focus, the review does not aim to cover all the challenges to solid tumor CAR-T therapy. Rather, it argues that a metabolism-based synthesis can provide a more comprehensive explanation for the dysfunction and more practical guidance around the design of next generation CAR-T cells with the potential to have durable activity against solid tumors[8]. Considering metabolism, the large biological and translational differences between hematologic and solid tumor CAR-T therapy are described in Table 1.

Table 1: Biological and Translational differences between hematologic and solid tumor CAR-T therapy**.**

Dimension	Hematologic malignancies	Solid tumors	Relevance to metabolic exhaustion	Key refs
Tumor architecture	Dispersed disease; easier immune-cell access	Spatially restricted lesions with stromal and hypoxic barriers	Limits infiltration, nutrient access, and repeated productive killing	9
Antigen landscape	Often more uniform lineage antigens	Greater heterogeneity and incomplete tumor eradication	Favors persistent sublethal antigen engagement and chronic signaling	10
Nutrient environment	Less extreme nutrient competition in circulation/lymphoid tissues	Glucose/glutamine restriction, lactate accumulation, acidosis, hypoxia	Drives bioenergetic insufficiency and accelerates exhaustion programming	11
Persistence/ expansion	Robust in vivo expansion and clinically meaningful persistence are common	Expansion and persistence are often limited or short-lived	Weak persistence is both a cause and consequence of metabolic dysfunction	12
Dominant failure mode	Relapse often linked to antigen escape or inadequate durability	Multifactorial failure with TME-driven dysfunction	Metabolic attrition becomes a central unifying mechanism	9
Clinical result	Multiple durable responses and established approvals in blood cancers	Clinical efficacy remains inconsistent in solid tumors	Supports the need for metabolism-focused engineering and biomarker strategies	9

Biological Basis of CAR-T Cell Exhaustion

Definition and hallmarks of T-Cell Exhaustion

T-cell exhaustion is an advanced adaptive stage of T-cell dysfunction characterized by chronic stimulation of persistent antigens seen in cancerous conditions and persistent infection. It does not equate with mere hypo-responsiveness or terminal inactivity. It is a dissociable differentiation stage defined by the gradual loss of terminal effector function, the persistent expression of a range of inhibitory receptors, a modified state of metabolism, and long-lasting changes to the transcriptional and epigenetic landscape. The lack of terminal effector function and the persistent expression of inhibitory receptors are characteristic to T-cell exhaustion, and are not seen in terminal inactivity. During a normal immune response, the temporary expression of inhibitory receptors (e.g. PD-1 and CTLA-4) does not cause irreversible T-cell dysfunction. In acute T-cell activation, the expression of these receptors serves as homeostatic mechanisms to prevent excessive T-cell-mediated inflammation. The response to T-cell exhaustion, however, is a state of persisting and permanent uninhibited signaling. Inhibitory signaling in T-cell exhaustion is characterized by a persistent state of low T-cell dysfunction, reduced secretion of pro-inflammatory cytokines (e.g. IL-2, TNF-α, IFN-γ), and loss of T-cell proliferative and cytotoxic function. Although T-cell exhaustion is characterized by a low state of optimal T-cell performance and is considered to be a persistent low functional state of T-cell activity, it is important to note that T-cell exhaustion is a heterogeneous condition. Understanding this is critical for CAR-T cell therapies, as it provides the point that T-cell exhaustion is a chronic low state of T-cell function that limits the efficacy of T-cell therapies in solid tumors[13].

Distinctive features of CAR-T exhaustion in solid tumors

Although CAR-T cells and endogenous tumor-reactive T cells share some features of T cell exhaustion, the phenomenon of T cell exhaustion is more complex in solid tumors, given the presence of both persistent engagement of the T cell receptor by solid tumor associated antigens, and multiple, CAR-related, stressors. First, in contrast to many of the successful treatments for hematologic malignancies, in which tumor cells in the periphery can be efficiently eliminated, allowing for robust expansion of CAR-T cells, in solid tumors, the CAR-T cells are subjected to continuous and, often, insufficiently sterilizing, peripherally stimulating, engagement by tumor-associated antigens, resulting in prolonged, incomplete tumor-associated signaling. Second, a solid tumor can create a hostile environment, which further enhances T cell exhaustion, including areas of limited oxygen and high acidity, a depletion of nutrients, an accumulation of lactic acid, and the presence of myeloid cells with suppressive phenotypes and inhibitory checkpoint ligands, which reduce the metabolic fitness and the effector function of T cells following tumor infiltration. Third, CAR-T cells are engineered to express receptors that contribute to exhaustion. Multiple lines of evidence show that CAR design also plays a role in T cell exhaustion, and that certain designs, including enhanced tonic signaling and certain configurations of costimulation, are associated with an accelerated state of exhaustion. Consequently, in solid tumors, T cell exhaustion is a highly complex phenomenon caused by persistent stimulation by tumor-associated antigens, a hostile tumor microenvironment, and stressors related to the continuous signaling of the CAR T cell. This is a factor that likely explains the limited expansion, rapid reduction in the persistence, and declining function of solid tumor CAR-T cells, even when there is effective migration and engagement of the tumor-associated antigens. In this context, T cell exhaustion in solid tumors should be treated as a direct and primary challenge to CAR-T cell function[14].

Functional consequences of exhaustion

The functional impact of exhaustion on CAR-T cells makes it relevant to the collapse of key features of CAR-T efficacy against solid tumors. Notably, the expansion of exhausted CAR-T cells is impaired after they come into contact with the target antigen. Consequently, an exhausted CAR-T cell population is incapable of adequately expanding to sustain the population present within the tumor. Exhausted CAR-T cells also downregulate the secretion of effector cytokines (e.g., IL-2, TNF-α, IFN-γ) and this directly diminishes effector function and the ability of CAR-T cells to elicit immune amplification within the tumor microenvironment. There is also the erosion of the cytotoxic function of CAR-T cells, characterized by impaired degranulation, decreased engagement in the killing of target cells and an inability to functionally respond to target antigen under prolonged and harsh conditions of metabolic stress. Consequently, exhausted CAR-T cells are deprived of the long-term goal of tumor immuno-surveillance. Most importantly, exhaustion of CAR-T cells creates a population of cytotoxic effectors that are functionally blunted and unable to sustain interaction with tumor cells. Recent studies have shown that the restoration of metabolic CAR-T cell support, and the reduction of cell exhaustion directly leads to improvements in mitochondrial integrity of CAR-T cells along with increased secretion of cytokines and enhanced cytotoxicity, resulting in significantly improved tumor clearance, with sustained protection from tumor growth. From this perspective, rather than simply being a phenotypic exhaustion of CAR-T cells, it describes the functional collapse of CAR-T cells, which integrates chronic antigen stimulation in solid tumors with lack of CAR-T cell persistence and poor long-term control of solid tumors[15].

Metabolic Determinants of CAR-T Cell Dysfunction in Solid Tumors

Glucose deprivation and nutrient competition

Glucose deprivation is a significant challenge faced in the performance of CAR-T cells when dealing with solid tumors. Tumor cells that proliferate rapidly can maintain high levels of glycolysis, characteristically increasing the transport of nutrients through oncogenic pathways of hyperactive PI3K–mTOR, MYC, and HIF. These pathways enable the aggressive consumption of tumor cell glucose even in conditions that are not limiting in oxygen. This leads to a hypercompetitive landscape of nutrients for effector T cells, translating to a severe disadvantage for these cells that are reliant on glycolysis for activation. Solid tumors exhibit both a competitive landscape and a passive feature of the tumor microenvironment; this is a mechanism of immune exclusion that impedes CAR-T cells and other immune effector cells from carrying out their function. This phenomenon has been demonstrated experimentally through engineered T cells, where the lack of sufficient glucose significantly impaired CAR-T cell activation, proliferation, and effector function. Furthermore, in low-glucose environments, selective upregulation of Glut3 (high-affinity transporter) instead of Glut1 (low-affinity transporter) improves the function and antitumor efficacy of CAR-T cells. This underlies the notion that nutrient competition impairs T cell function, with impaired glycolysis leading to decreased ability to respond to signaling, low levels of cytokine secretion, and lack of persistence in T cells. In this context, the glycolytic disadvantage of CAR-T cells in solid tumors manifests as an earlier, distinct metabolic defect that triggers and perpetuates exhaustion, as opposed to being a coexisting phenomenon[16].

Hypoxia and HIF-driven stress

While glucose competition constrains the potential fuel supply available for CAR-T cells, hypoxia further alters how that limited supply can be utilized. Hypoxic niches comprise poorly perfused regions within the tumor that feature low oxygen tension and place a predominant metabolic strain on infiltrating lymphocytes as they must compete for limited resources. The hypoxia-inducible factor-1-alpha (HIF-1α) appears to be a master regulator of this adaptation by stimulating glycolytic metabolism and inhibiting

mitochondrial oxidant programs. While this response may provide a temporary survival advantage, chronic hypoxia in the presence of persistent antigen stimulation seems to lead more quickly to dysfunction than it maintains long-term effector function. Recent work has linked chronic stimulation under hypoxia to rapid mitochondrial stress and acquisition of exhausted features, indicating that oxygen deprivation cooperates with persistent signaling to push T cells into a hypofunctional state. Hypoxia also compromises tumor control indirectly by reducing intratumoral motility and limiting access to tumor-cell-rich regions; indeed, mitochondrial metabolism has been shown to be critical for T-cell migration in three-dimensional tumor contexts, and hypoxic areas are associated with reduced T-cell movement. In addition, hypoxia can reinforce suppressive differentiation programs in exhausted T cells, including CD39-dependent suppressor features that further blunt antitumor immunity. For CAR-T cells, the net effect is a progressive loss of persistence, impaired spatial deployment within tumor tissue, and a reduced ability to sustain cytotoxic function in the very regions where effector pressure is most needed. Thus, hypoxia should be understood not as an isolated environmental stressor, but as a major organizer of exhaustion-promoting metabolic stress in solid tumors [17].

Lactic acid and acidic microenvironment

The effects of tumoral glycolysis shed far-reaching metabolic consequences that go beyond the depletion of glucose, to include lactic acid accumulation and extracellular acidosis both highly relevant in CAR-T dysfunction. Lactate is now recognized as not just a waste product but rather in fact an immunoregulatory metabolite that can modulate the tumor microenvironment through its effects on pH and also direct T-cell dysfunction. Tumor acidic microenvironments negatively regulate antigen recognition, cytokine production, proliferation, chemotaxis, and cytotoxicity leading to a scenario where CAR-T cells can extravasate into tumors but may not be fully functional thereafter. This finding is especially of note as it reveals that exhausted CD8 T cells gain an enhanced sensitivity to lactic acid by virtue of a changed transporter biology. Studies showed that terminally exhausted T cells uniquely upregulate MCT11 during progression from progenitor to terminal exhaustion, enabling lactic acid flux and rendering these cells hypersensitive to the lactate-rich microenvironment. MCT11 expression is strengthened by chronic TCR stimulation and hypoxia, and its overexpression accelerates functional exhaustion, whereas MCT11 blockade improves polyfunctional cytokine production and enhances long-term tumor control, including in human CAR-T settings. These data are highly informative for solid-tumor CAR-T biology because they show that lactate does not merely suppress effector cells non-specifically; rather, exhaustion alters transporter expression in ways that make dysfunctional cells even more vulnerable to metabolic toxicity. The acidic tumor milieu therefore acts both as a direct brake on effector function and as a selective pressure that stabilizes exhausted cell states [18].

Amino-acid depletion and metabolic restriction

Beyond carbohydrates and oxygen, solid tumors also impose profound amino-acid restriction on infiltrating lymphocytes. Tumor cells, stromal components and myeloid suppressor subpopulations cannibalize or utilize limiting amino acids (glutamine, arginine, and tryptophan), each contributing to T-cell fitness through related but distinct mechanisms. Within activated T cells, glutamine supports anabolic metabolism and mitochondrial function while also sustaining the biosynthetic programs required for effector differentiation and expansion. Arginine is essential for T-cell activation and proliferation, and its depletion by ARG1-expressing myeloid cells adversely affects antitumor immunity. Likewise, tryptophan depletion, frequently due to IDO action, reinforces this suppression by eliminating a key substrate and producing kynurenine that stimulates dysfunction via stress-response and aryl hydrocarbon receptor-linked signaling pathways. Reviews of tumor-infiltrating T-cell metabolism published in the past three years explain that these amino-acid restrictions are not subtle functional modifiers, creating direct impact by directly limiting proliferation, reducing cytokine output and preventing maintenance of biosynthetic competence under chronic stimulation. In the case of CAR-T cells in solid tumors, amino-acid scarcity can be especially deleterious since engineered cells are required to survive multiple rounds of antigen-driven responses while remaining proliferative and cytotoxic in a nutrient-poor microenvironment. This meta-restriction provides a potent form of metabolic restriction where both cellular fuel, building blocks and the signaling inputs for cellular function are provided in parallel thus increasing susceptibility to exhaustion and long-term tumor control loss[19].

Mitochondrial dysfunction and oxidative stress

These upstream substrate constraints converge upon the organelle most intimately associated with long-lasting T-cell viability: the mitochondrion. Mitochondrial integrity regulates much more than just energy supply such as spare respiratory capacity, redox balance, calcium handling, survival signaling, motility as well as the ability to escape terminal differentiation under chronic stress. Mitochondrial dysfunction can ultimately compromise cell life in response to antigen exposure, hypoxia, glucose deprivation, and acidic stress and also Robust metabolic responses to maintain high ATP-generating capacity are compromised by the combination of persistent antigen exposure and these perturbations causing a progressive transition from adaptive bioenergetics to mitochondrial insufficiency. The transition of it is a sign of metabolic exhaustion. Studies by Scharping et al demonstrated that hypoxia-mediated continued stimulation accelerates mitochondrial stress and drives exhaustion, while recent reports confirmed that mitochondrial function is critical for optimal intratumoral T-cell trafficking. CD8 T-cell motility in 3D tumor settings was mediated mainly by glucose and glutamine fueled TCA-cycle, where mitochondrial ATP and mitochondrial ROS functioned as needed complements for migration;

pharmacologic enhancement of mitochondrial activity improved CAR-T infiltration into tumor islets which translated to markedly increased tumor control as demonstrated by Simula et al. However, this does not mean that more ROS is necessarily useful. Tight regulation on mitochondrial ROS level can have a positive effect in activating and promoting motility, while chronic oxidative stress contributes to macromolecule damage, redox imbalance and bioenergetic failure. Such collapse is manifested in CAR-T cells as a diminished capacity to persist, reach the tumor site, produce cytokine and ultimately, undergo terminal dysfunction. Consistent with this, metabolic interventions that promote mitochondrial fitness such as succinate-supported mitochondrial resilience or enhanced glucose acquisition are linked to robust antitumor immunity. So, mitochondrial dysfunction is both the biochemical endpoint of multiple microenvironmental insults and a central mechanistic bridge towards stable exhaustion[20].

Interaction between metabolism and exhaustion signaling

The key conceptual point here is that metabolism does not constitute a parallel problem alongside exhaustion, but rather is integrated into the exhaustion program. Prolonged antigen stimulation, checkpoint signaling, inflammatory stress and metabolic deprivation act as a network that rewires the cellular transcriptome and epigenome landscape of transporters and the bioenergetic state of the cell. Recent data is consistent with a feed-forward model whereby continuous stimulation and hostile tissue environments initially induce metabolic adjustments that then enhance inhibitory and exhaustion-associated programs. The story for MCT11 is indicative: expression is enhanced by transition toward terminal exhaustion, correlates with BATF and TOX occupancy at the locus, and is augmented by persistent stimulation and hypoxia showing that transcriptional circuitry associated to exhaustion can encode directly metabolic vulnerability. On the other hand, then exposure to lactic acid, hypoxia and nutrient restriction exacerbates dysfunction, reduces polyfunctionality and stabilizes the exhausted phenotype. Broader synthesis articles explicitly depict metabolites, cytokines, and checkpoint pathways as convergent regulators of T-cell metabolism together with chromatin and transcription factor networks. This framework goes some way to explaining why single inhibitory receptor blockade in solid tumors often produces only a partial recovery: metabolic insufficiency and epigenetic exhaustion are linked such that refunctionalization becomes less sustainable. For CAR-T cells, this indicates that durable rescue may only be achieved through simultaneous intervention in both signaling and metabolic state. Thus, the biological problem in solid tumors is not simply that CAR-T cells are prevented from proliferating in a nutrient-reduced environment, but rather that the environment increasingly professionalizes them into cells with metabolism and exhaustion circuitry that synergize[12,21]. Table 2 summarises some of these metabolically restrictive signals, which all come together to create a feed-forward exhaustion program.

Table 2: Metabolic determinants of CAR-T dysfunction in solid tumors

Metabolic stressor	Dominant mediators/features	Effect on CAR-T biology	Suggested readouts/biomarkers	Key refs
Glucose deprivation / nutrient competition	Tumor glycolysis, limited extracellular glucose, transporter competition	Reduced activation, proliferation, cytokine production, and serial killing	Glucose uptake, GLUT1/GLUT3, ECAR, viability after rechallenge	11
Hypoxia	Poor perfusion, HIF-driven metabolic stress, reduced oxygen availability	Accelerated exhaustion, impaired mitochondrial fitness, weaker intratumoral motility	HIF-associated signatures, OCR/SRC, intratumoral localization, hypoxia markers	22
Lactate accumulation / acidosis	High extracellular lactate, low pH, MCT11-linked lactate sensitivity	Suppressed cytokine secretion, impaired cytotoxicity, stabilization of terminal dysfunction	Extracellular lactate, pH, MCT11, polyfunctionality assays	23
Amino-acid depletion	Glutamine, arginine, and tryptophan restriction; ARG1/IDO-associated metabolic pressure	Poor biosynthetic fitness, reduced proliferation, diminished effector competence	Amino-acid transporter status, ARG1/IDO context, proliferation and cytokine readouts	11
Mitochondrial dysfunction	Low spare respiratory capacity, depolarization, altered mtROS balance	Weak persistence, reduced migration, impaired tumor access and killing	MitoTracker/TMRE, basal and maximal OCR, SRC, mtROS, antioxidant reserve	12
Metabolism–exhaustion coupling	Chronic antigen signaling, TOX/NFAT/BATF-linked transcriptional remodelling, checkpoint co-expression	Converts transient stress into stable dysfunction	Multiplex PD-1/TIM-3/LAG-3/TIGIT with functional and metabolic assays	24

Functional Impact of Metabolic Exhaustion on Antitumor Activity

Loss of expansion and persistence

A major effect of metabolic exhaustion is the decreased in vivo expansion and persistence which are crucial for long-term CAR-T activity against solid tumors. Upon infusion, CAR-T cells need to sustain proliferation while also retaining a stem-like or memory capable compartment able to replenish effector cells over time. In metabolically challenged tumors, however, chronic stimulation is superimposed on glucose lack, hypoxia, oxidative stress and acidification; conditions which favour dysfunctional differentiation of CAR-T cells over durable self-renewal. Data supporting this claim come from experimental evidence that demonstrates a loss in CAR-T viability (but not cell strength) on exposure to glucose deprivation and more than 30-fold lower expansion in response to an equivalent number of transfused cells whereas metabolic interventions that preserve fitness improve persistence. Experimental Evidence demonstrated that manufacturing-induced metabolic priming reduced glycolytic bias, added central-memory features and enhanced the therapeutic persistence of GD2 TRAC-CAR T cells in vivo while FOXO1-driven reprogramming to promote mitochondrial fitness, stem-like phenotype, and environmental persistence in models of solid tumour. Also, IL-15-autocrine CAR-T cells demonstrated enhanced long-term survival and proliferation, characterized by the presence of a T_SCM-like subset within the tumor microenvironment. Together these data suggest that the suboptimal expansion and short longevity are not merely secondary technical limitations, but rather direct biological consequences of metabolic insufficiency and exhaustion programming[12,25].

Impaired cytotoxicity and cytokine production

Metabolic exhaustion also affects the effector arm of CAR-T therapy through impaired tumor killing and reduced cytokine polyfunctionality. Antigen recognition alone is not sufficient for effective cytotoxicity, as this process goes on to require bioenergetics and biosynthetic capacity required to support degranulation, serial target-cell killing and rapid synthesis of inflammatory mediators. In chronic infection, conventional CAR-T cells become increasingly dysfunctional as they lose effector cytokine production (e.g IFN-γ and TNF), a process that is associated with T cell exhaustion. But recent mechanistic studies revealed that metabolically optimized CAR-T cells maintain these functions better. GLUT1 overexpression enhanced glucose uptake and glycolysis, oxidative phosphorylation, cytokine secretion, and specific in vitro cytotoxicity as well as improved tumor control and persistence in vivo. In a similar way, FOXO1-based reprogramming also retained IFN-γ, TNF and granzyme-associated killing potential upon serial stimulation. On the other hand, MCT11-dependent uptake of metabolites by exhausted antitumor T cells driven in part by lactate impairs effector function and tumor immunity, which shows that metabolically stressed cells do not maintain effective responses in the tumor bed. In this sense, diminished Type 1 IFN and IL-2/TNF-like responses are not just phenotypic signs of exhaustion but rather functional defects that directly suppress the local reinforcement, regulation and implementation of tumoral immunity[13].

Clinical consequence: transient response or non-response

Such cellular defects directly impact the efficacy of therapy for individual solid tumors as occurs in patients. Clinically, CAR-T therapy has established a leading role in hematologic oncology, but no CAR-T product has been approved by FDA or EMA for solid tumors yet; Recent reviews indicate that insufficient expansion, clinical short persistence and diminished durability are preserving aspects within this space. However, this has important implications as trafficking or simple first tumor contact is not the essential step for clinical efficacy. Despite arriving at the tumor, recognizing antigen and initiating signaling, a CAR-T cell that lacks the metabolic capacity to expand, survive and kill multiple times within a hypoxic, nutrient-deprived lactate-rich microenvironment will ultimately also fail. In this situation, the therapeutic course could be presented by a transient radiographic regression, temporary stabilization of the disease or total nonresponse, which is dependent on functional fitness exhaustion kinetics after activation of intratumoral CAR-T cells. Biomarker studies establishing metabolic fitness, memory-state composition, and cytokine polyfunctionality as intrinsic CAR-T features most closely associated with efficacy power this interpretation, while engineering approaches that enhance metabolic competence similarly augment tumor control in vivo. The clinical implication is thus straightforward: metabolically unfit CAR-T cells not only fail to reach tumor sites but also cannot maintain an antitumor program once they do. Thus, metabolic exhaustion provides a mechanistic link between cell-intrinsic dysfunction and the disappointing trend towards incomplete, short-lasting or absent responses that continues to hamper solid-tumor CAR-T therapy [26,27].

Biomarkers of Metabolically Exhausted CAR-T Cells

Tumor microenvironmental biomarker validation in solid tumors reveals that CAR-T cells with exhausted metabolism convey the best information when interpreted as a whole-states rather than individual readouts. In practice, not a single marker reliably describes exhaustion, due to chronic antigen exposure and simultaneous remodelling of phenotype/function/metabolism by tonic CAR signalling, hypoxia/hypercapnia/oxyglycemia (often led by metabolic competition in the microenvironment), and oxidative stress. Thus, a translational biomarker framework should unify surface phenotype, retained effector function, mitochondrial fitness and anatomical compartment from which cells were sampled. The integrated perspective is critical in solid tumors that cannot identify the stress programs active in the tumor microenvironment by blood-based measurements[28]. Table 3 illustrates a working biomarker platform that could help in detecting metabolically exhausted CAR-T cells.

Table 3: Biomarker framework for metabolically exhausted CAR-T cells in solid tumors

Biomarker tier	High-value markers/assays	What it indicates	Best sample source	Key refs
Phenotypic	PD-1, TIM-3, LAG-3, TIGIT co-expression; loss of memory markers; CD39/CD57 where feasible	Chronic stimulation and progression toward dysfunctional states	Tumor biopsy/FNA preferred; blood supportive but incomplete	[9]
Functional	IFN-γ, TNF-α, IL-2 after restimulation; CD107a; serial-kill assays; fold-expansion/Ki-67	Whether phenotype has translated into true loss of antitumor function	Fresh product, paired blood, and tumor-site cells	[29]
Metabolic	Glucose uptake, OCR/ECAR, spare respiratory capacity, mitochondrial potential, mtROS	Bioenergetic competence and rescue potential	Fresh infusion product or viable tumor-site CAR-T cells	[12]
Compartment-specific	CAR persistence in blood plus tumor-local profiling (biopsy, CSF, cavity fluid, aspirates)	Distinguishes systemic persistence from intratumoral dysfunction	Paired blood + local compartment	[30]
Pre-infusion product-quality	T_SCM/T_CM enrichment, stem-like transcriptional programs, favourable pre-infusion states	Predicts durability and persistence potential	Infusion product	[12]
Integrated interpretation	Combine phenotype + function + metabolism + compartment	No single marker captures metabolic exhaustion adequately	Multimodal workflow	[30]

Phenotypic exhaustion markers

A simple phenotypic panel focused on inhibitory receptor expression continues to be the most translationally accessible entry point for potential identification of metabolically dysfunctional CAR-T cells. PD-1, TIM-3, LAG-3 and TIGIT have been found to be amongst the most informative markers when they are co-expressed rather than independently assessed. This distinction is important since PD-1 positivity alone can be present after activation and supports a recent antigen experience, while concurrent upregulation of multiple inhibitory receptors better reflects chronic stimulation and progression to dysfunctional states. Higher burdens of these receptors have repeatedly tracked with poorer expansion, persistence and disease control in CAR-T settings, while reduced expression has been associated with improved product quality. Importantly, receptor profiling could not simply be viewed as a checklist, these phenotypes gain greater significance when considered with differentiation context, as exhaustion in solid tumors frequently occurs alongside attenuated early-memory features and acquisition of stress-associated/pre-terminal phenotypes (e.g. CD57 positivity). In line with this, the most translationally useful phenotypic signature is not "PD-1+ only" but rather a composite profile of multi-checkpoint co-expression plus reduced memory-like identity, which more faithfully recapitulates durable dysfunction and represents a more actionable target for product optimization and on-treatment monitoring [31].

Functional biomarkers

Functional biomarkers identify whether an exhausted phenotype has become a functionally relevant loss of antitumor efficacy. Metabolically impaired CAR-T cells are defined by: decreased polyfunctionality through the decreased production of IFN-γ, TNF-α and IL-2 in response to antigen restimulation; poor proliferative expansion upon successive challenges; and decreased killing ability during repeated target encounter. This is particularly important because receptor-positive cells may remain partially active, and early loss of function often occurs prior to true terminal collapse. The most informative functional package for translational purposes, therefore, fuses cytokine output with a dynamic stress test (eg short rechallenge assays), degranulation or killing readouts and some measure of proliferation e.g. Ki-67, fold-expansion post-repeated stimulation. This approach is further supported by recent CAR-T studies that show interventions improving metabolic fitness similarly rescue cytokine secretion, persistence and tumor control, indicating that preserved function is a primary rather than secondary endpoint in determining metabolic resilience. Functional biomarkers are therefore essential to discriminate reversible metabolic stress from irreversible exhaustion with little likelihood of rescue through downstream engineering or combination therapy[32,33].

Metabolic biomarkers

The hallmark feature of many solid tumors is metabolic dysregulation, which typically leads to bioenergetic limitations underlying CAR-T failure in the tumor microenvironment rather than simply loss of target antigen recognition. The informative highest tier of human metabolism combines mitochondria status, glycolytic capacity, and oxidative phosphorylation potential. Mitochondrial mass is best read in concert with membrane potential (ΔΨm). Increase in mitochondrial mass without conservation of ΔΨm supports proliferation of damaged

organelles, whilst very high ΔΨm can signal glycolytic potentiation and vulnerability to ROS resembling an effector state more than a true durability exercise. Static mitochondrial abundance is therefore less informative than basal oxygen consumption, maximal respiration and spare respiratory capacity. This implies that glycolytic competence should not be characterized as a stable property but rather an inducible one, thus measurements of basal glucose uptake or ECAR-based reserve should be the preferred metric instead of a static resting value, because CAR-T cells often experience exhaustion under repeated metabolic demand. Hence, a feasible translational panel might include Mito Tracker-based mass/potential or glucose uptake assays or GLUT1-related readouts in addition to OCR/ECAR profiling (when possible) and oxidative stress markers like mitochondrial ROS or glutathione status. Recent CAR-T work demonstrates that metabolic biomarkers are mechanistic indicators of rescue potential, enhanced glucose handling can augment ECAR, OCR, spare respiratory capacity, mitochondrial potential and antioxidant defenses while diminishing exhaustion-associated transcriptional programs[20,34].

Translational sampling strategies

The sampling strategy is the most important factor that determines clinical relevance of exhaustion biomarkers. The relatively non-invasive nature of collecting peripheral blood makes it a useful resource for serial monitoring, as we can assess CAR persistence and broader changes in cytokines and circulating analytes easily, and liquid biopsy approaches may be able to detect persisting CAR-T cells at very low frequencies over time. But blood is not the full story for identifying metabolic exhaustion in solid tumors, due to the localized nature of the key pressures leading to dysfunction, such as hypoxia and nutrient deprivation, suppressive myeloid interactions, and spatial exclusion.

This limitation is illustrated clearly in locoregional IL-13Rα2 CAR-T therapy for recurrent high-grade glioma, where cerebrospinal fluid and tumor-cavity fluid displayed marked IFNγ-pathway induction after treatment, whereas serum showed only modest changes; notably, pretreatment intratumoral CD3 density also correlated with survival. For solid-tumor CAR-T trials, the most informative design is therefore paired-compartment sampling: blood for feasibility and longitudinal tracking, together with at least one local specimen, such as fresh biopsy, fine-needle aspirate, resection tissue, cavity fluid, or CSF when anatomically relevant. Analyzing these paired specimens with multiparameter flow cytometry, transcriptomics, or spatial profiling yields a far more faithful picture of whether CAR-T cells are merely present systemically or are actually becoming metabolically incapacitated within the tumor niche [35].

Engineering Strategies to Overcome Metabolic Exhaustion

Because metabolic exhaustion in solid tumors is driven by persistent antigen signaling, nutrient deprivation, hypoxia, adenosine accumulation, and chronic inflammatory stress, restorative engineering must go beyond increasing short-term cytotoxicity. The most promising modern strategies aim instead to build CAR-T cells that enter the tumor in a less differentiated, bioenergetically economical state, retain the capacity to switch into high-output effector metabolism only when needed, and receive support signals in a spatially restricted manner that limits systemic toxicity. In this sense, current CAR-T engineering is moving from simple activation enhancement toward programmable metabolic durability[36]. Representative engineering strategies that restore metabolic fitness and their main trade-offs are summarized in Table 4.

Metabolic rewiring of CAR-T cells

As metabolic rewiring directly targets the bioenergetic bottlenecks that underlie terminal dysfunction, it is taking center stage as the most immediate approach to restoring optimal CAR-T fitnes. One of the central design principles is that the best CAR T cells should not be constitutively hyperglycolytic at baseline preserving a stem-like oxidative state prior to infusion with acquisition of inducible glycolysis and biosynthetic activity after tumor engagement. This principle is demonstrated through FOXO1-based engineering. In solid-tumor models, mice with FOXO1-overexpressing CAR-T cells enriched a more stem-like population when FOXO1 expression was appropriately tuned in such a way as to improve mitochondrial fitness, maximal respiratory capacity and spare respiratory capacity while also mitigating features associated with exhaustion but maintaining their effector functionality after restimulation [12].

Complementary approaches attempt to reprogram nutrient access at the tumor core. Selective metabolic refuelling with ADA1 and D26, utilize hypoxic adenosine-rich tumor niches to convert immunosuppressive adenosine into inosine and a fuel source which enhances CAR-T persistence and anti tumor function via diminished metabolic inhibition [44]. More broadly, findings from recent work suggest that metabolic engineering should be assessed on flexibility rather than absolute pathway activationccas durable cells are those that sustain mitochondrial competence, antioxidant reserves and the capacity to engage glycolysis at will - not fully committed to sustained anabolic drive. This difference is particularly relevant for solid tumors in which premature overcommitment to glycolysis can spur differentiation and collapse in conditions of nutrient deprivation. In this regard, we should consider metabolic rewiring as means for flexible energy maintenance throughout the whole CAR-T life cycle (e.g., during manufacturing and tumor residence) rather than a simplistic modality of “healthier” metabolism raised in abstract[45].

Table 4: Engineering strategies to restore metabolic fitness in solid-tumor CAR-T cells

Strategy	Representative approach	Mechanistic rationale	Expected benefit	Main limitation / risk	Key refs
Metabolic rewiring	GLUT3 overexpression	Improves low-glucose uptake under nutrient competition	Better activation, proliferation, cytokine output, and tumor control	Could increase anabolic demand if not balanced	37
Metabolic rewiring	FOXO1 overexpression	Preserves stem-like identity and mitochondrial fitness	Better persistence, stemness, and efficacy in solid tumors	Over-bias toward quiescence may blunt immediate effector output if not tuned	12
Alternative fuel support	ADA1 + CD26 refuelling	Converts suppressive adenosine into inosine and supports CAR-T metabolism	Improved function in adenosine-rich TME	Complexity of construct design and metabolic context dependence	38
Cytokine armouring	IL-15-armoured GPC3 CAR-T	Enhances survival, expansion, and intratumoral persistence	Stronger expansion and response signals in solid tumors	Higher CRS risk; requires safety control	39
Cytokine survival module	Constitutive IL-7 receptor (C7R)	Supports cytokine-independent survival and persistence	Improved activity in pediatric CNS tumor setting	Neuroinflammatory toxicity/CRS must be monitored	40
Transcriptional reprogramming	BATF/IRF4 reinforcement	Counteracts exhaustion-associated transcriptional drift	Better tumor persistence and function	Biological effect depends on interaction balance and construct context	41
Transcriptional reprogramming	c-Jun overexpression	Restores AP-1 activity and exhaustion resistance	Better proliferation and sustained function	Potential signaling overactivation if not controlled	42
Hypoxia-adapted design	Hypoxia-sensing / hypoxia-responsive CARs	Restrict or optimize CAR activity in low-oxygen tumor zones	Improved selectivity, reduced exhaustion, better solid-tumor efficacy	Depends on reliable hypoxia sensing across heterogeneous tumors	43
Gene editing	MED12/CCNC targeting; CRISPR CELLFIE hits such as RHOG/FAS/PRDM1	Reprograms persistence, apoptosis resistance, and fitness pathways	Better expansion, function, and in vivo efficacy	Manufacturing/regulatory complexity for multiplex editing	38
Manufacturing optimization	Memory-preserving, less glycolysis-biased expansion conditions	Avoids pre-infusion terminal differentiation	Better post-infusion persistence and metabolic flexibility	Requires standardization in GMP workflows	9

Cytokine support modules

Another competing solution is to put sufficient cytokine support right into the CAR-T product, thereby alleviating entirely reliance on the hostile tumor microenvironment for long term persistence and metabolic maintenance. The reasoning is not complicated: Homeostatic cytokines (e.g., IL-7 and IL-15) promote survival, memory maintenance, mitochondrial integrity, and long-term proliferative capacity while dependency on exogenous cytokines or pure dominance of IL-2 driven expansion can steer cells to a terminally differentiated fate. The clinical translation of constitutively active IL-7 receptor signaling has already begun with C7R-engineered GD2. CAR T cells to provide a microenvironment for survival independent of cytokines in pediatric CNS cancers [46,47]. Similarly, IL-15 armoring has also been advantageous specifically in solid tumors. In a first In-human study of GPC3 CAR-T cells, IL-15 co-expression increased cell expansion, intratumoral survival, and antitumor activity, indicating that cytokine armouring can meaningfully improve the persistence problem that has limited solid-tumor CAR-T therapy[48]. Preclinical work with T cell-restricted IL-15 and IL-21 similarly suggests that cytokine support is most effective when it is cell-confined and cooperative rather than systemic, because that architecture promotes fitness while reducing the risk that broad cytokine exposure will trigger uncontrolled

inflammation. Thus, the most translationally attractive cytokine modules are not simply "stronger" cytokine programs, but spatially and quantitatively restrained circuits that reinforce memory and metabolic resilience without converting the product into a constitutively inflamed cell state [49].

Gene-editing and transcriptional reprogramming

Gene-editing and transcriptional reprogramming approaches are increasingly designed around a mechanistic goal: shifting CAR-T cells away from fixed exhaustion programs and toward a state that can preserve memory features while repeatedly reacquiring effector function. A major axis of this effort is to target the transcriptional imbalance induced by chronic CAR stimulation. BATF and IRF4 Counteract NFAT-Dominated Exhaustion Programs in Tumor-Infiltrating CAR-T Cells to Shape a More Functional Transcriptional Landscape Under Chronic Stimulation (Jain et al., 2023). Also, c-Jun-based reprogramming has revealed that restoration of AP-1 activity enhances cytokine production, costimulatory molecule expression, proliferative fitness and tumor control (but not always with the simple reduction in canonical inhibitory receptors which operates depending on the tumor context [50].Extending this concept, FOXO1 engineering imposes a regulatory program associated with memory that retains stemness and mitochondrial competence without irreversibly blocking activation-induced effector differentiation if expression of the protein is appropriately calibrated [12].Now, extending this strategy systematically is enabled by CRISPR-based discovery platforms. SCREENING TOOLS CELLFIE screens identified perturbations that enhance CAR-T performance across clinically relevant dimensions (RHOG, FAS and PRDM1 loss) while previous work showed that disrupting either MED12 or CCNC can improve CAR expansion, metabolic fitness and the ability to control tumour's [51]. Together, these studies suggest that the next generation of CAR-T editing will no longer be cantered on one or two individual "magic" genes, but rather rationally edited circuitry controlling exhaustion entry, metabolic restraint, and transcriptional plasticity when faced with chronic tumour stress [52].

Hypoxia-adapted or microenvironment-responsive CAR designs

Well-conditioned CAR-T cells can also fail if they are designed with an assumption of a permissive environment that is not present in solid tumors. This has inspired hypoxia-adapted and microenvironment-responsiveness CAR frameworks that preferentially initiate function in hostile tumor supportive niches. This is nicely illustrated by the development of hypoxia-sensing CAR systems that transform an inherent obstacle of solid tumours into a triggering signal. In preclinical models, hypoxia-sensing CAR-T cells limited CAR expression in low-oxygen tumor areas, enhancing tumor specificity and decreasing off-tumor toxicity even with broadly expressed antigens[53]. More recently, hypoxia-responsive designs have also been associated with less exhaustion and enhanced proliferation as well as beneficial metabolic reprogramming compared to conventional CAR-T cells; in need of the possible common denominator of environmental gating tuned similarly to safety and fitness instead of only antigen discrimination [50].Similar designs are now starting to incorporate other metabolic information from the tumor such as glutamine availability in order for sustained function under nutrient stress [54]. A further application of this was localized immunomodulation, as in the case of CAR-T directed to deliver PD-L1-targeted cytokine fusion proteins that act preferentially in the tumor environment. In addition to enabling control over the local and systemic immune contexture, we found that αPD-L1–IL-12 CAR-T cells were superior in enhancing infiltration into solid tumors and tumor control whilst also reducing toxicities associated with systemic exposure to IL-12 when compared with less spatially constrained cytokines [55] in prostate and ovarian cancer models. These strategies are particularly appealing for the treatment of solid tumors since they link functional potency to contextual sensing, thus minimizing the potentially excessive metabolic burden and toxicity associated with non-targeted protein stimulation [56].

Manufacturing strategies to preserve stemness and fitness

In addition, due to how much of metabolic exhaustion is pre-programmed before infusion, manufacturing represents a true engineering lever (not just a logistical step). Less differentiated T-cell states, particularly enriched for T memory stem cell and central memory-like subsets have repeatedly been shown to persist more effectively long-term than heavily differentiated products. Therefore, contemporary manufacturing increasingly seeks to maintain stemness by reducing culture time in vitro, limiting over-activation and replacing traditional IL-2-dominant expansion conditions with combinations of cytokines such as IL-7, IL-15, or IL-21 that more effectively preserve memory potential [57]. These conclusions are further supported by studies into metabolic conditioning, which demonstrate that T cells can be pushed through a metabolically compromised and glycolysis-biased state during standard expansion conditions before they even see tumour. Redirecting glucose flux during ex vivo expansion thus presents as a ready opportunity to produce superior epigenetically and metabolically competent cells with enhanced mitochondrial capacity and function post-infusion [58]. Mechanistically, manufacturing should strive to generate CAR-T cells that are oxidative and developmentally plastic at baseline, not fully "activated" products that look powerful in very short-term assays but had already taken a step toward exhaustion. Even in the absence of these, a key challenge for the field lies in reconciling the conditions that preserve such stemness into scalable manufacturing pipelines without compromising on reproducibility, release testing or dose precision.

Limits and safety trade-offs

Despite such characteristics, more effective metabolic engineering does not necessarily yield better clinical therapy. Consequently, any manipulation can augment

inflammatory toxicity if it improves persistence, cytokine responsiveness or tumor-site accumulation. This has been demonstrated with cytokine-armoured approaches: GPC3 CAR-T cells expressing IL-15 are capable of greater expansion and intratumoral activity, but at the cost of increased incidence of cytokine release syndrome (CRS) (feasible managed by use of IL-1/IL-6 blockade or safety-switch activation)[48]. Similarly, C7R-GD2. However, CART cells also illustrated the clinical benefit of IL-7 support as well as eliciting neuroinflammatory toxicities that needed intensive treatment in the CNS [59]. Second, it is important to note that not all metabolic changes predict lasting clinical efficacy as tumor exhaustion in solid tumors represents only one component of an overarching systems level problem coupled with antigen heterogeneity, poor trafficking, stromal exclusion and tolerogenic myeloid networks. Third, some programs require both fine tuning and maximum activation. The FOXO1 study demonstrated a similar relationship, with active FOXO1 increasing stemness but initially reducing human effector cytokine production, highlighting the importance of locally calibrating gene expression since significant shifts toward quiescence may temporarily prohibit effective tumor killing[12]. Lastly, rapid or extreme manufacturing may maintain stemness; however, it is at the cost of quality control and infusion-dose standardization. These and other such considerations are driving an evolution of the field towards tunable, conditionally regulated safety switches paired with more multidimensional release criteria that assess not just potency but also developmental state, metabolic plasticity, and toxicity risk.

Translational and Clinical Implications

Metabolism as a Key Trial Endpoint

Metabolism is most appropriate as a formal endpoint in solid-tumor CAR-T trials since data limited to objective response rate does not sufficiently address whether an administered product was able to attain durable biological fitness in vivo. Bioactivity can be apparent even in early-phase studies without evidence of sustained, clinically meaningful benefit, suggesting that a hallmark of antitumor activity does not delineate a metabolically competent treatment. While half of evaluable patients treated with IL-13Rα2 CAR-T in the phase I trial for recurrent high-grade glioma achieved stable disease or better, most informative correlates of benefit were neither simply radiographic nor biomarker-driven per-compartment: intratumoral CD3+ T-cell abundance, product fitness and CNS cytokine induction aligned with outcomes cogently suggesting that cell state and local immune activity are major determinants of efficacy. Likewise, single-cell multi-omic profiling of clinical CAR-T products has shown that durable persistence is strongly linked with pre-infusion functional programs, reaffirming the more general idea that the biology of the cell product dictates therapeutic success rather than response rate alone. This means trials of solid tumors should prospectively assess metabolic fitness as a mechanistic endpoint in parallel with safety and efficacy since persistence, adaptability under nutrient stress, and resistance to exhaustion are likely better surrogate measures of durable benefit than radiographic response evaluated independently [60,61].

Integrating Metabolism-Focused Engineering with Complementary Strategies

Engineering focused on metabolism alone is unlikely to succeed if not integrated with strategies addressing trafficking and extrinsic suppression in the tumor micro-environment. A CAR-T cell can be metabolically fitter, but will still fail to perform if it cannot reach tumour nests, penetrate the stroma or maintain function against suppressive myeloid cells, hypoxia and checkpoint signalling. This is why contemporary next-generation strategies are more and more combining fitness-sustaining designs with chemokine receptor matching, locoregional delivery, cytokine armoring, checkpoint-resistance modules or microenvironment-reprogramming methods. In translational terms, metabolic engineering should be considered the intracellular half of a two-part problem, where trafficking and tumor-site remodeling tackle the other anatomical-ecological half. The practical consequence for trial design is that combination products should be favored when the principal obstruction is unambiguously multifactorial, an obvious approach with poorly infiltrated or highly fibrotic tumors where restoration of mitochondrial or glycolytic competence alone will not result in lasting tumor control [6,62].

Key Measurements for Future Trials

Future trials should advance toward a multi-dimensional biomarker paradigm that will inform whether CAR-T cells persist, and function at the tumor site, remain metabolically competent and avoid transiting to fixed exhaustion states. At a minimum, persistence should be assessed serially in blood and if pathology-appropriate, also in nearby tumor somatic compartments (e.g. cerebrospinal fluid, cavity fluid or repeat biopsy specimens) because local sampling can show treatment biology that the peripheral blood does not capture. Tumor-site function should be evaluated through evidence of local immune activation, including regional-specific cytokine induction and direct demonstration of CAR-T distribution or expansion in the relevant tissue compartment. Metabolic monitoring is another area that warrants more careful thought, as we will eventually need feasible surrogate readouts of mitochondrial fitness (i.e. oxidative capacity and nutrient utilization), stress adaptation etc., instead of just conventional phenotyping. Third, the link between exhaustion and a lack of functionality could be further strengthened through combining inhibitory receptor co-expression with transcriptomic or high-dimensional immune-state analyses since durable responses are associated with products that maintain memory-like features and resist terminal dysfunction. The future trial schema that can be set up most informatively in practical terms will be one which integrates pre-infusion product profiling with longitudinal post-infusion compartment-specific sampling, sequentially

associating manufacturing quality and in vivo persistence, local function and metabolic state, to clinical outcome within the same overall study design [63,64].

CONCLUSION

In conclusion, this review highlights metabolic exhaustion as a common and central feature of CAR-T cells that leads to poor efficacy in solid tumors. Instead of representing a downstream result of treatment failure, metabolic stress is identified as a mechanistic axis connecting chronic antigen stimulation, tumor ecology in adverse environments, injury to mitochondria and progressive loss of effector competence. Glucose restriction, hypoxia, lactic acidosis and depletion of amino-acids synergize to reprogram CAR-T cells into a reversible state associated with reduced proliferation capacity, impaired production of cytokines and cytotoxicity, compromised persistence, and loss of memory potential. Thus, limited success of CAR-T therapy in solid tumors can be interpreted not only as a problem of antigen recognition or tumor infiltration but mainly as an inability to maintain cellular fitness after engaging the tumor. This framework also elucidates how the field will move into translation. The best of those advances preserves developmental plasticity prior to infusion, maintain mitochondrial resilience in the tumor microenvironment, and link effector activation to contextual environmental cues rather than constitutive hyperactivation. Thus, future advances will rely on a comprehensive biomarker approach that integrates phenotype, function, metabolic competence and tumor-site activity with clinical trial designs in which both metabolic fitness and persistence are treated as formal endpoints. In summary, restoring metabolic fitness should be considered not simply as an optimization strategy, but rather as a prerequisite to establishing CAR-T therapy into a robust and clinically efficacious therapeutic platform against solid malignancies.

Author Contribution Statement:

Balakoti Erothi has performed the literature review, data analysis and drafted the original manuscript.

Sai Janardhan has provided supervision, critical insights and reviewed the final manuscript.

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