



**INTERNATIONAL JOURNAL OF
PHARMACEUTICAL SCIENCES**
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: <https://www.ijpsjournal.com>



Review Article

Gut Microbiota Derived Short-Chain Fatty Acids as Immunometabolic Modulators of CAR-T Cell Therapy in Non-Lymphoma Cancers.

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ARTICLE INFO

Published: 06 May 2026

Keywords:

CAR-T Cell Therapy, Gut Microbiota, HDAC Inhibition, Solid Tumours, Short-Chain Fatty Acids, Tumour Microenvironment.

DOI:

10.5281/zenodo.20055146

ABSTRACT

By employing chimeric antigen receptors (CAR)-engineered T cell therapy, the treatment of haematologic malignancies has been revolutionised, introducing a personalised, durable immunotherapy strategy. However, due to the challenging impediments of the tumour microenvironment (TME), such as metabolic blockade, physical sequestration, and T cell exhaustion, the therapy's outcome against solid tumours remains limited. Meanwhile, recent studies have shown that the gut microbiome is a key factor in modulating overall immunity, and its metabolites have a primary impact on the therapeutic outcomes. This review integrates these two areas, highlighting the pivotal role of short-chain fatty acids (SCFAs), particularly butyrate and propionate, as highly potent immunometabolic mediators that could augment the efficacy of CAR-T cells. We depict their biochemical contents, pharmacokinetic characteristics, and mechanism of action, which involve tissue homing, the induction of memory phenotypes, and the regulation of the mTOR pathway and histone deacetylase inhibition. Alongside, evaluations show that butyrate and propionate both produce cooperative and antagonistic influences on T cells. Finally, we mention translational methods such as ex vivo CAR-T conditioning and in vivo microbiome modulation to employ SCFAs as metabolic adjuvants to enhance the efficacy and stability of CAR-T cell therapy against solid tumours.

INTRODUCTION

1. The Paradox of CAR-T Cell Efficacy: Advances in Lymphoma Versus Tribulations in Solid Cancers

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



The concept and clinical development of chimeric antigen receptor CAR-T cell therapy is probably one of the most notable stories in the field of translational oncology. It is a story that highlights the fusion of synthetic biology, immunology, and cellular engineering.¹ This treatment approach is based on the genetic reprogramming of the patient's autologous T lymphocytes that express a synthetic receptor. This receptor is a perfect combination of the antigen, binding specificity of a monoclonal antibody single-chain variable

fragment (scFv) and the strong, intracellular signalling domains typical of T cell activation, generally CD3 and one or more co-stimulatory molecules such as CD28 or 4, 1BB.² Once infused into the patient, the modified lymphocytes initiate a targeted attack against tumour cells expressing the same tumour-associated antigen recognised by the antibody. They even acquire the ability of a massive clonal expansion and long-lasting immunological memory.

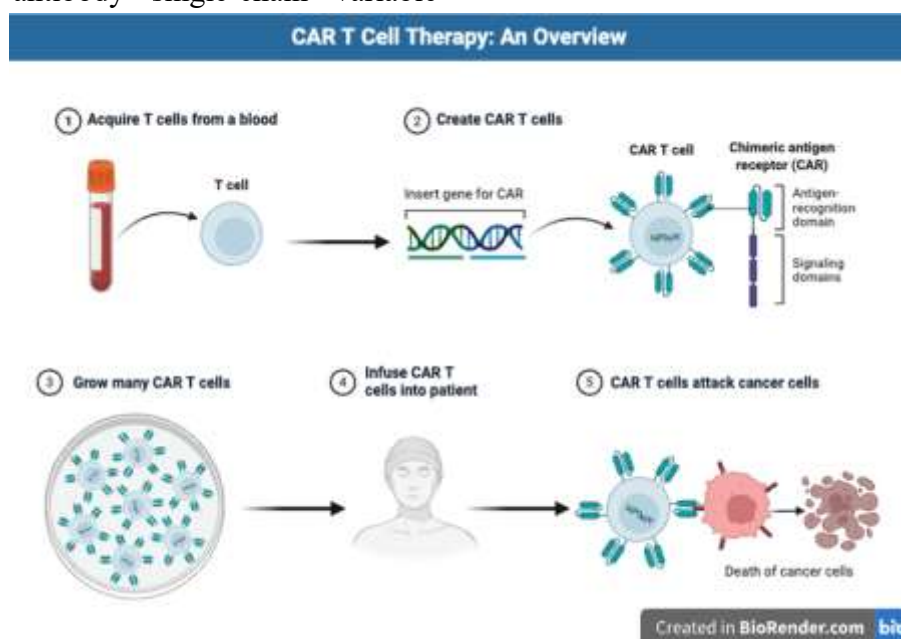


Figure 1: Overview of CAR T-Cell Therapy

The clinical validation of this strategy was clearly demonstrated in relapsed/refractory B-cell acute lymphoblastic leukaemia (ALL) and diffuse large B-cell lymphoma (DLBCL), and drugs like tisagenlecleucel and axicabtagene ciloleucel recorded complete response rates of 40, 80% in very heavily pretreated patients, thus facilitating their historic regulatory approvals.³ The successful outcomes, thus, fundamentally confirmed the great concept that the human immune system can be externally modified to achieve long-lasting cancer control.⁴

Nevertheless, the picture becomes more challenging when the concept is extended beyond

liquid tumours to the broad and highly heterogeneous group of solid tumours, including lung, breast, colon, pancreatic, and prostate carcinomas, as well as glioblastomas and sarcomas. The delivery of CAR-T efficacy in these types of malignancies has been disappointingly slow, with a glaring lack of the spectacular, long-lasting remissions that have been the hallmark of haematological settings.⁵ The difference in efficacy between the two settings is so pronounced that the explanation of it not being by chance is pretty much self-evident, the main reason being the very different biological characteristics of the leukaemias/lymphomas living in the circulatory, lymphoid, or bone

marrow niches and the architecturally complex, three-dimensional tissue structures of solid tumours.⁶

The latter is a real stronghold, basically one that, through evolutionary selection, has been built to escape immune surveillance, and as such, one of which it does this is by showing sequential and often synergistic barriers at the CAR- T cells, so that collectively these constraints kill the CAR- T cells' therapeutic potential.⁷ The first and very often the final hurdle of the treatment is a problem of physical access and tumour homing. Malignant B cells can move, circulate freely and also be found in well-vascularized lymphoid organs. At the same time, solid tumours may have aberrant

and dysfunctional vasculature: their blood vessels have a complex, chaotic architecture, blood flow is intermittent, there is high interstitial fluid pressure, and these characteristics can be observed in combination.⁸ The above mentioned defective vasculature blocks not only the extravasation of CAR- T cells administered intravenously, but also, by developing a hypoxia and nutrient deprivation gradient from the outside to the inside of the tumour, furthermore worsens the tumour microenvironment.⁹ Besides, the majority of solid tumours are covered by a dense, highly cross-linked extracellular matrix (ECM) laden with collagens, fibronectin, and hyaluronic acid, all of which are products of the cancer-associated fibroblasts (CAFs).¹⁰

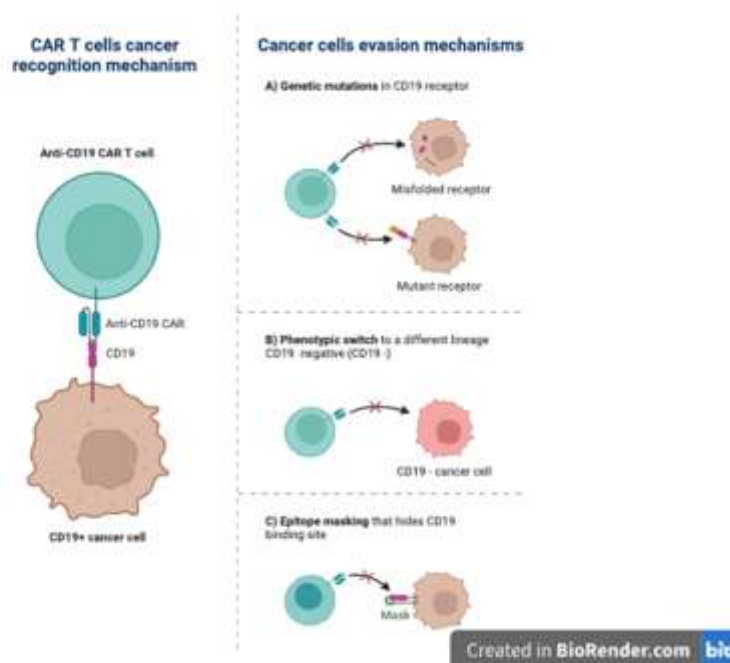


Figure 2: Mechanism of Action of CAR-T Cells

This desmoplastic stroma, a type of connective tissue growth, constitutes a very dense physical barrier that hinders T cell penetration and their movement. Intravital microscopy based studies show CAR- T cells getting hindered at the tumour periphery and are not able to penetrate the malignant nest.¹¹ Even if the infiltration is successful, the spatial distribution is mostly patchy

and restricted to perivascular areas, thus most parts of the tumour are left untargeted. CAR-T cells are the immunosuppressive metabolic and cellular landscape designed to suppress effector immunity and are metabolically antagonistic: the tumour cells that proliferate rapidly and suppressive immune populations consume glucose and amino acids

voraciously, thus these spots are subjected to extreme nutrient competition.¹² Hypoxia, which is almost a universal condition, leads to the stabilisation of hypoxia-inducible factors (HIFs) that, among other things, induce the expression of immune checkpoint ligands like PD-L1 on tumour cells and thus favour the recruitment and function of regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs)¹³

These suppressive populations possess a wide range of inhibitory tools that enable them to execute their functions. Among these mechanisms is the secretion of anti-inflammatory cytokines (e.g., TGF, IL, 10), expression of surface enzymes that deplete essential metabolites (e.g., CD73/CD39 producing immunosuppressive adenosine), and direct cell, contact mediated suppression.¹⁴ The impact on CAR-T cells is that they become functionally paralysed or 'exhausted.' One of the main features of CAR, T cell exhaustion is the continuous incline in the expression of several inhibitory receptors (e.g., PD, 1, TIM, 3, LAG, 3), decline in proliferation and cytokine secretion (IFN, TNF, IL, 2), along with a metabolic shift toward a dysfunctional state relying on glycolysis and impaired oxidative phosphorylation.¹⁵ Exhaustion is not simply an adaptive downregulation; it is a specific epigenetic and transcriptional state that is fixed and makes cells refractory to reactivation.¹⁶

In addition to these challenges, antigen variability and loss represent a significant obstacle. Solid tumours are highly genomically unstable and subject to clonal evolution, resulting in a diversity of antigen expression patterns.¹⁷ CAR-T cells that recognise only one antigen put a very strong selective pressure on the tumour that can eliminate antigen-positive cells very quickly and thus lead to the emergence of antigen-negative tumour cell clones that can escape immune recognition and

cause disease relapse.¹⁸ This is a phenomenon where less frequent in CD19, which is an essential element of the tumour lineage. Moreover, CAR-T cell activation in the tumour microenvironment can lead to activation, induced cell death (AICD) or terminal differentiation, thereby exhausting the long-lived, self-renewing memory cell pool that is necessary for continuous immune surveillance.¹⁹

The synergic effect of these obstacles has been seen in the clinical trial data of solid tumour CAR-T therapies. Although the first phase of trials showed that there were some objective responses and the biological activity evidence was very strong (for example, tumour, infiltrating lymphocyte engraftment, cytokine release), these responses were generally short-lived and limited in the number of patients. The percentage of patients with complete and lasting remissions is still very low, which indicates that the simple transfer of the CAR construct technology from B-cell malignancies to solid tumours is not sufficient.²⁰ Without simultaneously modifying the T cell product so that it can survive and function in a completely different environment, the therapy will not work.²¹ The awareness about this fact has led to a huge shift in the field to "armouring" strategies, which are genetic modifications that make cells resistant to suppression or improve their inherent fitness.

However, an alternative and potentially synergistic strategy may not focus on additional synthetic modification of the CAR structure, but rather on leveraging the body's own physiological mechanisms to activate and strengthen T cells. Within this framework, the gut microbiota and its diverse metabolic capacity stand out as a powerful and highly intricate. Modulatory system.

2. The Gut Microbiota: An Intrinsic Pharmacopoeia for Systemic Immune Regulation



The human digestive system is a dwelling place for an enormous number of diverse and continuously changing communities of microorganisms, the gut microbiota, which in total contain millions of genes (the microbiome) that far outnumber the host's own genetic material.²² This microbial community, no longer regarded as mere passive residents, has been identified as a major factor influencing the host physiology, thereby this impacts a variety of processes, including nutrient metabolism and vitamin production, as well as the immune system development and function.²³

One of the main ways the microbiota impacts the host's physiology at a distance is by releasing a variety of small-molecule metabolites that are taken up by the portal blood, become systemically available, and act as signalling molecules at the various sites.²⁴

This effectively defines the gut as an endocrine organ in its own right, whose microbial inhabitants function as distributed bioreactors churning out a diverse pharmacopoeia of immunologically active compounds. This landmark finding of the gut microbiome composition can profoundly regulate the effectiveness of cancer immunotherapy, in particular immune checkpoint inhibitors (ICIs) targeting PD-1/PD-L1 or CTLA-4, is the definitive proof of this systemic axis.²⁵ Groundbreaking work revealed that patients with metastatic melanoma or non-small cell lung cancer who were responsive to ICIs had microbial signatures with distinct features that were enriched with certain taxa (e.g., *Faecalibacterium*, *Bifidobacterium*, *Akkermansia muciniphila*),

whereas non-responders typically had a microbial dysbiosis or were on broad-spectrum antibiotics that neutralise the therapeutic effect.²⁶

Transplanting faecal microbiota from responders to germ free or antibiotic treated mice has been shown to restore anti PD-1 efficacy, hence confirming the causality.²⁷ This discovery left no room for doubt that the microbiome was a major modifiable factor influencing the therapeutic results, which then became a subject of intense research to identify the specific microbial products responsible for the phenomenon. The microbiota's immune and modulatory effects are facilitated via various intertwined routes.²⁸ Direct contact with immune cells residing in the gut, thus determining the local inflammation and the differentiation of particular T helper cell subsets (e.g., Th1, Th17, Tregs) systemic activation of innate immunity, especially by means of training bone marrow myeloid progenitors and altering the antigen, presenting cell function; Cell signalling through metabolites, whereby small molecules either diffuse or they are transported into the circulation to directly interact with the receptors on peripheral immune cells. This last route is the one that current methods of pharmaceutical intervention can most easily be targeted, thus linking the microbiome to cell therapies such as CAR-T cell therapy.²⁹ There is increasing clinical data suggesting that the microbiome is directly responsible for the therapeutic outcomes of CAR-T cells. In 2022, Smith et al. conducted a landmark study in which they examined the stool microbiome of patients who had undergone anti-CD19 CAR T therapy for B-cell cancers.

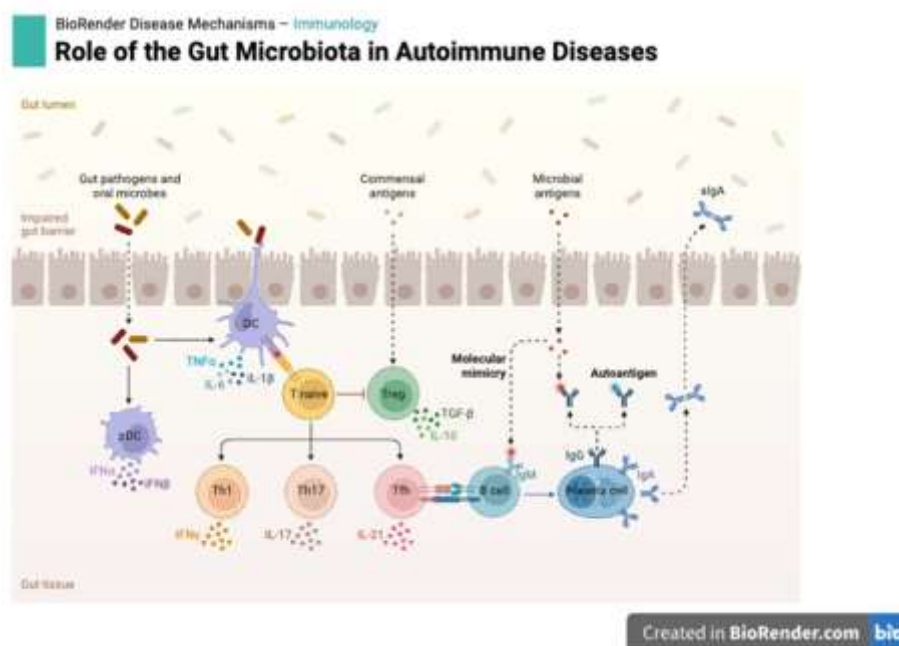


Figure 3: Role of Gut Microbiota in Modulating CAR-T Responses "Gut microbiota derived metabolites modulate immune responses influencing CAR-T efficacy."

It is found that a microbial signature characterised by the presence of butyrate, producing bacteria (e.g., Ruminococcaceae, Lachnospiraceae) was highly associated with increased *in vivo* CAR, T cell expansion, as well as longer progression, and free survival.³⁰ On the contrary, a dysbiosis state that was mainly dominated by *Enterococcus* species was found to significantly contribute to the increased occurrence of severe immune effector cell-associated neurotoxicity syndrome (ICANS) and lesser expansion. Although this association does not confirm that metabolites themselves directly caused the effect, it certainly provides a robust clinical framework for the development of mechanistic hypotheses. It is implied that a local, SCFA rich microbial community may systemically 'prime' the host to be more receptive to the engraftment, expansion, and functional longevity of administered therapeutic T cells.

This could occur through multiple non-mutually exclusive mechanisms: conditioning of the lymphoid niche, modulation of systemic inflammation, or direct epigenetic or metabolic

effects on the T cells themselves, either *in vivo* after infusion or potentially during *ex vivo* manufacturing if metabolites are present in-patient serum.³¹

Therefore, the gut microbiome transcending its role in digestive health to become a crucial variable in the pharmacokinetics and pharmacodynamics of cellular immunotherapies. This thought opens a transformative therapeutic avenue; rather than attempting to overcome the suppressive solid TME solely through genetic engineering of the CAR-T cell, we can simultaneously modulate the host's physiological state using targeted microbial or metabolite-based interventions. Among the myriad microbial metabolites, short-chain fatty acids (SCFAs) stand out for their well characterised biosynthesis, potent and pleiotropic immunomodulatory properties, and emerging direct relevance to T cell biology, positioning them as prime candidates for adjuvant development in CAR-T therapy for solid cancers.³²

3. Biochemical Genesis and Pharmacokinetics of Butyrate and Propionate

Short-chain fatty acids are saturated aliphatic carboxylic acids containing one to six carbon atoms, with acetate (C2), propionate (C3), and butyrate (C4) representing more than 95% of the SCFAs in the colonic lumen and systemic circulation. It is anaerobic and solely depends on the fermentative activity of the resident colonic bacteria on dietary substrates that are not digested

in the small intestine, mainly complex carbohydrates and fibres identified as "microbiota, accessible carbohydrates" (MACs).³³ These are resistant starches, inulin, pectin, beta-glucans, and arabinoxylans, whose chemical features decide which bacterial taxa and metabolic pathways are used for their degradation. Hence, the absolute amount and molar ratio of the formed SCFAs are not constant but are changing results of diet composition, microbial community structure, gut transit time, and host physiology.³⁴

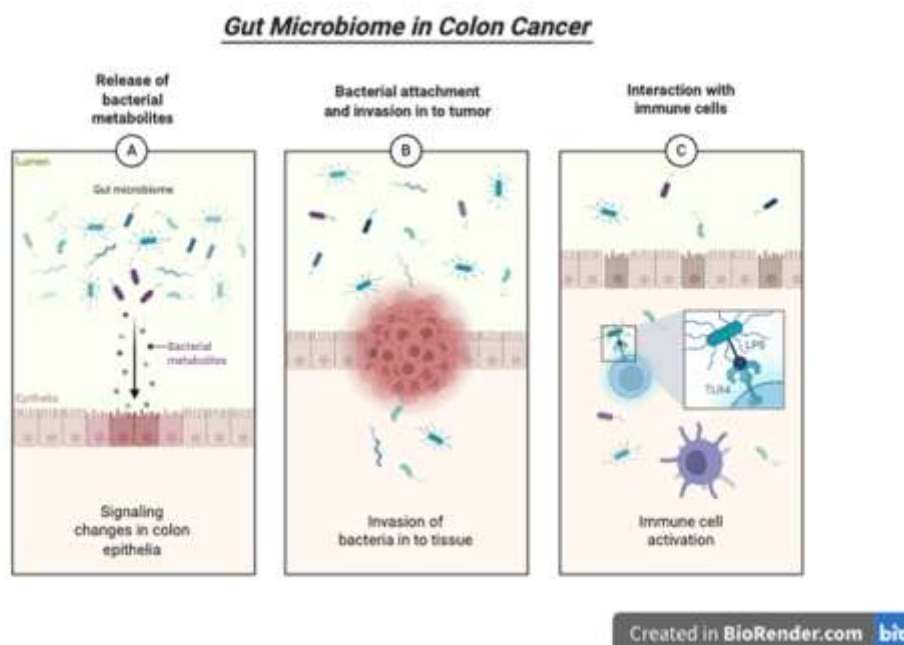


Figure 4: Gut Microbiome in Colon Cancer

Butyrate synthesis happens through a tightly regulated metabolic pathway. The key pathway in the human colon is the butyryl CoA: acetate CoA transferase pathway, which is used by Firmicutes of Clostridium clusters IV and XIVa, the major butyrogenic species *Faecalibacterium prausnitzii* and *Roseburia* spp.³⁵ In this process, two acetyl CoA molecules are combined to form acetoacetyl CoA, which is then reduced to which is finally changed to butyrate by CoA transfer to an outside acetate molecule. The butyrate kinase chain is an alternative, but less frequently used pathway. Importantly, butyrate is far from being a simple

waste product; it is the main energy source for colonocytes, providing 60- 70% of their oxidative ATP requirements, that explains its major role in maintaining colonic epithelial integrity, controlling mucosal inflammation, and helping barrier function.³⁶ The extremely high first pass metabolism rate in the colonic mucosa limits its systemic bioavailability considerably. After that, the luminal produced butyrate can only partially reach the portal vein, and it is still subjected to hepatic clearance, so in the peripheral circulation, the concentrations are in the range of low nanomolar to low micromolar, under normal conditions.³⁷ The pharmacokinetic behaviour

poses a major limitation to its use as a systemic drug. However, this profile also suggests that methods of enhancing its delivery, for example, prodrugs or targeted release formulations, could result in greater biological effects.

There are at least three different bacterial routes for propionate biosynthesis; a more diverse phylogenetic set of microbes contributes to the production of propionate. The dominant pathway of Bacteroidetes (e.g., *Bacteroides* spp.) is the succinate pathway, where polysaccharides are broken down to succinate, which is further converted to propionate by decarboxylation.³⁸ Firmicutes like *Roseburia inulinivorans* and *Coprococcus catus* use the acrylate pathway by which lactate is converted to propionate, while other members use the propanediol pathway to ferment fucose or rhamnose. Propionate, unlike butyrate, is not a fuel for colonocytes; it does so only to a very small extent. It is very well absorbed, takes the portal vein path to the liver, and there it is used mainly for gluconeogenesis.³⁹ As a result, the level of propionate in the systemic circulation is higher than that of butyrate, and it is an important component of the gut-brain axis, controlling satiety and metabolism.⁴⁰

The different destinies of these two SCFAs deeply impact their potential for use as immunomodulatory adjuvants. Butyrate might be strongest in the immediate colonic and portal environments, thus affecting the immune cells that travel through the gut, associated lymphoid tissue (GALT) and liver, both major immune sites. If systemic effects on CAR, T cells in the blood or after infusion are desired, ways to avoid the latter's rapid elimination are needed. Since propionate has a higher systemic level, it could be more appropriate for direct modulation of peripheral immune cells.⁴¹ A clear hold of these pharmacokinetic properties is essential to

rational design intervention strategies, e.g., changing production ratios by diet, giving purified compounds, or using engineered delivery systems.

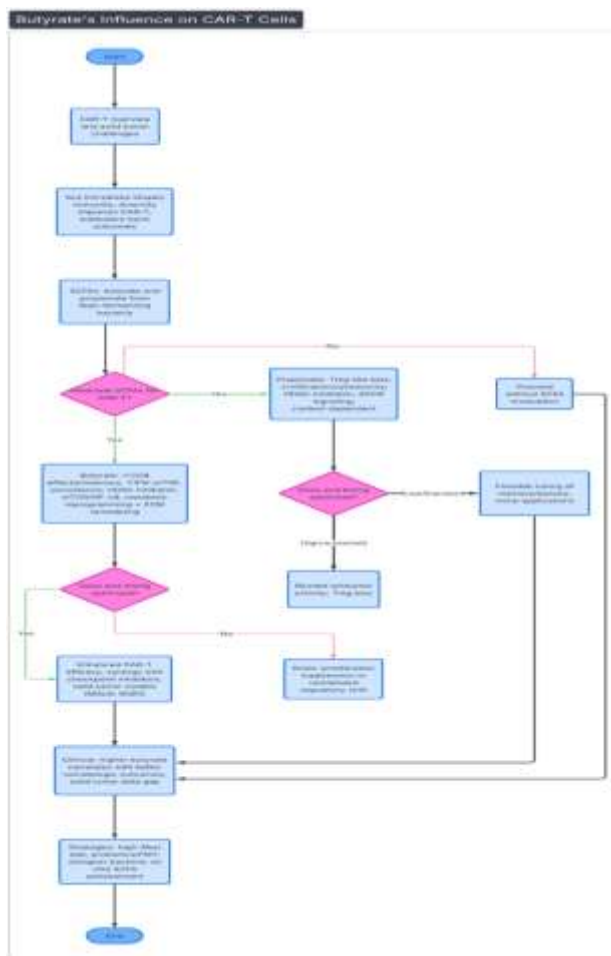


Figure 5: Strategic Model for Enhancing CAR-T Cell Efficacy Using Butyrate

4. Explaining the Molecular Symphony: How Butyrate Influences CAR-T Cell Biology. The idea that a very small, four-carbon fatty acid can radically change the complex biology of a genetically engineered T cell is based on the fact that butyrate can interact with the key points of cell regulation. Its effects are diverse because they stem from both its primary function as an epigenic regulator and the downstream impacts on cell metabolism, signalling, and differentiation. For CAR T cells designed to target the solid tumour microenvironment, the results of this are

multipronged strategies that could be transformative.

4.11. Epigenetic Reprogramming: Activating Gene Expression Potential via HDAC Inhibition

The main, and best understood, mechanism of action of butyrate is its role as a selective, non-competitive inhibitor of zinc dependent histone deacetylases (HDACs), specifically Class I (HDACs 1, 2, 3, 8) and Class IIa (HDACs 4, 5, 7, 9).⁴² At physiological concentrations (micromolar range), butyrate interacts with these enzymes by attaching to the catalytic pocket, thus it prevents the deacetylation of lysine residues on histone tails. As a result, there is a buildup of hyperacetylated histones (e.g., H3K9ac, H3K27ac) that changes the nucleosome structure, loosening chromatin and making DNA more accessible to transcription factors and RNA polymerase II.⁴³ This allows for more gene transcription. However, the specific genes that get turned on or off depend on the previous chromatin state and the set of transcription factors in the cell. Butyrate has been found to promote memory and stemness transcriptional program in T cells through HDAC inhibition. The research (Bachem et al) revealed that when CD8 T cells were exposed to butyrate during their in vitro activation, their memory potential was significantly enhanced upon adoptive transfer.⁴⁴ This was attributed to increasing the expression of the genes for transcription factors that play a pivotal role in memory T cell development and maintenance, such as TCF7 (which encodes TCF1) and LEF1.⁴⁵

TCF1 is one of the most important regulators of stem cells, like CD8 T cells. The role of TCF1 is essential in self renewal, maintenance, and generation of effector cells during response to chronic infection or tumour. If CAR T cells, during their ex vivo expansion and also upon strong antigen stimulation in vivo, very frequently and

rapidly differentiate into terminal effector cells, then butyrate pretreatment may act as a brake on their differentiation.

Epigenomic changes by which memory genes are maintained, butyrate may help to develop a CAR, T product that is rich in stem cell memory (TSCM) or central memory (TCM) phenotypes, marked by the elevated expression of CD62L and CCR7.⁴⁶ These two memory subsets have greater proliferative burst capacity, longevity, and metabolic flexibility compared to their terminally differentiated effector (TEFF) counterparts and these features, which have been directly linked to enhanced antitumor effectiveness in preclinical models.⁴⁷ Moreover, HDAC inhibition has the potential to undo the epigenetic mechanisms underlying T cell exhaustion directly. Exhausted T cells are associated with a tightly repressive chromatin configuration that comprises specific histone methylation patterns (for example, H3K27me3 deposited by Polycomb Repressive Complex 2).⁴⁸

One possibility for butyrate induced hyperacetylation is to block the suppressive chromatin environment and thus keep the genes necessary for effector function and proliferative capacity accessible. As for vorinostat, an HDAC inhibitor, it could lower the expression of several inhibitory receptors (PD-1, TIM-3, LAG-3) on tumour infiltrating lymphocytes.⁴⁹ Despite direct butyrate evidence in CAR-T cells still being gathered, the strong mechanistic background indicates that butyrate will be able to assist CAR-T cells in staying in a more functional, less exhausted state within the TME, which is suppressive by nature.



4.2. Metabolic Reprogramming: Fueling Persistence through mTOR Modulation and Oxidative Metabolism

T cell function is inextricably linked to cellular metabolism. Upon activation, inexperienced T cells undergo a metabolic switch from catabolic oxidative phosphorylation (OXPHOS) to anabolic aerobic glycolysis to support rapid biomass accumulation and proliferation, a process driven by the PI3K-AKT-mTOR signalling axis.⁵⁰ However, sustained intense glycolytic flux is associated with terminal effector differentiation and reduced longevity. In contrast, memory T cells rely more on fatty acid oxidation (FAO) and OXPHOS, which support long term survival and self renewal.⁵¹ The hostile, nutrient-poor TME of solid tumours further stresses this metabolic duality, often disabling the glycolytically addicted effector cells.

Butyrate intersects with this metabolic circuitry at multiple levels. First, as a short-chain fatty acid, it can be imported into T cells via monocarboxylate transporters (MCTs) and directly oxidised in the mitochondria to yield acetyl-CoA, entering the tricarboxylic acid (TCA) cycle and producing ATP, also reducing the equivalents (NADH, FADH₂) for OXPHOS.⁵² This provides an auxiliary energy source that could supplement or spare glucose, a critical advantage in glucose depleted tumour regions.

Second, butyrate's HDAC inhibitory activity modulates the mTOR pathway. The relationship is

complex and concentration dependent. Some reports indicate that butyrate can suppress mTORC1 activity, potentially by upregulating negative regulators like REDD1 or through acetylation of key pathway components.⁵³ Inhibition of mTORC1 shifts cellular metabolism away from robust glycolysis and protein synthesis toward a more catabolic, OXPHOS dependent state. Precisely, the metabolic profile associated with memory T cells. This metabolic reprogramming enhances "spare respiratory capacity," a measure of mitochondrial fitness that allows cells to respond to sudden energy demands and is a hallmark of long lived, persistent T cells.⁵⁴ By promoting this metabolic state during *ex vivo* activation or early after infusion, butyrate could engineer CAR-T cells that are intrinsically more resilient to the metabolic hardships of the TME.

4.3. Functional Enhancement: Cytotoxicity, Homing, and Survival

Beyond epigenetics and metabolism, butyrate influences specific functional attributes of T cells relevant to CAR-T efficacy. *In vitro* studies consistently report that butyrate treatment during T cell activation augments their subsequent effector response. For instance, Park et al. showed that butyrate-conditioned CD8⁺ T cells produced significantly higher levels of IFN- γ and TNF- α upon re-stimulation.¹³ This enhanced polyfunctionality could translate to more potent tumour cell killing by CAR-T cells.

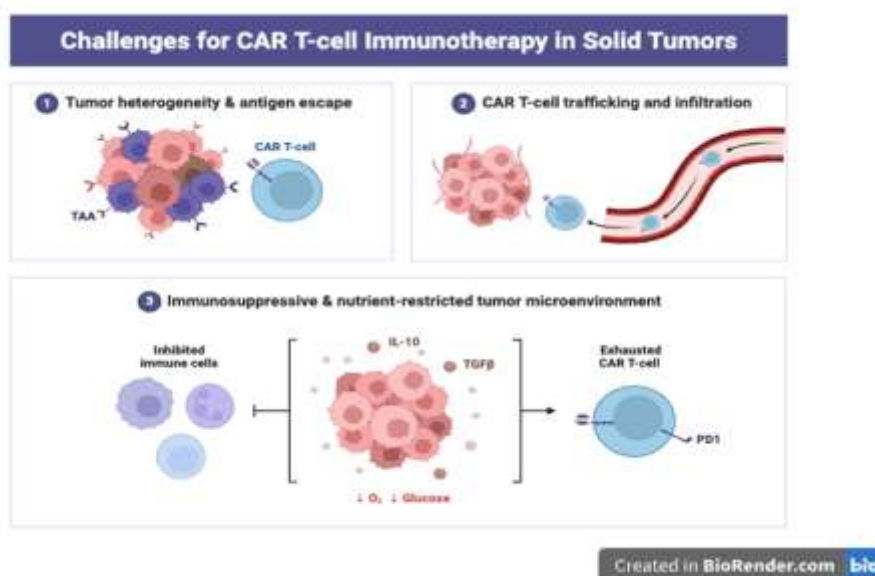


Figure 6: Challenges in CAR T-Cell Immunotherapy for Solid Tumors

A particularly intriguing and less-explored area is butyrate's potential impact on tumour homing. Efficient infiltration into solid tumours requires the coordinated expression of specific chemokine receptors (e.g., CXCR3 for ligands like CXCL9/10/11) and adhesion molecules (integrins). The TME often fails to produce adequate chemokines or presents physical barriers that downregulate these homing molecules on T cells. There is emerging evidence that HDAC inhibition can modulate the expression of such receptors.⁵⁵ Butyrate could potentially upregulate CXCR3 or other trafficking molecules on CAR-T cells, improving their chemotaxis toward the tumour. Additionally, by suppressing the differentiation of T cells toward highly migratory effector phenotypes that may traffic poorly, and instead promoting a central memory phenotype that efficiently homes to secondary lymphoid organs for optimal activation before migrating to tissues, butyrate may orchestrate a more effective trafficking strategy.⁵⁶

Finally, butyrate can influence T cell survival through modulation of apoptosis pathways. It can induce apoptosis in rapidly dividing cancer cells, but in normal lymphocytes, the effects are more

context dependent and promote survival. By promoting a memory like, metabolically fit state and potentially upregulating anti-apoptotic proteins like Bcl-2, butyrate may enhance the persistence of CAR-T cells *in vivo*.⁵⁷

In synthesis, butyrate acts not as a simple "on" switch for T cells, but as a sophisticated reprogramming agent. It pushes the transcriptional and metabolic identity of the cell away from a fragile, terminally differentiated state and toward a more durable, flexible, and functionally competent state precisely the engineering goal for CAR-T cells intended to conquer solid tumours.

5. Propionate: A Distinct yet Complementary Immunomodulatory Profile

Propionate, while structurally similar to butyrate, engages the immune system through a partially distinct mechanism, offering complementary or potentially contrasting effects, so that it must be carefully considered in any adjuvant strategy. Its primary signalling mechanism is thought to be via engagement of specific G-protein-coupled receptors (GPCRs), particularly GPR41 (FFAR3) and GPR43 (FFAR2), which are expressed on

various immune cells, including T lymphocytes, neutrophils, and macrophages.⁵⁸ These receptors couple to Gi/o proteins, inhibiting adenylate cyclase and reducing intracellular cAMP levels, which in turn influences an abundance of downstream effectors, including MAPK pathways, calcium flux, and NF- κ B signaling.⁵⁹

The immunological consequences of propionate signalling through these receptors are context-dependent and can appear contradictory. In the intestinal lamina propria, propionate signalling through GPR43 on colonic T cells and innate lymphoid cells has been shown to promote the generation and function of regulatory T cells (Tregs) and the production of the anti-inflammatory cytokine IL-10, contributing to the maintenance of mucosal tolerance.⁶⁰ However, in systemic contexts and on different cell types, propionate can also promote effector responses. For example, in neutrophils, GPR43 activation enhances chemotaxis, phagocytosis, and inflammatory mediator production.⁶¹

Regarding direct effects on conventional T cells and, by extension, CAR-T cells, the data are more scant and sometimes conflicting compared to butyrate. Propionate's HDAC inhibitory activity is significantly weaker, meaning its epigenetic impact is likely less pronounced.⁶² Therefore, its primary influence may be through GPCR-mediated metabolic and signalling changes. Some studies suggest that propionate can inhibit T cell proliferation and IFN- γ production,⁶³ while others indicate it may have minimal direct effect or even support certain effector functions depending on the cytokine.⁶⁴ This ambiguity highlights a critical gap in knowledge: a direct comparison of butyrate and propionate on human CAR-T cell phenotype, metabolism, and *in vivo* anti-tumour function is urgently needed.

The differential receptor usage suggests that the combined application of both SCFAs might yield synergistic benefits. Butyrate could provide the strong epigenetic push toward memory and metabolic fitness via HDAC inhibition, while propionate, through GPCR signalling, could refine the inflammatory tone, perhaps mitigating excessive cytokine release syndrome (CRS) or modulating the activity of suppressive myeloid cells in the TME. Alternatively, their sequential application might be considered using propionate to modulate the host environment pre-infusion and butyrate to condition the CAR-T product *ex vivo*.

6. Translational pathways from Preclinical research to clinical approach: Clinical Correlations and Translational Adjuvant Strategies.

The mechanistic plausibility of SCFA-mediated CAR-T enhancement gains substantial weight from emerging clinical correlative data. As previously noted, Smith et al. found that a favourable microbiome enriched in butyrate producers correlated with improved CAR-T expansion and outcomes in lymphoma.³⁰ While this study did not measure serum SCFA levels directly, it provides a strong ecological link. More direct evidence comes from a 2023 study by Stein-Thoeringer et al., which not only confirmed the negative impact of dysbiosis but also identified that high intestinal concentrations of the disaccharide lactose could drive enterococcal expansion and increase neurotoxicity risk, indirectly suggesting that specific microbial metabolic pathways are directly relevant to CAR-T pharmacology.⁶⁵

Extrapolating these findings to solid tumours requires bridging studies. Currently, few trials have systematically collected microbiome or metabolome data from patients receiving solid tumour directed CAR-T cells. However, the

biological rationale is arguably even stronger for solid cancers, given the greater need for T cell persistence and metabolic fitness. Preclinical models offer compelling evidence-based studies. For instance, priming of tumour specific T cells or CAR-T cells with HDAC inhibitors like valproic acid or entinostat has been shown to enhance their anti tumour efficacy in murine models of solid tumours, correlating with increased persistence and reduced exhaustion.^{21,66} While these are pharmaceutical HDAC inhibitors, they validate the target pathway and suggest that the endogenous HDAC inhibitor, butyrate, could be harnessed for a similar effect, potentially with a superior safety profile.

Several translational strategies can be envisioned to leverage butyrate and propionate as adjuvants:

1. *Ex Vivo* Conditioning During CAR-T Manufacturing:

This is the most direct and controllable approach. Sodium butyrate or other derivatives could be added to the culture media during the T cell activation and expansion phase. This would directly imprint the desired epigenetic and metabolic program onto the CAR-T product before infusion. A key consideration is timing and concentration; prolonged exposure might overly skew toward memory at the expense of initial effector function, while insufficient exposure may have no effect.⁶⁷ Patent literature reflects commercial interest in this space, with claims covering methods for enhancing persistence of therapeutic cells through culture with metabolic modulators or HDAC inhibitors.²²

2. *In Vivo* Microbiome Priming via Prebiotic/Postbiotic Administration:

This strategy aims to boost endogenous SCFA production in the patient before lymphodepleting

chemotherapy and CAR-T infusion. Administration of prebiotic fibres (e.g., high-amylose maize starch, inulin, pectin) could selectively nourish SCFA producing bacteria.⁶⁸ Alternatively, "postbiotic" formulations of purified SCFAs or their prodrugs (e.g., tributyrin, a triglyceride of butyrate) could be administered orally or via colon targeted delivery systems to directly elevate systemic levels.⁶⁹ The goal would be to create a systemic metabolic environment conducive to CAR-T engraftment and function.

3. *Systemic Pharmacological Administration of SCFA Analogues or Delivery Systems:*

To overcome butyrate's poor pharmacokinetics, engineered prodrugs or nanocarriers are being developed. For example, polymeric nanoparticles designed for colon specific release or lipid based carriers that enhance systemic absorption could provide controlled, sustained delivery of butyrate.⁷⁰ This approach would allow precise dosing to achieve therapeutic systemic concentrations without gastrointestinal distress.

Each strategy carries distinct advantages and challenges. *Ex vivo* conditioning offers precision but doesn't address the host systemic environment. *In vivo* modulation is systemic but faces inter-individual variability in microbiome composition and response. The optimal approach may be a combination. Manufacturing a butyrate-conditioned CAR-T product and simultaneously priming the host with a prebiotic or probiotic regimen to support its continued function after infusion.

Pharmacokinetic and Formulation Barriers:

As detailed, the native pharmacokinetics of butyrate are ill-suited for systemic immunomodulation. Achieving stable, therapeutic plasma concentrations requires advanced



formulation science.⁷¹ Oral administration of free sodium butyrate leads to rapid absorption in the upper GI tract, potential gastric discomfort, and swift hepatic clearance. Colon targeted delivery systems (e.g., pH-sensitive or microbially degradable coatings) can deliver butyrate to its site of production but may not optimally increase systemic levels. Prodrugs like tributyrin are hydrolysed throughout the body, offering a more sustained release but less control.⁷² Developing clinically viable, reproducible, and scalable delivery platforms is a non trivial prerequisite.

Context Duality and Dose Optimisation:

The immunomodulatory effects of SCFAs are famously context dependent. The same metabolite that promotes anti-inflammatory in the gut may be required to promote inflammatory Th1 responses systemically for cancer control.⁷³ The dose response curve is likely biphasic and cell-type specific. An inappropriate dose or timing could theoretically blunt the very CAR-T response it aims to enhance or exacerbate immunosuppression by expanding Tregs. Therefore, extensive preclinical work in immunocompetent, tumour bearing models is essential to map the therapeutic window and define optimal administration schedules relative to CAR-T infusion.

Inter-Individual Variability:

The baseline gut microbiome is highly personalised, influenced by diet, genetics, antibiotics, and disease state.⁷⁴ This means the endogenous production of SCFAs and the host's responsiveness to prebiotic interventions will vary dramatically between patients. A one-size-fits-all fibre supplement may have unpredictable effects.

Future approaches may need to be personalised, guided by baseline metagenomic and metabolomic profiling, or may bypass the microbiome entirely through direct administration of defined postbiotic formulations.

Integration with CAR-T Design and Other Armouring Strategies:

SCFA modulation should not be viewed in isolation but as one component of a multi-faceted engineering strategy. How does butyrate conditioning synergise with CARs incorporating 4-1BB versus CD28 co-stimulation, given their differential effects on metabolism and persistence?

⁷⁵ Would it enhance or interfere with "armoured" CARs secreting cytokines like IL-12 or IL-15?⁷⁶ Systematic combination studies are needed. Furthermore, SCFAs might be particularly synergistic with strategies that target the TME's metabolic constraints, such as enzymes that degrade adenosine or provide alternative nutrients.

Definitive Clinical Correlation in Solid Tumours:

The most immediate need is for prospective clinical trials in solid tumour CAR-T therapy that incorporate rigorous microbiome and metabolome analysis. Serial sampling of stool, blood, and even tumour tissue to measure SCFA levels and correlate them with CAR-T pharmacokinetics (expansion, persistence), pharmacodynamics (cytokine release, tumour infiltration), and clinical outcomes (response, toxicity, survival) will be indispensable.⁷⁷ Such studies will move the field beyond association to a deeper understanding of causation and identify which patients are most likely to benefit from adjuvant SCFA strategies.

Table 1: Differential Effects of Butyrate and Propionate on CAR-T Cell Function

Feature	Butyrate	Propionate
Primary Metabolic Source	Bacterial fermentation of resistant starches via Acetyl-CoA route.	Fermentation of lactate/threonine via succinate or acrylate pathways.



HDAC Inhibition Potency	High Potency: Strong inhibition of Class I HDACs.	Intermediate Potency: weaker than butyrate, stronger than acetate.
T-Cell Phenotype Promoted	Effector/Memory (Tem): Promotes CD8 ⁺ cytotoxic phenotype and memory precursors.	Regulatory/Tolerogenic: often shifts toward FoxP3 ⁺ Treg-like phenotype.
Key Molecular Targets	Increased H3K27ac at Pcd1/Cd28 promoters; Increased mTORC1/C2 signaling.	Increases S6K phosphorylation; modulates FoxP3, ROR γ t transcription factors.
Cytokine Profile	Pro-inflammatory: Increases IFN- γ , TNF- α , IL-2.	Anti-inflammatory: Suppresses IL-17, IFN- γ , and degranulation.
Tumor Microenvironment Effect	Enhances ECM remodeling (collagen degradation) to improve infiltration.	Preserves junctional integrity/barrier function; reduces infiltration.
Clinical Implication	Enhances persistence and cytotoxicity (especially in solid tumors).	Potential toxicity management (tissue preservation) but risks blunting anti-tumor efficacy.

In conclusion, the modulation of CAR-T cell therapy by gut microbiota-derived short-chain fatty acids represents a frontier where immunology, microbiology, and metabolism intersect. Butyrate and propionate offer a powerful, evolutionarily honed set of tools to epigenetically and metabolically re-engineer therapeutic T cells, endowing them with the resilience, persistence, and functional competence needed to thrive within the hostile sanctuary of solid tumours. While significant translational challenges remain, the mechanistic rationale is robust, and the potential payoff a broadly applicable, low-toxicity adjuvant that could unlock the promise of CAR-T therapy for a vast array of common cancers is immense. The future of this field lies in a disciplined, iterative cycle of mechanistic discovery, sophisticated bioengineering, and rigorous clinical validation, ultimately aiming to harness the power of our microbial companions to conquer one of oncology's greatest challenges.

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HOW TO CITE: Christine Anna Anil, Edlin Domini. T, Ratchambika S, Vaishnavi Pawar, Dr. Ponni Sivaprakasam, Gut Microbiota Derived Short-Chain Fatty Acids as Immunometabolic Modulators of CAR-T Cell Therapy in Non-Lymphoma Cancers, *Int. J. of Pharm. Sci.*, 2026, Vol 4, Issue 5, 1173-1192. <https://doi.org/10.5281/zenodo.20055146>

