Review

Exploring the potential impact of non-invasive modifications of the Gut Microbiome on Cancer Immunotherapy – a narrative review

Adi David * and Shaked Lev-Ari

Posted Date: 8 January 2024

doi: 10.20944/preprints202401.0594.v1

Keywords: ICB; irAE; CTCAE; microbiome; biomarkers; Diet; metabolism

Review

Exploring the Potential Impact of Non-Invasive Modifications of the Gut Microbiome on Cancer Immunotherapy—A Narrative Review

Adi David [1,*] and Shaked Lev-Ari [2]

[1] Tal Center for Integrative Medicine, Institute of Oncology, Chaim Sheba Medical Center, Ramat-Gan, 52621, Israel
[2] Ella Lemelbaum Institute For Immuno-Oncology, Chaim Sheba Medical Center, Ramat-Gan, 52621, Israel
* Correspondence: adi.david@sheba.health.gov.il

Abstract: The following narrative review embarks on a comprehensive exploration of the pivotal role played by the gut microbiome within the Diet-Microbiota-Immunity (DMI) tripartite, aiming to enhance anti-cancer immunotherapy efficacy. While revolutionizing cancer treatment, resistance to immunotherapy and immune-related adverse events (irAEs) remain challenges. The tumor microenvironment (TME), shaped by cancer cells, influences immunotherapy resistance. The gut microbiome, influenced by genetics, environment, diet, and interventions, emerges as a critical player in TME reshaping, thereby modulating immune responses and treatment outcomes. Dietary patterns like the Mediterranean diet, caloric restriction modifications, and specific nutritional components show promise in influencing the tumor microenvironment and gut microbiome for better treatment outcomes. Antibiotics, disrupting gut microbiota diversity, may compromise immunotherapy efficacy. This review emphasizes the need for tailored nutritional strategies to manipulate microbial communities, enhance immune regulation, and improve immunotherapy accessibility while minimizing side effects. Ongoing studies investigate the impact of dietary interventions on cancer immunotherapy, pointing towards promising developments in personalized cancer care. This narrative review synthesizes existing knowledge and charts a course for future investigations, presenting a holistic perspective on the dynamic interplay between dietary interventions, the gut microbiome, and cancer immunotherapy within the DMI tripartite.

Keywords: ICB; irAE; CTCAE; microbiome; biomarkers; diet; metabolism

Introduction

Immunotherapy has significantly advanced cancer treatment and is continually breaking new ground. Anticancer immunotherapy, which employs T lymphocyte-mediated antigen-directed cytotoxicity, is pivotal for engaging the immune system against cancer through approaches like adoptive cellular therapy, anti-cancer vaccines, and Immune Checkpoint Blockade (ICB) [1–3]. However, initial resistance or eventually acquired resistance to immunotherapy is common. Moreover, the blocked natural immune checkpoint cascade can lead to powerful immune-related adverse events (irAEs) with varying severity (15–90%), involving overactive memory T cells infiltrating organs and causing inflammation. Severe immune-related adverse events (irAEs), classified as grade 3-4 according to the Common Terminology Criteria for Adverse Events (CTCAE) - the widely accepted standard classification and severity grading scale for adverse events in cancer therapy - pose potential life-threatening risks, affecting 15-30% of patients undergoing anticancer immunotherapy [2]. Currently, practical biomarkers serving as surrogates to predict treatment response are being examined in the context of irAEs, signaling the engagement of the immune system.

Resistance to immunotherapy is attributed to different pathways, including the presence of an immunosuppressive and immune-evasive tumor microenvironment (TME). In their strive to support growth and proliferation, Cancer cells' high metabolic demands and their main dependency on

 doi:10.20944/preprints202401.0594.v1

glycolysis (Warburg effect) lead to nutrients deficiency and a hypoxic state. In response, immune and stromal cells release cytokines and chemokines, and deposit an extracellular matrix that promotes cytotoxic T and NK cells exhaustion, while supporting the proliferation of immunosuppressive immune cells, more suited for this environment. A key mechanism in cancer cells-induced immune evasion exploits the immune systems' tight regulation by inhibitory checkpoints to avoid collateral damage and autoimmunity. For instance, An overexpression of PDL-1 on tumor cells and in the TME reduces the activation of PD-1 positive cells (i.e., Activated T-cells, Natural-Killer [NK] cells, B cells, macrophages, Dendritic Cells [DCs]). In 2021, Verma et al. demonstrated that post ICB initiation, cancer cells'-induced absence or poor presentation of tumor antigenic proteins by antigen-presenting cells (APCs), may result in dysfunctional T cells (CD8+PD-1+CD38^{hi} phenotype) [4–7].
Altogether, these findings justify further research into factors that could promote a balance between the cytotoxic and regulatory elements of the immune system, resulting in an effective treatment response while reducing irAEs.

The gut microbiome, consisting of bacteria, viruses, fungi, and protozoans, has recently gained recognition as a significant factor in immunotherapy. Increasingly recognized for its role in host health, the gut microbiome participates in host nutrition, metabolism and physiology, protection against pathogens, and the development of the immune system, as well as producing vital vitamins (B and K) from otherwise undigestible molecules. Roughly 90% of all gut bacteria belong to the *Firmicutes* and *Bacteroidetes* phyla, while the remaining 10% are distributed among *Verrucomicrobe, Proteobacteria*, and *Actinobacteria [8].* Gut dysbiosis, marked by changes in its composition and function, is linked to various diseases, including increased autoantibody production, leading to autoimmune disorders alongside other gastrointestinal issues, metabolic disorders, neurological conditions, and cancer development [3,8].

Emerging evidence indicates the manipulability of the gut microbiome. It becomes evident, that distinct gut microbiota plays a crucial role in the immune response, tumor microenvironment (TME), and tumor characteristics, that ultimately impact immunotherapy outcomes [4,9–17]. The complex interplay between the microbiome, host systems, and tumor immune surveillance is influenced by genetics, environment, diet, and various interventions like antibiotics, probiotics, and lifestyle changes. Although the precise nature of these interactions remains largely unexplored, these data emphasize the need for the development of safe and viable strategies for modulating the microbiome in favor of immunotherapy.

Numerous studies have extensively investigated the impact of dietary patterns on gut microbiome composition and functionality, revealing rapid microbiome alterations with dietary changes. Short-term dietary interventions result in transient microbiome modifications, while long-term diets, especially those high in fermentable nutrients or animal proteins, demonstrate gut microbiome persistent changes, associating with distinct enterotypes, such as *Bifidobacteria* or *Bacteroides* and *Clostridia*, respectively [3,8].

Consequently, a deeper understanding of microbiome-host interactions and exploring non-invasive microbiome modifications, crucially underpin the development of strategies to enhance anticancer immunotherapy. In this review, we delve into the established mechanisms that explain how such influences on the gut microbiome—via factors like dietary regimens, specific dietary components, or antibiotics—may favorably affect anti-cancer immunotherapy.

The "microbiome-immunity" axis

The bidirectional interplay between the gut microbiota and the host's immune system maintains a delicate equilibrium for microbiota and guards against potential pathogens. Commensal gut bacteria shape the host immune response through two primary mechanisms. Firstly, they prompt cell differentiation in innate or adaptive immune cells, allowing them to function locally in the gut or at extraintestinal sites. This process involves the secretion of antimicrobial peptides and IgA into the gut lumen in response to dendritic cell (DC) activation by bacteria or microbial peptides, ultimately restructuring the gut microbiota. DC migration to draining lymph nodes activates naive T cells, transforming them into effector T cells, including regulatory (Tregs), cytotoxic, and helper T cells

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 January 2024 doi:10.20944/preprints202401.0594.v1

(TH), which may then return to the gut mucosa or enter systemic circulation. Tregs foster an anti-inflammatory cytokine environment, while TH17 cells boost Paneth cell production of antimicrobial peptides and play a role in recruiting polymorphonuclear leukocytes (PMNs) from the bloodstream. Secondly, the concept of "molecular mimicry" comes into play, signifying a resemblance between the gut microbiome and tumor neoantigens [13,14,18–23].

Specific bacterial taxa linked to immunotherapy response aren't well-established yet [3,8], but, several clinical studies, including recent phase-1 trials involving fecal microbiota transplants (FMT) from responder to non-responder patients to ICBs, exhibit promising results. Those studies correlate both microbiome taxa richness (α diversity) and certain taxa compositions (β diversity) with favorable changes in immune cell infiltrates and gene expression profiles, both in the gut lamina propria and the tumor microenvironment [8,14,19,24]. Moreover, a recent study correlates specific gut microbiome taxa with risk of irAEs. In this study, *Bacteroides Vulgatus* was correlated with a lower risk of irAE's, while *Bacteroides Dorei* was correlated with a high risk of irAE's in ICB treatment for metastatic melanoma [24]. These data align with pre-clinical data.

Given the variability observed in studies linking particular bacterial strains to positive responses in immunotherapy, metabolomics (the study of end products of microbial metabolism, in feces and serum) combined with metagenomics (the study of structure and function of all genetic sequences isolated from microbial samples) are now widely used to further study the effect of microbiome on cancer immunotherapy, and promote the discovery of microbiota-linked biomarkers for response prediction of ICBs (e.g., indole, aldehydes, and short-chain fatty acids), which could be a promising target for precision medicine [25,26].

The Diet-Microbiota-Immunity (DMI) tripartite

Healthy diet not only influences cancer risk through associations with obesity, inflammation, and carcinogenesis but also shapes anti-cancer treatment outcomes. Plant-based diets demonstrate anti-carcinogenic effects by inhibiting carcinogens' activation, angiogenesis, and inflammation, and promoting cell cycle and cell signaling regulation. This contrasts the detrimental impact of diets high in refined cereals, sugary beverages, alcohol, and red/processed meat. Cancer therapies frequently induce malnutrition and weight loss, jeopardizing treatment efficacy and quality of life. Thus, ensuring proper nutrition in cancer patients is imperative to prevent malnutrition and optimize therapy outcomes [8]. In this exploration, we delve into diverse dietary patterns showcasing a positive impact on the gut microbiota to bolster improved outcomes in anti-cancer immunotherapy.

Mediterranean diet (MD)

MD is a highly recommended nutrition regimen characterized by increased plant fiber, vegetable-to-animal protein ratio, and a favorable polyunsaturated-to-saturated fat ratio. Studies show that saturated fatty acids, animal proteins, and certain vitamins affect inflammatory responses by activating TLR4, increasing trimethylamine N-oxide (TMAO) synthesis, resulting in gut epithelial barrier permeability, metabolic endotoxemia and inflammation [8].

Clinical research has demonstrated that adhering to the MD is associated with reduced systemic inflammation, heightened insulin sensitivity, and improved absorption of essential micronutrients (such as iron, vitamin D3, and folic acid). These positive effects align with the diverse functions of various gut bacteria, producing beneficial metabolites (such as Short-chain fatty acids - SCFAs) that can potentially enhance intestinal health, metabolism, and immunity [8,27].

SCFAs, stemming from dietary fiber fermentation, Comprising acetate (C2), propionate (C3), and butyrate (C4), uphold gut epithelial balance, boost nutrient absorption, fortify antimicrobial defenses, and activate metabolic pathways, profoundly influencing mucosal and systemic immunity. Butyrate, in particular, promotes regulatory T cell differentiation, generating anti-inflammatory interleukin-10 (IL-10), CTLA-4, and tumor growth factor-β (TGF-β). This immune regulation is mediated via interaction with G-protein coupled receptor 43 (GPR43) and inhibition of histone deacetylase production. Furthermore, Dietary fibers influence the immune system through Bacteroidetes bacteria induced glycans. For example, polysaccharide A, produced by Bacteroides

 doi:10.20944/preprints202401.0594.v1

fragilis, acts as a toll-like receptor-2 (TLR2) ligand, fostering regulatory Tregs differentiation [22,27–30]. Indeed, the MD-induced adjustments in microbiota profiles exhibit elevated levels of fiber-degrading bacterial populations, including Bacteroidetes, Clostridium cluster XIVa, Faecalibacterium prausnitzii, Lactobacilli, and Bifidobacteria, or reduced levels of other Firmicutes and Proteobacteria [21,27,31].

Significantly, Bacteroides fragilis (mentioned before) and F. prausnitzii, both upregulated in some of the aforementioned studies, have been acknowledged for their ability to stimulate CD4+ T cells, and the anti-inflammatory interleukin-10, thereby reducing inflammation [27,32,33]. These results were echoed in a recent study that evaluated the connections between microbiome clusters, response to ICB and toxicity in 218 melanoma patients. In this study, responders' taxa were enriched with *F. prausnitzii, Butyricicoccus pullicaecorum* and *Akkermansia muciniphila* [21]. Previous studies have demonstrated that an increase in *Akkermansia spp*. is associated with metabolic improvements, including decreased liver triglyceride accumulation and alleviated intestinal inflammation [34].

Recently, an observational study published in JAMA Oncology in 2023 revealed that among patients with advanced melanoma treated with ICB (n=91), higher adherence to the MD was associated with an increased likelihood of treatment response (probability of 0.77 for ORR; P = .02; false discovery rate, 0.032; effective degrees of freedom, 0.83; probability of 0.74 for PFS-12; P = .01; false discovery rate, 0.021; effective degrees of freedom, 1.54) [31].

Dietary interventions that exploit the metabolic vulnerabilities of cancer cells

Reduced caloric intake, restricted meal timing, low-carbohydrate diets like the Ketogenic diet, or even complete fasting share a disruptive metabolic effect on the organism's nutrient availability and mediate a differential stress response. In stress conditions, normal cells prioritize repair and maintenance over growth and proliferation, while cancer cells lack this mechanism, and thus become vulnerable to the combination of diet and cancer treatments [35–38]. Moreover, such unfavorable conditions dynamically shift the delicate balance between anti-cancer immunogenicity and immune evasion through a direct effect of starvation on the tumor microenvironment and post-starvation favorable gut microbiome changes [35–46].

Energy Restriction (ER) diets

ER modalities, i.e., Short-Term Water Fasting (STWF) diet, Time-Restricted Eating (TRE, circadian-based) , fasting mimicking diets (FMD), or the Ketogenic Diet (KD), have shown efficacy in reducing cancer development risk. They also exhibit positive effects chemotherapy-induced AEs, including fatigue, weakness, gastrointestinal and hematological toxicity, ultimately contributing to an enhanced patients' quality of life (QoL). ER's impact on treatment outcomes has been substantiated in both preclinical and clinical studies of chemotherapy for cancer [3,6,41,47,48]. Despite the wealth of accumulated knowledge, ER modalities' effects on immunotherapy treatments are on the beginning of exploration.

STWF consists of total food abstinence for 72 to 120 hours, without limiting hydration; FMD consists of five to six days of caloric restriction, that is low in protein, high in healthy fats, and complex carbohydrates, as well as essential vitamins and minerals. KD is a high-fat, low protein/carbohydrate diet. FMD mimics the STWF and KDs' effects while avoiding potential side effects of lack of essential nutrients; TRE consists of 16h of fasting followed by 8h of Norma-caloric diet every 24h. Though TRE is the most feasible method to implement lack of nutrients' stress during cancer treatments, STWF and FMD might be more effective [3,6,46].

Recent studies indicate that interventions involving STWF or FMD bring about altered release of hormones and growth factors, decreased inflammation, and restructuring of both tumor vasculature and extracellular matrix, to influence the differentiation and accumulation of immune cells, leading to the depletion of regulatory T cells (Tregs) and stimulation of cytotoxic cells. Post-ER positive metabolic changes - lower plasma glucose, insulin, and IGF-1, and increased ketones - correlate with a decline in peripheral monocytes and specific immunosuppressive cell types like Monocytic-derived suppressor cells (M-MDSCs), polymorphonucler myeloid-derived suppressor

 doi:10.20944/preprints202401.0594.v1

cells (PMN-MDSCs), and Tregs. Starvation-induced autophagy boosts the formation of tumoricidal M1 macrophages, enhancing antitumor immunity and promoting cytotoxic T cells, cytolytic NK cells, and activated/memory CD8+ cells. These systemic shifts align with beneficial metabolic and immunologic alterations in the TME. ER-induced reductions in nutrient availability impede tumor survival, counteract tumor-driven nutrient diversion, and modify the typical TME conditions marked by high lactate and low pH. This shift prevents the exhaustion of cytotoxic T and NK cells while hindering the proliferation of immunosuppressive cells. Additionally, these TME changes increase the expression of MHC class II molecules in antigen-presenting cells (APCs). The decreased metabolic activity of tumor cells reduces the release of factors like adipokines (leptin, adiponectin), IL-6, CCL2, and CCL5, known contributors to a dense extracellular matrix that hinders T cell infiltration into the TME [39–44,46,47]. This positive impact extends beyond the cellular level, as alterations in the post-starvation gut microbiome further shape immune responses.

Both clinical trials and observational research on ER protocols, including Intermittent Fasting (IF), have uncovered striking similarities in microbiome and immune system characteristics when compared to clinical trials involving Fecal Microbiota Transplantation (FMT). In the FMT trials, these shared features correlated with enhanced responses to ICBs in patients with advanced melanoma. Notable examples include an increase in gut microbiome α diversity and elevated levels of *Firmicutes*, particularly the butyric acid-producing *Lachnospiraceae* within the *Clostridium* cluster [3,14,16,19,47,49–51]. Decreased levels of the potentially pathogenic *Proteobacteria* alongside increased levels of *Akkermansia muciniphila* were also demonstrated in some of these observational studies [3].

Currently, several ongoing studies are further investigating the effects of ER on cancer immunotherapy in melanoma, Non-Small Cell Lung Cancer (NSCC), and Triple-Negative Breast Cancer (TNBC). These studies are registered under the identifiers NCT04387084, NCT03700437, NCT05582538, and NCT05763992, respectively.

Ketogenic Diets

The ketogenic diet involves restricting carbohydrates, which leads to lowered insulin levels and heightened cortisol levels. This, in turn, triggers the production of ketone bodies, enabling the body to shift its primary energy source from carbohydrates to fat, thus mimicking a state of fasting [3]. Ketone bodies can inhibit histone deacetylases (HDACs), and it is Therefore hypothesized that ketosis may help slow tumor growth and promote differentiation via epigenetic chromatin modifications [52,53]. In a recent in-vivo study, Weber et al. demonstrated that Ketogenic diets slow melanoma growth in vivo regardless of tumor genetics (mutations in BRAF, NRAS or wild type melanoma) and metabolic plasticity [54].

It's worth noting that the National Cancer Institute (NCI) recommends the Ketogenic Diet (KD) as a safe and feasible adjunct therapy for Glioblastoma (GBM), and research has explored its therapeutic potential for other cancers. However, due to the challenges associated with adhering to a ketogenic diet, ongoing studies are investigating alternative ER-based diets, particularly Fasting Mimicking Diets (FMD), with the goal of achieving similar benefits [55].

Specific dietary components

Specific food components, such as tryptophan and indole, urolithin A, vitamin A, vitamin D and a balanced omega 6:3 ratio help maintaining intestinal intra-epithelial homeostasis either directly or through microbiota modulation. Out of those, Vitamin D levels and Omega 6:3 ratio, often disturbed in cancer patients, will be further discussed [22,25]. [8]

Pro and Pre-biotics, High dietary fiber diet

Probiotics are defined as beneficial live microorganisms that can be consumed through commercially available supplements or fermented foods, such as kimchi, yogurt, kefir, pickled vegetables, and sauerkraut [3]. The most commonly used probiotics include *Bifidobacterium*, *lactobacillus*, and yeast. Probiotics exhibit immunomodulatory properties, through direct actions such

 doi:10.20944/preprints202401.0594.v1

as boosting the activity of macrophages or NK cells, and influencing the secretion of immunoglobulins or cytokines, and indirect mechanisms like fortifying the gut epithelial barrier, modifying mucus secretion, and competitively excluding other (pathogenic) bacteria [23]. In a study conducted by Dizman et al. in 2021, it was demonstrated that supplementation with a *Bifidobacterium*-based probiotic yogurt could modulate the gut microbiome of patients with metastatic renal cell carcinoma who were receiving Vascular Endothelial Growth Factor Receptor Tyrosine Kinase Inhibitor (VEGF-TKI) therapy. The study found that *Akkermansia muciniphila*, *Bacteroides caccae*, and *F. prausnitzii* were elevated in the majority of samples from patients who experienced clinical benefits [56].

On the other hand, Antibiotics use, while controlling infections, disrupt the gut microbiota diversity, possibly leading to gut dysbiosis, that may reduce immunotherapy efficacy. A 2020 meta-analysis by Wilson et al. found a significant correlation between antibiotic use and decreased Progression-Free Survival (PFS) and Overall Survival (OS) in 2,889 cancer patients (most commonly lung (59%), renal cell carcinoma (RCC) or urothelial carcinoma (16.3%), and melanoma (18.7%)). Those without antibiotic exposure showed prolonged OS, especially when the exposure was defined as 42 days prior to ICB initiation (HR 3.43, 95% CI 2.29-5.14, $p < 0.0001$). PFS was also longer in patients who did not receive antibiotics (pooled HR 1.65, 95% CI 1.3-2.1, $p < 0.0001$). This was later confirmed by two 2021 observational studies. Mohiuddin et al.'s study of 568 immunotherapy-receiving patients demonstrated a shorter median PFS in antibiotics receiving patients (mPFS: 27.4 vs. 43.7 months). In NcQuade et al.'s study of 128 patients on ICB, a pronounced benefit was observed in patients with sufficient dietary fiber intake and no probiotic use [13,23]. Nevertheless, one study claims that antibiotics supplementation had no effect on response to ICBs in NSCLC patients [23]. This conflicting data warrants a further investigation as to antibiotics' effect on immunotherapy treatments.

In a recent study, published in Science magazine in 2021, (n=132) researchers investigated the correlations between higher dietary fiber intake and probiotic consumption and the response to ICBs in melanoma patients, hypothesizing that both dietary factors could influence the gut microbiome. The study revealed that a high dietary fiber intake and no probiotic use were associated with an improved response to ICB (with 82% responders compared to 59% in those with lower dietary fiber intake), as well as an extended Progression-Free Survival (PFS) (median PFS not reached versus 13 months). The study suggests that these results may be primarily attributed to the microbiome within the tumor microenvironment (TME). The beneficial bacteria identified in this study align with recent findings, with microbial α diversity, the *Ruminococcaceae* family, and *Faecalibacterium* genus abundances being numerically higher in patients with sufficient dietary fiber intake and no probiotic use [13]. It is worth mentioning, that a recent systematic review and meta-analyses of prospective studies and randomized controlled trials emphasized the importance of nondigestible fiber to human health, suggesting a notable decrease in all causes of cardiovascular-related mortality, type 2 diabetes, and colorectal cancer when comparing high- and low-fiber consumers [57].

A current study being conducted at the M.D. Anderson Cancer Center (ClinicalTrials.gov Identifier: NCT04645680) is also aiming to evaluate gut microbiome characteristics following a high dietary fiber diet and the diet's effect on ICB treatments in advanced melanoma patients.

These findings suggest that while dietary fibers should probably be recommended, at least as a part of a healthy, mostly plant-based diet, there are still challenges to address regarding the safety of using commercially available probiotics in the context of Immunotherapy treatments.

Dietary supplements

Omega-3 fatty acids, Omega 6:3 fatty acids ratio

Omega-3 polyunsaturated fatty acids (PUFAs), found in fish, seafood, nuts, and seeds, exhibit chemo and immune-protective effects. These involve suppressing nuclear factor-κB, activating AMPK/SIRT1, modulating cyclooxygenase (COX) activity, and up-regulating anti-inflammatory lipid mediators. Pre-clinical studies indicate that dietary fish oil rich in Omega-3 PUFAs enhances

 doi:10.20944/preprints202401.0594.v1

spleen B cell numbers, cytokine/IgM production, and reduces autoantibodies in various conditions. In individuals at risk for rheumatoid arthritis, Omega-3 PUFAs supplementation and higher levels in red blood cell membranes associate with reduced anti-cyclic citrullinated peptide and rheumatoid factor positivity [8]. These beneficial effects are partially attributed to its influence on gut epithelial barrier integrity. Omega-3 PUFAs mitigate gut and systemic inflammation by promoting SCFA-producing bacteria and engaging GPR120, particularly in macrophages, thereby suppressing TNF-α and IL-6 and mitigating tissue inflammation. In mice, maintaining a balanced tissue omega-6:omega-3 PUFA ratio enhances intestinal alkaline phosphatase production, which suppresses LPS-producing microbiome members. Simpson et al.'s study links omega-3 fatty acids with higher α diversity [8,21,22].

The researched reported dosages of omega-3 s vary from 1.5 to 3 g/day of EPA via oral administration, for periods of 8 to 12 weeks. Omega-3 fatty acids are well tolerated, with no serious adverse events reported [58].

Vitamin D

Calcitriol, the active form of vitamin D (VD), plays a crucial role in the bidirectional relationship between gut microbiota and immunoregulation. VD deficiency is linked to gut dysbiosis and autoimmune diseases like inflammatory bowel disease, multiple sclerosis, type one diabetes, and systemic lupus erythematosus [59,60]. VD exhibits immunoregulatory characteristics by dampening T-cell-mediated immune responses. Given that irAEs likely stem from an abnormal T and B cell activation and an overall heightened inflammatory response, resembling hyper-immune reactions seen in autoimmune patients, the immunoregulatory attributes of VD, coupled with substantial evidence of its therapeutic benefits in autoimmunity, imply potential effectiveness in mitigating irAEs. VD enhances the integrity of the gut mucosa, influencing elements such as Paneth cells, tight junctions, and pro-inflammatory cytokines. In the gut microbiome, higher VD levels are associated with an increase in the plant glycans' degrader *Prevotella* phylum and reduced *Haemophilus* and *Veillonella* [3]. Low VD levels correlate with fewer tolerogenic dendritic cells and more T cell receptor $\alpha\beta$ cells in the lamina propria. VD intake in humans is tied to lower circulating levels of lipopolysaccharides (LPS) [3,22]. In pre-clinical studies, VD supplementation induces the expression of PD-1 and CTLA-4 on gut epithelial cells and PD-1 on immune cells. These studies also reveal reduced IFNγ synthesis and increased IL-4 production when influenced by calcitriol [3]. The accumulative data suggest that the dual impact of VD, involving an adrenal-steroid-like suppression of excessive lymphocytic activity and a concurrent upregulation of PD-1 antibodies on immune cells, may confer potential benefits in the context of immunotherapy treatment. While the mentioned positive outcomes are promising, prospective clinical studies have not demonstrated a substantial impact on the progression of various cancer types or overall survival (OS). As a result, routine screening for vitamin D deficiency or vitamin D supplementation is not currently recommended in the context of cancer treatment [61].

Conclusion and possible study pathways

The intricate interplay between tumor cells, immunotherapy, and the gut microbiome underscores the challenges and opportunities in the realm of cancer treatment. Tumor cells orchestrate an immunosuppressive microenvironment, hampering immunotherapy effectiveness, while immunotherapy itself can lead to systemic immune overactivity and severe side effects. The pivotal role of the gut microbiome as a mediator of these dual effects has been elucidated through diverse studies, highlighting the impact of fecal microbiota transplantation (FMT), antibiotics, and probiotics. The evolving understanding of the complex connections involving nutrition, gut microbiome composition, microbiome diversity, and microbiome by-products present a yet another promising avenue for transformative research. The Mediterranean diet emerges as a potential ally, fostering anti-inflammatory states and supporting a gut microbiome conducive to balanced immunogenicity. Specific nutritional interventions, such as the ketogenic diet and fasting, exploit high metabolic tumor cells' demands in order to diminish the unfavorable TME immunoediting.

Additionally, dietary components like the omega 6:3 ratio and vitamin D, often deficient in cancer patients, may influence immune responses through their anti-inflammatory properties. As we navigate through this intricate landscape, clinicians and researchers must consider tailored non-invasive strategies to manipulate microbial communities and metabolites, aiming to counter dysbiosis and enhance immune regulation. This holistic approach calls for a paradigm change which may potentially broaden the accessibility of immunotherapy for a wider patient population while mitigating the severity of treatment-related side effects.

Author Contributions: We hereby declare that both Adi David and Shaked Lev-Ari have made substantial contributions to the conception and writing of this work; AND has approved the submitted version; AND agrees to be personally accountable for the author's own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and documented in the literature.

Funding: This research received no external funding

Conflicts of Interest: The authors declare no conflict of interest

References

1. DePeaux K, Delgoffe GM. Metabolic barriers to cancer immunotherapy. Nat Rev Immunol. 2021 Dec 29;21(12):785–97.
2. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. Nat Rev Immunol. 2020 Nov 20;20(11):651–68.
3. Szczyrek M, Bitkowska P, Chunowski P, Czuchryta P, Krawczyk P, Milanowski J. Diet, Microbiome, and Cancer Immunotherapy—A Comprehensive Review. Nutrients. 2021 Jun 28;13(7):2217.
4. Wang S, Xie K, Liu T. Cancer Immunotherapies: From Efficacy to Resistance Mechanisms – Not Only Checkpoint Matters. Front Immunol. 2021 Jul 21;12.
5. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. Cell. 2017 Feb;168(4):707–23.
6. Cortellino S, Longo VD. Metabolites and Immune Response in Tumor Microenvironments. Cancers (Basel). 2023 Jul 31;15(15):3898.
7. Verma V, Shrimali RK, Ahmad S, Dai W, Wang H, Lu S, et al. PD-1 blockade in subprimed CD8 cells induces dysfunctional PD-1+CD38hi cells and anti-PD-1 resistance. Nat Immunol. 2019 Sep 29;20(9):1231–43.
8. Russo E, Nannini G, Dinu M, Pagliai G, Sofi F, Amedei A. Exploring the food-gut axis in immunotherapy response of cancer patients. World J Gastroenterol. 2020 Sep 7;26(33):4919–32.
9. Zheng D, Ratiner K, Elinav E. Circadian Influences of Diet on the Microbiome and Immunity. Trends Immunol. 2020 Jun;41(6):512–30.
10. Nova E, Gómez-Martinez S, González-Soltero R. The Influence of Dietary Factors on the Gut Microbiota. Microorganisms. 2022 Jul 7;10(7):1368.
11. Zhang P. Influence of Foods and Nutrition on the Gut Microbiome and Implications for Intestinal Health. Int J Mol Sci. 2022 Aug 24;23(17):9588.
12. Sgro M, Kodila ZN, Brady RD, Reichelt AC, Mychaisuk R, Yamakawa GR. Synchronizing our clocks as we age: the influence of the brain-gut-immune axis on the sleep-wake cycle across the lifespan. Sleep. 2022 Mar 14;45(3).
13. Spencer CN, McQuade JL, Gopalakrishnan V, McCulloch JA, Vetizou M, Cogdill AP, et al. Dietary fiber and probiotics influence the gut microbiome and melanoma immunotherapy response. Science (1979). 2021 Dec 24;374(6575):1632–40.
14. Baruch EN, Youngster I, Ben-Betzalel G, Ortenberg R, Lahat A, Katz L, et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. Science (1979). 2021 Feb 5;371(6529):602–9.
15. McQuade JL, Daniel CR, Helmink BA, Wargo JA. Modulating the microbiome to improve therapeutic response in cancer. Lancet Oncol. 2019 Feb;20(2):e77–91.
16. Su J, Wang Y, Zhang X, Ma M, Xie Z, Pan Q, et al. Remodeling of the gut microbiome during Ramadan-associated intermittent fasting. Am J Clin Nutr. 2021 May;113(5):1332–42.
17. Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. Nat Rev Immunol. 2016 Jun 27;16(6):341–52.
18. Gopalakrishnan V, Helmink BA, Spencer CN, Reuben A, Wargo JA. The Influence of the Gut Microbiome on Cancer, Immunity, and Cancer Immunotherapy. Cancer Cell. 2018 Apr;33(4):570–80.

19. Davar D, Dzutsev AK, McCulloch JA, Rodrigues RR, Chauvin JM, Morrison RM, et al. Fecal microbiota transplant overcomes resistance to anti–PD-1 therapy in melanoma patients. Science (1979). 2021 Feb 5;371(6529):595–602.
20. Shaikh FY, Gills JJ, Sears CL. Impact of the microbiome on checkpoint inhibitor treatment in patients with non-small cell lung cancer and melanoma. EBioMedicine. 2019 Oct;48:642–7.
21. Simpson RC, Shanahan ER, Batten M, Reijers ILM, Read M, Silva IP, et al. Diet-driven microbial ecology underpins associations between cancer immunotherapy outcomes and the gut microbiome. Nat Med. 2022 Nov 22;28(11):2344–52.
22. Zmora N, Suez J, Elinav E. You are what you eat: diet, health and the gut microbiota. Nat Rev Gastroenterol Hepatol. 2019 Jan 27;16(1):35–56.
23. Xu X, Ying J. Gut Microbiota and Immunotherapy. Front Microbiol. 2022 Jul 1;13.
24. Usyk M, Pandey A, Hayes RB, Moran U, Pavlick A, Osman I, et al. Bacteroides vulgatus and Bacteroides dorei predict immune-related adverse events in immune checkpoint blockade treatment of metastatic melanoma. Genome Med. 2021 Dec 13;13(1):160.
25. Lobel L, Cao YG, Fenn K, Glickman JN, Garrett WS. Diet posttranslationally modifies the mouse gut microbial proteome to modulate renal function. Science (1979). 2020 Sep 18;369(6510):1518–24.
26. Yoon SJ, Lee C Bin, Chae SU, Jo SJ, Bae SK. The Comprehensive "Omics" Approach from Metabolomics to Advanced Omics for Development of Immune Checkpoint Inhibitors: Potential Strategies for Next Generation of Cancer Immunotherapy. Int J Mol Sci. 2021 Jun 28;22(13):6932.
27. Nagpal R, Shively CA, Register TC, Craft S, Yadav H. Gut microbiome-Mediterranean diet interactions in improving host health. F1000Res. 2019 May 21;8:699.
28. Belkaid Y, Hand TW. Role of the Microbiota in Immunity and Inflammation. Cell. 2014 Mar;157(1):121–41.
29. Xiong RG, Zhou DD, Wu SX, Huang SY, Saimaiti A, Yang ZJ, et al. Health Benefits and Side Effects of Short-Chain Fatty Acids. Foods. 2022 Sep 15;11(18):2863.
30. Malczewski AB, Navarro S, Coward JI, Ketheesan N. Microbiome-derived metabolome as a potential predictor of response to cancer immunotherapy. J Immunother Cancer. 2020 Oct 30;8(2):e001383.
31. Bolte LA, Lee KA, Björk JR, Leeming ER, Campmans-Kuijpers MJE, de Haan JJ, et al. Association of a Mediterranean Diet With Outcomes for Patients Treated With Immune Checkpoint Blockade for Advanced Melanoma. JAMA Oncol. 2023 May 1;9(5):705.
32. Meslier V, Laiola M, Roager HM, De Filippis F, Roume H, Quinquis B, et al. Mediterranean diet intervention in overweight and obese subjects lowers plasma cholesterol and causes changes in the gut microbiome and metabolome independently of energy intake. Gut. 2020 Jul;69(7):1258–68.
33. Rinott E, Meir AY, Tsaban G, Zelicha H, Kaplan A, Knights D, et al. The effects of the Green-Mediterranean diet on cardiometabolic health are linked to gut microbiome modifications: a randomized controlled trial. Genome Med. 2022 Mar 10;14(1):29.
34. Rao Y, Kuang Z, Li C, Guo S, Xu Y, Zhao D, et al. Gut Akkermansia muciniphila ameliorates metabolic dysfunction-associated fatty liver disease by regulating the metabolism of L-aspartate via gut-liver axis. Gut Microbes. 2021 Jan 1;13(1).
35. Deligiorgi M V., Liapi C, Trafalis DT. How Far Are We from Prescribing Fasting as Anticancer Medicine? Int J Mol Sci. 2020 Dec 1;21(23):9175.
36. Turbitt WJ, Demark-Wahnefried W, Peterson CM, Norian LA. Targeting Glucose Metabolism to Enhance Immunotherapy: Emerging Evidence on Intermittent Fasting and Calorie Restriction Mimetics. Front Immunol. 2019 Jun 25;10.
37. Eriau E, Paillet J, Kroemer G, Pol JG. Metabolic Reprogramming by Reduced Calorie Intake or Pharmacological Caloric Restriction Mimetics for Improved Cancer Immunotherapy. Cancers (Basel). 2021 Mar 12;13(6):1260.
38. Clifton KK, Ma CX, Fontana L, Peterson LL. Intermittent fasting in the prevention and treatment of cancer. CA Cancer J Clin. 2021 Nov 12;71(6):527–46.
39. Bader JE, Voss K, Rathmell JC. Targeting Metabolism to Improve the Tumor Microenvironment for Cancer Immunotherapy. Mol Cell. 2020 Jun;78(6):1019–33.
40. Collins N, Han SJ, Enamorado M, Link VM, Huang B, Moseman EA, et al. The Bone Marrow Protects and Optimizes Immunological Memory during Dietary Restriction. Cell. 2019 Aug;178(5):1088-1101.e15.
41. Vernieri C, Fucà G, Ligorio F, Huber V, Vingiani A, Iannelli F, et al. Fasting-Mimicking Diet Is Safe and Reshapes Metabolism and Antitumor Immunity in Patients with Cancer. Cancer Discov. 2022 Jan 1;12(1):90–107.
42. Sadeghian M, Rahmani S, Khalesi S, Hejazi E. A review of fasting effects on the response of cancer to chemotherapy. Clinical Nutrition. 2021 Apr;40(4):1669–81.
43. Ajona D, Ortiz-Espinosa S, Lozano T, Exposito F, Calvo A, Valencia K, et al. Short-term starvation reduces IGF-1 levels to sensitize lung tumors to PD-1 immune checkpoint blockade. Nat Cancer. 2020 Jan 13;1(1):75–85.

44. de Gruil N, Pijl H, van der Burg SH, Kroep JR. Short-Term Fasting Synergizes with Solid Cancer Therapy by Boosting Antitumor Immunity. Cancers (Basel). 2022 Mar 9;14(6):1390.
45. Zhao R, Zhao X, Wang X, Liu Y, Yang J, Jiang S, et al. Fasting promotes acute hypoxic adaptation by suppressing mTOR-mediated pathways. Cell Death Dis. 2021 Nov 3;12(11):1045.
46. Cortellino S, Longo VD. Metabolites and Immune Response in Tumor Microenvironments. Cancers (Basel). 2023 Jul 31;15(15):3898.
47. Mao YQ, Huang JT, Zhang SL, Kong C, Li ZM, Jing H, et al. The antitumour effects of caloric restriction are mediated by the gut microbiome. Nat Metab. 2023 Jan 16;5(1):96–110.
48. Brandhorst S. Fasting and fasting-mimicking diets for chemotherapy augmentation. Geroscience. 2021 Jun 7;43(3):1201–16.
49. Guo Y, Luo S, Ye Y, Yin S, Fan J, Xia M. Intermittent Fasting Improves Cardiometabolic Risk Factors and Alters Gut Microbiota in Metabolic Syndrome Patients. J Clin Endocrinol Metab. 2021 Jan 1;106(1):64–79.
50. Maifeld A, Bartolomaeus H, Löber U, Avery EG, Steckhan N, Markó L, et al. Fasting alters the gut microbiome reducing blood pressure and body weight in metabolic syndrome patients. Nat Commun. 2021 Mar 30;12(1):1970.
51. Forslund SK. Fasting intervention and its clinical effects on the human host and microbiome. J Intern Med. 2023 Feb 22;293(2):166–83.
52. Newman JC, Verdin E. Ketone bodies as signaling metabolites. Trends in Endocrinology & Metabolism. 2014 Jan;25(1):42–52.
53. Nencioni A, Caffa I, Cortellino S, Longo VD. Fasting and cancer: molecular mechanisms and clinical application. Nat Rev Cancer. 2018 Nov 16;18(11):707–19.
54. Weber DD, Aminzadeh-Gohari S, Thapa M, Redtenbacher AS, Catalano L, Capelôa T, et al. Ketogenic diets slow melanoma growth in vivo regardless of tumor genetics and metabolic plasticity. Cancer Metab. 2022 Dec 18;10(1):12.
55. Nutrition in Cancer Care (PDQ®)–Health Professional Version.
56. Dizman N, Hsu J, Bergerot PG, Gillece JD, Folkerts M, Reining L, et al. Randomized trial assessing impact of probiotic supplementation on gut microbiome and clinical outcome from targeted therapy in metastatic renal cell carcinoma. Cancer Med. 2021 Jan;10(1):79–86.
57. van der Hee B, Wells JM. Microbial Regulation of Host Physiology by Short-chain Fatty Acids. Trends Microbiol. 2021 Aug;29(8):700–12.
58. Ma YJ, Yu J, Xiao J, Cao BW. The Consumption of Omega-3 Polyunsaturated Fatty Acids Improves Clinical Outcomes and Prognosis in Pancreatic Cancer Patients: A Systematic Evaluation. Nutr Cancer. 2015 Jan 2;67(1):112–8.
59. Galus Ł, Michalak M, Lorenz M, Stoińska-Swiniarek R, Tusień Małecka D, Galus A, et al. Vitamin D supplementation increases objective response rate and prolongs progression-free time in patients with advanced melanoma undergoing anti–PD-1 therapy. Cancer. 2023 Jul 24;129(13):2047–55.
60. Sun L, Arbesman J, Piliang M. Vitamin D, autoimmunity and immune-related adverse events of immune checkpoint inhibitors. Arch Dermatol Res. 2021 Jan 9;313(1):1–10.
61. NCCN Guidelines Version 4.2023 Colon Cancer. 2023.