Research Trends Through Bibliometric Analysis of Fecal Microbiota Transfers for Immunotherapy

Astri Dewayani[1*], Ricky Indra Alfaray[2,3], Takashi Kobayashi[4,5] and Firas Farisi Alkaff[1,6*]

[1]Department of Anatomy, Histology and Pharmacology, Faculty of Medicine, Universitas Airlangga, Surabaya 60131, Indonesia

[2]Institute of Tropical Disease, Universitas Airlangga, Surabaya 60115, Indonesia

[3]Department of Environmental and Preventive Medicine, Faculty of Medicine, Oita University, Yufu 879-5593, Japan

[4]Department of Infectious Disease Control, Faculty of Medicine, Oita University, Yufu 879-5593, Japan

[5]Center for GLOBAL and LOCAL Infectious Diseases (RCGLID), Oita University, Yufu, 879-5593, Japan

[6]Division of Nephrology, Department of Internal Medicine, University of Groningen, University Medical Center Groningen, Groningen 9713 GZ, The Netherlands

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*Corresponding authors: astri.dewayani@fk.unair.ac.id; f.f.alkaff@umcg.nl

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Abstract

Objectives: Cancer immunotherapy has become a vital component of cancer treatment. However, the innovative approaches developed have shown varying levels of efficacy, with only a small percentage of patients benefiting from the treatment. A significant amount of ongoing research suggests that modulating the intestinal microbiota through fecal microbiota transplants (FMT) may help improve response rates and minimize complications. This study presents an analysis of research evolution within FMT in cancer immunotherapy to identify trends, hotspots and potential themes.

Methods: We searched for articles relating to FMT in immunotherapy on Scopus covering publications between 2014 and 2024. Bibliometric analysis was performed using R Biblioshiny and VoSviewer. Data on top authors, journals, and most cited articles are displayed. Keyword analysis was further explored through co-occurrence networks and thematic mapping.

Results: A total of 557 articles were included. The annual publications' growth rate was 53.58% over the past 10 years. *Frontiers in Immunology* had the highest number of publications. The top contributing countries were China (973 publications, 3,191 citations), the USA (941 publications, 11,965 citations), Italy (372 publications, 1,081 citations), and France (306 publications, 9,045 citations). Six clustering labels were generated from 76 keywords, with "immunotherapy" and "microbiome" being the most frequent.

Conclusions: China's high research output has become the most contributing; however, the USA had a higher proportion of inter-country collaboration and citation. Current trends in FMT research are exploring pharmacomicrobiomics and immune response functions. FMT is expected to continue requiring clinical integration, particularly considering microbiota differences due to demographic variations across countries.

Keywords: fecal microbiota transfer: immunotherapy; cancer treatment; publication trends

Introduction

Immunotherapy is a novel biotherapy designed to enhance immune response against cancer. Various immunotherapy drugs, including checkpoint inhibitors (ICIs), vaccines, or chimeric antigen receptor (CAR) T cells, have been developed to achieve effective treatments. The current widely used ICIs are cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4), programmed death-1 receptor (PD-1), and its ligand (PDL-1).[1] Even so, the efficacy varies widely among patients and can be discontinued due to primary and secondary immune resistance, even immune-related adverse events.[2,3] Therefore, determining methods to enhance immunotherapy efficacy and responsiveness is of utmost concern.

More than 2,000 different species of bacteria make up the adult gut microbiota, with increased density and diversity from the stomach to the colon. The healthy gut microbiota is a diverse, stable, resistant, and resilient microbial ecosystem.[4] Findings revealed that the crosstalk between the gut microbiome and the host immune system has evolved into an interrelated, multifold network. Modulation of the gut microbiota helps in the understanding of disorders related to its impairment.

Fecal microbiota transplantation (FMT) is a novel intervention technique that involves reshaping the host's gut microbiome by transferring fecal material containing gut microbiota from a healthy donor to a recipient who has a disrupted microbiota. It has gained increasing attention due to its safety and effectiveness in clinical settings.[5] Emerging evidence suggests that FMT holds promise beyond infectious diseases, potentially influencing the efficacy of immunotherapies.[2,6] As the utility of FMT continues to be uncovered, we collected evidence and ongoing research to explore global trends in fecal microbiota transplantation and immunotherapy, and to predict future directions in this emerging field.

Methods

Search strategy

We selected the Scopus database to search for relevant literature on 8 July, 2024. Scopus database was selected because it is one of the largest citation databases, larger than Web of Science, and covers a wide range of subjects.[7] The search strategy was the combination of fecal microbiota transplants and immunotherapy using the following words: #1 TS = TITLE-ABS-KEY ‘Fecal Microbiota Transplantation’ OR ‘Fecal Microbiota Transfer’ OR ‘Fecal Microbiota Transplants’) and #2 TS = TITLE-ABS-KEY (‘Immunotherapy’ OR ‘Immunotherapeutic’ OR ‘Immunotherapies’). The publication period was specified from 2014 to 2024. The type of literature was limited to articles and reviews, and the language was limited to English. We searched and screened all the papers within one day to ensure the consistency of the data. The data were exported from Scopus, including citation information, bibliographical information, abstract, keywords and references. Duplicates were identified and removed based on Scopus electronic identifier to ensure each publication was counted only once. Consistencies of the authors’ affiliation were checked by two authors (AD and RIA). The flowchart of the screening process is shown in Figure 1.

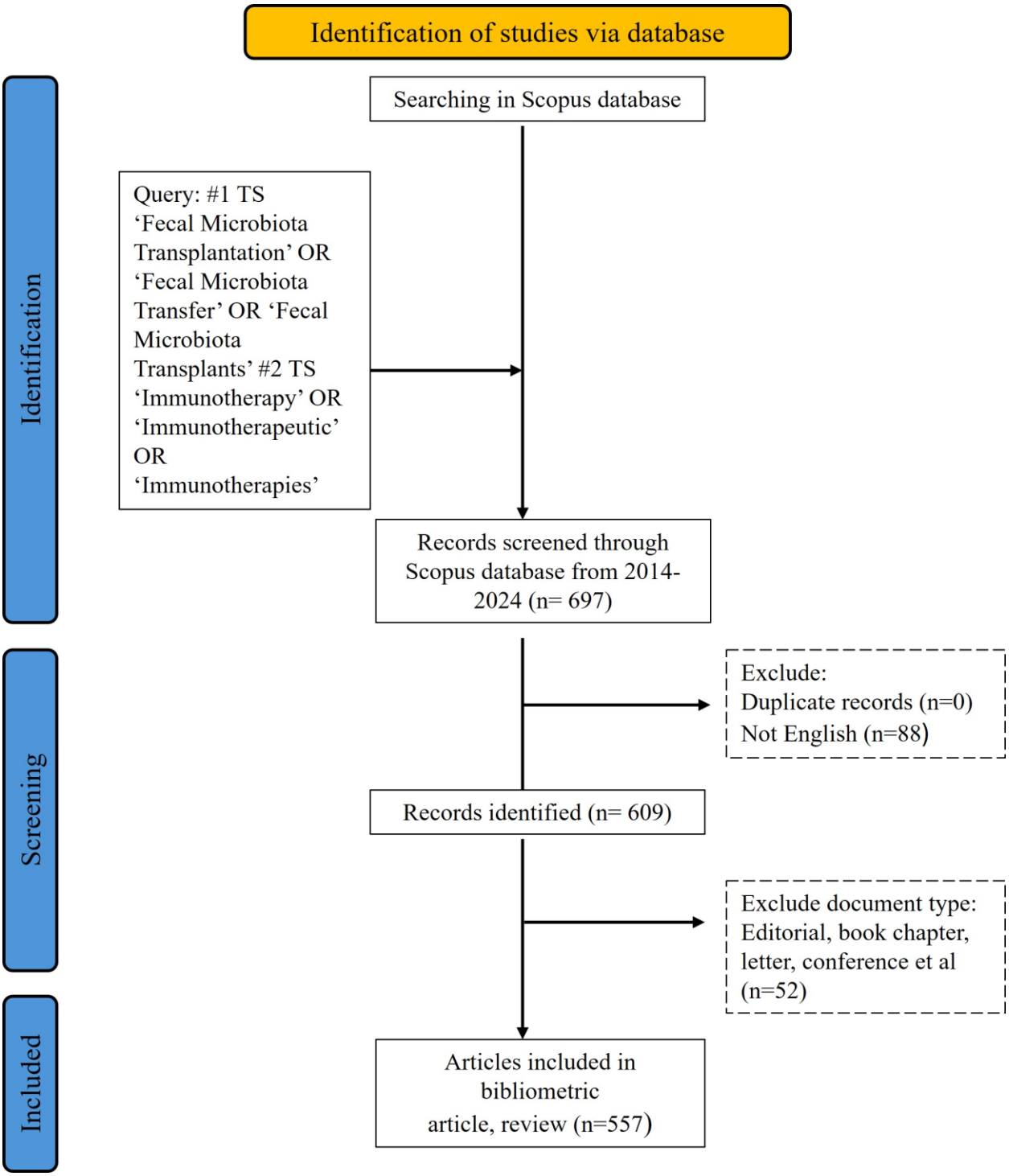

Figure 1: Flowchart of the literature screening.

Data analysis and visualization process

The bibliometric analysis software VOSviewer (ver. 1.6.20) was engaged to map the co-occurrence of keywords and collaboration link countries. The R-based Biblioshiny software package was used to visualize the publication trends, top productive authors and institutions in the field. The affiliation collaboration map and conceptual structure maps

help in indicating research patterns in a specific discipline or knowledge field over time, allowing for a more accurate understanding of the evolution of a given scientific frontier.[8]

Results

General analysis of publication trends

A total of 557 studies were included in the analysis (Fig. 1), encompassing 151 articles and 406 reviews. Over the past 10 years, the overall trends of publication have been rising with an annual growth rate of 53.58%.

There was a gradual increase in the number of publications from 2014 to 2018. Before 2018, the number of articles was less than 10 per year. Then it rapidly rose after 2019, with the peak of annual publications seems to be yet to come, reaching 123 in 2023. This trend demonstrates the growing recognition among scholars of the significance of gut microbiota's role through FMT in immunotherapy (Fig. 2a).

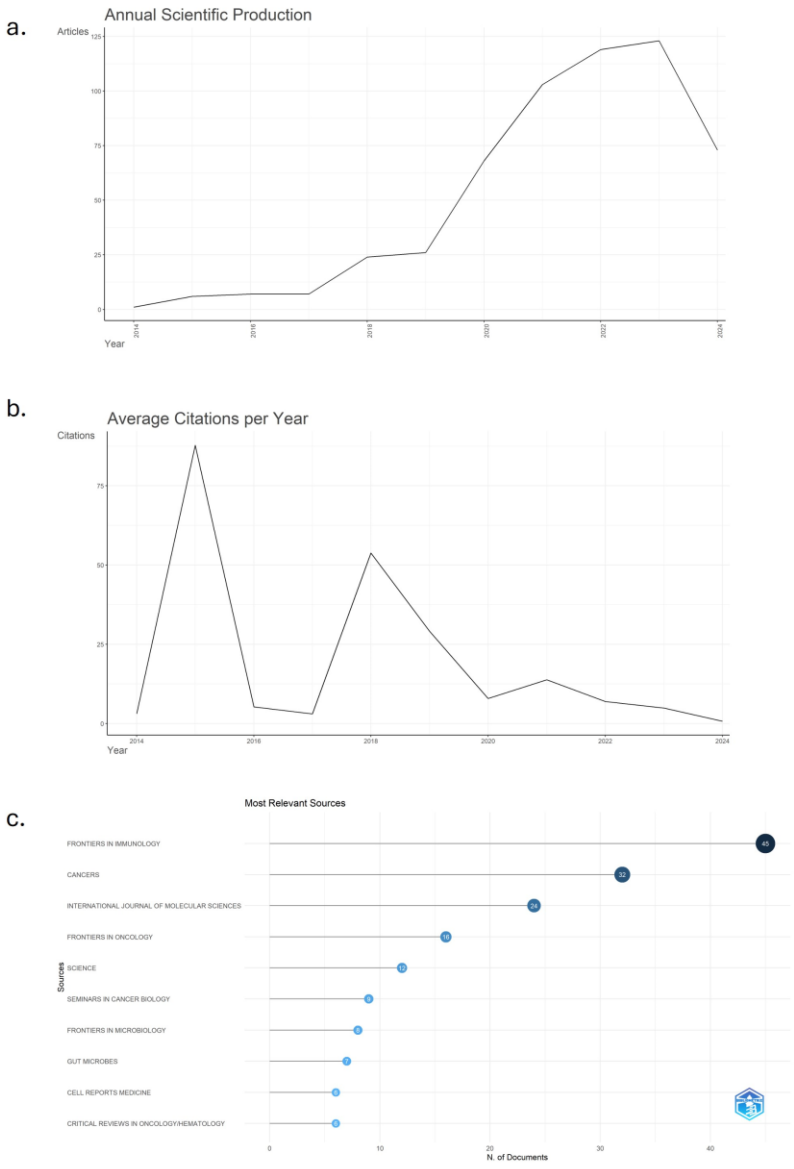

Figure 2: General publication trend related FMT and immunotherapy. (a.) Annually published scientific paper over the decade. (b.) Average citation per year. (c.) top 10 most relevant sources.

The average annual citation (Fig. 2b) shows a spike of cited papers in 2015 at 87.80, which indicates the beginning of attention toward the microbiota's role in disease treatment. Following that, it went low and spiked again in 2018 at 53.79. In 2020-2023, despite the volume of published papers showing an upward trend, the average citation of papers declined, depicting a decrease in the influence of papers in the field of fecal microbiota transplantation and immunotherapy.

The published journal articles concentrated on immunology, oncology and molecular areas. The top 10 relevant journals according to the publication volumes are shown in Fig. 2c. *Frontiers in Immunology* has the most relevant sources (n=45). However, other journals, such as *Science Immunology*, started to publish articles on fecal microbiota transplantation and immunotherapy from 2018 onwards.

Active countries, authors and affiliations

There were 2892 researchers who contributed to the research of this topic, with the top 10 authors collectively producing 108 papers or approximately 19.3% of the total collected publications (Fig.3a). The top 2 most number of publication contributors are in line with their affiliation as the most active one. University of Texas MD Anderson Cancer Center led the number of publications with 199 articles (Fig. 3b). The institutions are located in the USA, and the country became the second most corresponding (n=133), while China leads the publication volume as the most corresponding (n=147). Even so, the USA had a higher proportion of inter-country collaboration than China. This suggests that in the high-output research field, there is still a lack of collaboration between China and other countries (Fig. 3c). Outside the top five countries, the Asian region—including India, Japan, Hong Kong, and South Korea—each contributed fewer than 12 documents. Middle Eastern countries such as Iran and Israel had five documents each, as did Brazil from Latin America (Fig. 3c).

Figure 3: Analysis of authors and corresponding affiliation countries. (a.) Top ten writers according to published works, (b.) Top relevant author affiliation, (c.) Most relevant corresponding countries, red symbolizes intra-countries and green symbolize inter-countries collaboration (d.) Country production over time between 2014-2024, (e.) Top cited corresponding countries.

There was a significant increase in the number of publications from China, which grew rapidly from 2022, becoming the largest contributor (n=973), overtook the USA (n=941). Similarly, Italy (n=372) surpassed France (n=306) (Fig. 3d). Nevertheless, the USA and France have steadily continued to produce papers and remain the most cited countries (with 11,965 citations and 9,405 citations, respectively) (Fig. 3e). The number of publications from the top countries exceeds the total of 557 documents included in the current study. This discrepancy arises because the countries of all co-authors are counted, resulting in multiple country appearances per article.

Figure 4a displays the cooperation international network of countries/regions connected to each other. According to their cooperation, 28 countries are clustered into five groups. The USA was the most cooperative with 24

collaborating countries (total link strength (TLS) = 90), followed by France (TLS = 61) and Italy (TLS = 51). The cooperation cluster network of institutions connected to each other is shown in Fig. 4b.

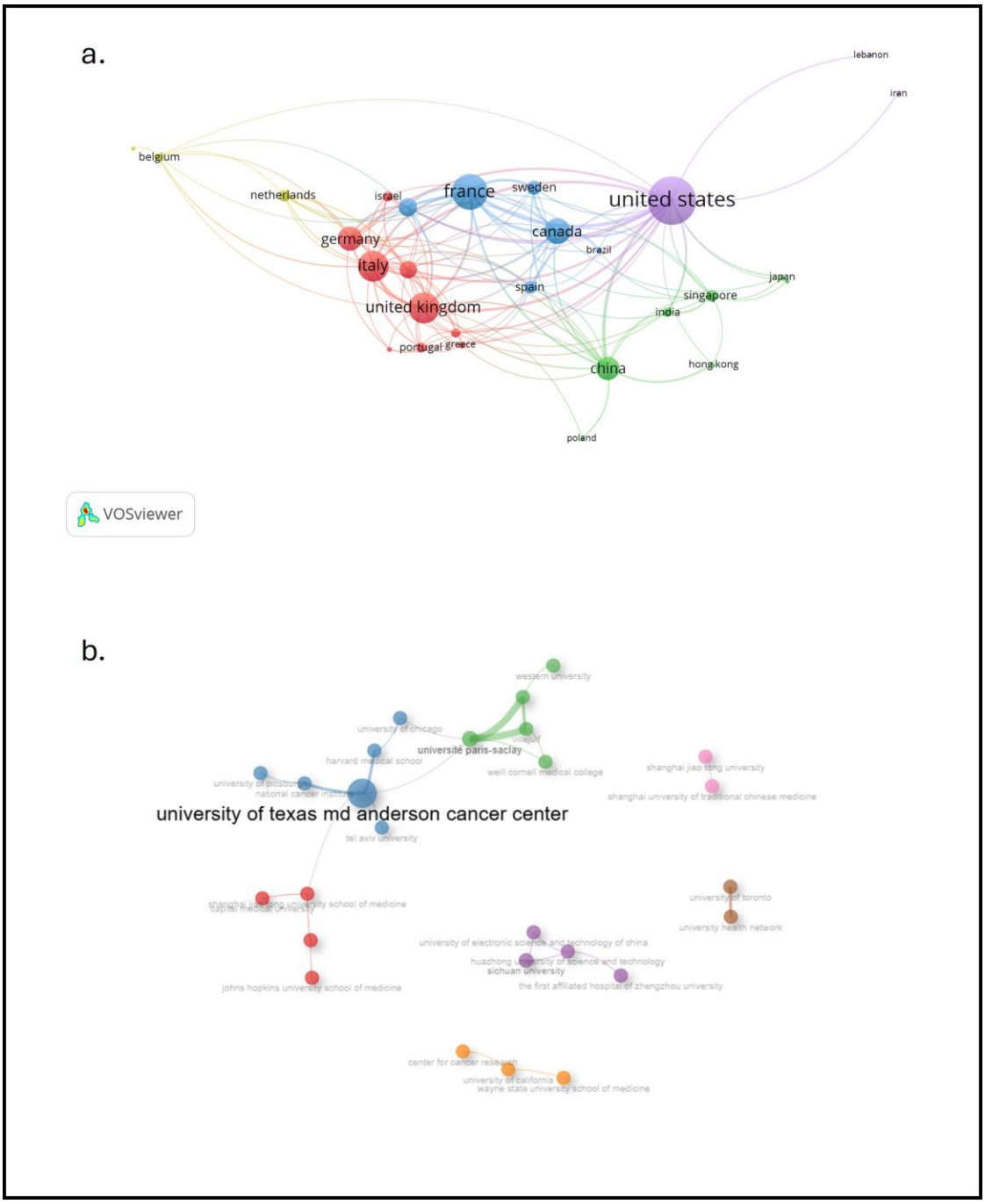

Figure 4: Corresponding affiliation link. Node size represents the influence and frequency, while the link shows cooperative relationship (a) Countries collaboration. (b) Institution collaboration.

Table 1 summarizes the top 10 cited papers in the field of fecal microbiota transplantation and immunotherapy. The top 4 papers were published in *Science Immunology*. Routy et al., published a paper entitled "Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors", and it was cited 3570 times. They demonstrated that fecal microbiota transplantation from ICI responded patients can reduce melanoma growth in

antibiotic-treated mice by enhancing PD-1 blockade efficacy. In addition to that, they identified that bi-colonized mice with *Akkermansia muciniphila* and *Enterococcus hirae* increased Th1-produced immune cells and CD4/FoxP3 ratios, which helped ameliorate the compromised PD-1 blockade.[9]

Table 1: Most cited articles.

Paper	Title	Total Citations	TC per Year
ROUTY, B. et al., 2018, SCIENCE[9]	Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors	3570	510.00
GOPALAKRISHNAN, V. et al., 2018, SCIENCE[26]	Gut microbiome modulates response to anti–PD-1 immunotherapy in melanoma patients	3012	430.29
SIVAN, A. et al., 2015, SCIENCE[21]	Commensal Bifidobacterium promotes antitumor immunity and facilitates anti–PD-L1 efficacy	2750	275.00
VÉTIZOU, M. et al., 2015, SCIENCE[27]	Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota	2469	246.90
GALON, J. AND BRUNI D, 2019, NAT REV DRUG DISCOV[10]	Approaches to treat immune hot, altered and cold tumours with combination immunotherapies	1995	332.50
GOPALAKRISHNAN, V. et al., 2018, CANCER CELL[28]	The Influence of the Gut Microbiome on Cancer, Immunity, and Cancer Immunotherapy	845	120.71
ZHAO, H. et al., 2021, SIGNAL TRANSDUCT TARGET THER[29]	Inflammation and tumor progression: signaling pathways and targeted intervention	843	210.75
BARUCH, E.N. et al,, 2021, SCIENCE[6]	Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients	791	197.75
DAVAR, D. et al., 2021, SCIENCE[30]	Fecal microbiota transplant overcomes resistance to anti–PD-1 therapy in melanoma patients	768	192.00
HELMINK, B.A. et al., 2019, NAT MED[31]	The microbiome, cancer, and cancer therapy	699	116.50

In essence, these significant studies primarily address the function of the use of FMT as an adjuvant therapy for solid tumors that are treated with ICI. A review by Galon and Bruni in 2019 classified solid tumors based on their immune infiltration and contexture in the tumor microenvironment: hot (highly infiltrated), altered and cold (less infiltrated). The hot type can be managed with T cell-targeting immunotherapies like ICIs, but it is still limited in cold and altered tumors treatment. Efforts in converting cold tumors into inflamed hot type to achieve better response to immunotherapy through combination treatments include microbiome modulation.[10]

Evolution of keywords

Keywords show high generalization of a study topic and content. Evaluating high-frequency keywords could provide a clear picture of the hotspots and research trends in the fields of immunotherapy and FMT. Fig. 5a shows the results of a cluster and co-occurrence analysis of terms. Using a minimum co-occurrence threshold of 5, a total of six clustering labels were produced from 76 keywords analysis using VOSviewer. Blue cluster primary keywords include "immunotherapy" and "microbiome". This illustrates the immunotherapy links to the greatest number of other keywords. The yellow cluster represents "fecal microbiota transplantation" linked to "dysbiosis" and "biomarker."

The keyword evolution is grouped in three timing frames (Fig. 5b). The flow between the nodes depicts the evolutionary orientation of the theme complexes, with certain topics gradually evolving and developing, with others

gaining importance. From 2014 to 2017, the primary keywords focused on bacteria and inflammatory bowel disease. During 2018 to 2020, with the increasing number of studies, keywords such as immunotherapy, gut microbiota, and immune checkpoints became more prominent. In 2021 to 2024, the most frequent keyword was immunotherapy, accompanied by the emergence of terms like vaccines, tumor microenvironment, and adoptive cell therapy.

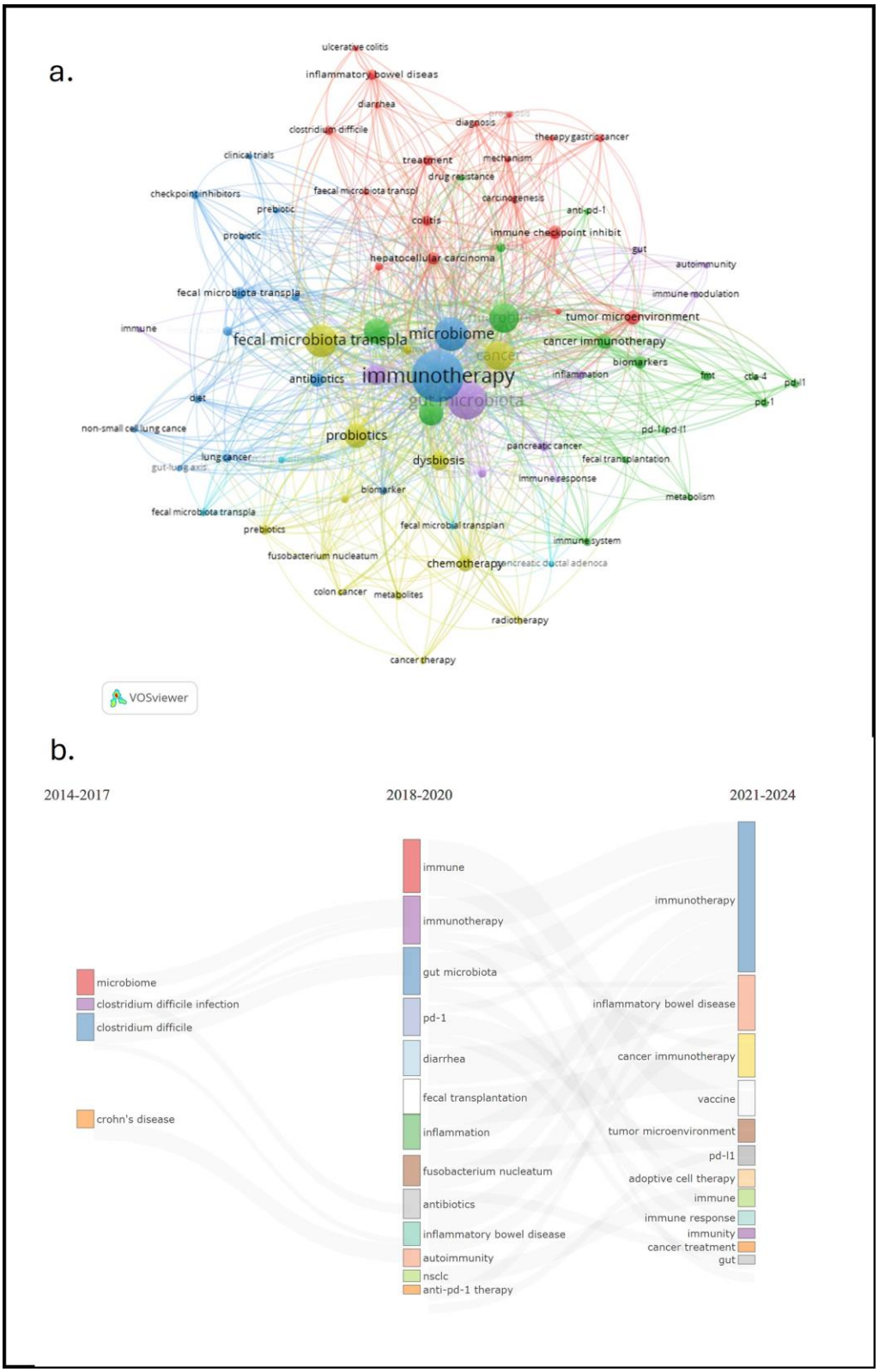

Figure 5: Analysis of keywords (a.) Co-occurrence frequency is represented by node size, network clustering indicated by color, and a closer association between words is shown by proximity to another node of the same color. (b.) Evolutionary timeline highlights the usage and emergence of various keywords

Thematic maps of keywords

The thematic map (Fig. 6) is a conceptual framework utilizing "author's keywords" as the variable, illustrating the present topic groups within the domains of "fecal microbiota transplantation" and "immunotherapy." The map is divided into four quadrants based on relevance and degree of development. The first quadrant in the upper-right represents the motor themes, which are characterized by both high density and centrality. These themes are mature and essential for organizing the topic. Researchers took an in-depth analysis in the use of FMT for immune checkpoint inhibitor and inflammatory bowel disease.

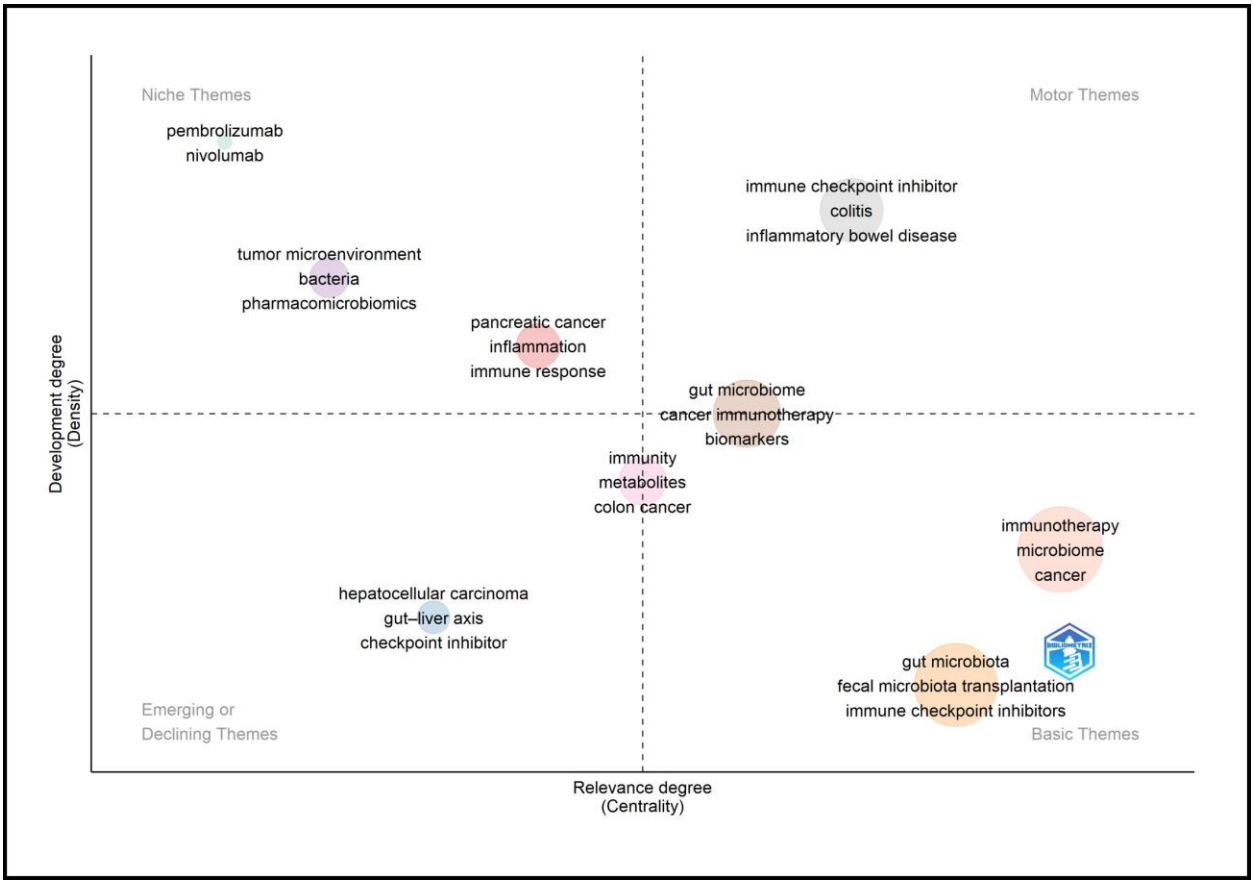

Figure 6: Thematic map of keywords. The motor theme represents the central topic in the field, the basic theme portrays foundational concepts, niche theme refers to highly specialized areas of research, and emerging or declining indicate emerging or previous declining themes.

Themes in the second quadrant exhibit a high degree of development but a lower degree of relevance, indicating robust development momentum but limited connection to mainstream research in the field. Themes such as pembrolizumab, tumor microenvironment and pharmacomicrobiomics are in the quadrant. Scholars have paid attention to the role of microbiota in anti-cancer pharmacokinetics besides their involvement with immune cells.[11,12]

The third quadrant, characterized by low density and centrality, shows that the direction will be either emerging or declining. The potential themes are the gut-liver axis and checkpoint inhibitors. The fourth quadrant, with high relevance but low development, represents basic themes that usually serve as the foundation for understanding a particular field. As seen in Fig. 6, microbiome, cancer, and gut microbiota are basic themes in the exploration area.

Discussion

In this study, we analyzed publications related to FMT and immunotherapy from 2014 to 2024. The findings indicate a significant rise in the number of annual publications starting from 2018, with an annual growth rate exceeding 50%. Two peaks and a subsequent decline in annual citation data reflect strong interest in this topic, although further exploration is needed to support advances in immunotherapy. Moreover, the gap in scientific influence is likely due to FMT still being in the early stages of exploration, requiring further experimental validation and clinical integration. Although the recent publication output is high in number, it has not yet received sufficient recognition, as reflected in the decline in average citations over the past three years.

The USA and France have long been leading contributors to research on immunotherapy and FMT; however, the predominant active contribution and corresponding author now comes from China, which has seen rapid growth in research output over the past two years, surpassing that of the USA. This trend aligns with the number of inter-country collaborations, the most-cited country, and the most active affiliations, which continue to be based in the USA. Such high levels of research output from China can be partly attributed to the high attention and financial support of the government and research community where the policies encouraging on paper quality rather than quantity of collaboration.[13]

From the perspective of top articles, several high-quality and most-cited papers were first published in *Science Immunology*, even though *Frontiers in Immunology* has seen a higher number of publications, especially with a significant increase in the last five years (Fig 2b). Additionally, based on the analysis of keyword co-occurrence clustering and highly cited literature, we discovered the research hotspots and frontiers are as follows:

FMT and pharmacomicrobiomics

Pharmacomicrobiomics explores the interaction between drugs and the microbiome, focusing on how gut microbiota influences drug efficacy, toxicity, and metabolism. FMT has been explored as an adjunct therapy in cancer treatment to enhance the response to immunotherapy and chemotherapy by modulating the gut microbiota. Mechanism in this ability: 1) drug metabolism and activation[11]; 2) enhancing drug efficacy[14]; 3.) reducing drug toxicity.[15]

Gut microbiota can activate or inactivate drugs, affecting their therapeutic outcomes. It can metabolize prodrugs, such as fluoropyrimidines, into their active forms, which are crucial for their cytotoxic effects against cancer cells. Furthermore, specific microbial metabolites like butyrate can enhance the efficacy of drugs like oxaliplatin by activating cytotoxic T cells.[11]

Improved PD-1/PD-L1 blocker efficacy has been linked to higher relative abundances of *Bifidobacterium*, *Fecalibacterium*, and other *Ruminococcaceae* taxa, probably due to increased antigen presentation and improved effector T cell activity in both systemically and locally in the tumor microenvironment.[9] Nevertheless, a high production of short-chain fatty acids (SCFAs) by *Ruminococcaceae* (propionate and butyrate) appeared to limit the antitumor activity of anti-CTLA-4, with reduced systemic inflammation and immune activation in tumor-bearing mice.[16]

FMT and immune response

The gut microbiota plays a crucial role in shaping the immune system. Dysbiosis in the gut microbiota is linked to various immune-related diseases, including inflammatory bowel disease (IBD), autoimmune disorders, and cancers. FMT can help reestablish a healthy microbial balance, potentially leading to improved immune regulation and reduced inflammation.[17] The mechanisms through which FMT influences the immune response are still being elucidated. It is believed that a diverse and balanced gut microbiota promotes the production of SCFAs and other metabolites that enhance regulatory T cell function and reduce pro-inflammatory cytokines, thus supporting a more effective immune response.[18]

The initial report on FMT was done to treat *Clostridium difficile* recurrent infection[17] The treatment was found to be able to restore IL-10-producing cells, including Tregs and DCs[19] or increase the production of antimicrobial peptides (Camp and S100A8) and mucins (Muc1 and Muc4) alongside expansion of innate lymphoid cells (ILC)-2 and ILC-3.[20] The potential is also shown to modulate cancer immunotherapy. Sivan et al. explored the crosstalk in melanoma mouse-model given fecal transfer. The mechanism involved augmented dendritic cell function, leading to enhanced CD8+ T cell priming and accumulation in the tumor microenvironment.[21] This condition gives higher IFN-γ gene expression and granzyme B to attack cancer cells. Moreover, FMT lowers the classic exhaustion marker PD-1 expression.[22]

FMT and immune checkpoint inhibitor

Despite increasing favorability to neoadjuvant PD-1/PD-L1 or CTLA-4, the use of ICIs can lead to the activation of autoreactive T cells, which induce immune-related adverse events.[3] Multiple studies have utilized the potential for microbiome modulation to favorably affect ICIs' efficacy and response. Shaikh et al. reported that FMT from a human responder patient to germ-free (GF) mice enhanced the anti-PD-L1 treatment compared to FMT from non-responder patients. This effect is more pronounced in the MC38 colon cancer model, which is more immunogenic and moderately responsive to ICIs than the B16F10 melanoma model. The observed Clostridium species were enriched in mice with a non-progressing tumor in both FMT source groups.[23] In a study by Routy et al., the mouse model was treated with oral supplementation of *A. muciniphila* or together with *E. hirae*, which refined the PD-1/PDL-1 blockage in the antibiotic-treated mouse.[9]

Human clinical trials indicated that combination treatment with FMT from a complete response donor helps alleviate ICIs refractoriness. The post-treatment condition had a relative abundance of Enterococcaceae, *Enterococcus*, and *Streptococcus australis*, and lower levels of *Veillonella atypica*. However, similar patterns were also observed in non-responders and pre-treatment samples, indicating no clear link between these taxa and clinical response.[6] Another case report demonstrated that metastatic FMT recipients recovered from ICIs enterocolitis.[24] FMT increased bacterial diversity and richness closer, similar to the donor microbiota profile. Nonetheless, no existing research has given a consistent and convincing group of bacteria as driving the ICI response in humans or mice. This challenges the identification of the optimal compositions for different conditions. The inconsistent result based on the pre-treatment of immunotherapy, including cancer treatment (chemotherapies, kinase inhibitors) or non-cancer treatment (antibiotics), might alter the gut microbiome. Furthermore, different microbiome profiling analyses and databases of transfer material or evaluation give different compositions. The two most common methods are 16sR ribosomal gene sequencing, which typically identifies up to the genus level, while metagenomics sequencing can identify the species. Bacteria species within the same genus can have contrasting effects. For example, *Bacteroides caccae* enrichment was positively correlated with better response of ICIs, while *Bacteroides ovatus* and *Bacteroides dorei* were associated with worse progression-free survival.[25]

Comprehensive bibliometric analysis can be used to track the research progress and emerging trends in FMT exploration in cancer immunology; however, this approach has limitations. To ensure precision in analysis, we only included English-written documents in the Scopus database results. A single database was used instead of merged databases because differences in metadata and citation metrics could slightly skew the results. Moreover, recently published high-quality research may not have received significant attention yet due to delayed citations.

Conclusion

The development of immunotherapy holds great promise for revolutionizing precision cancer therapy. Research on the role of the FMT in immunotherapy has become a hotspot area in recent decades. Using bibliometric and visualization methods, we observed the growth in the number of publications in the past 10 years. It is worth mentioning China's growth rate of publications; however, the USA remains the most influential in terms of affiliations and collaborative networks. The study identified three thematic topics related to FMT in immunotherapy, including fundamental themes, motor themes, and niche themes, which depict the direction of emerging trends.

Fecal microbiota plays a role through microbiota alterations, modulation of fatty acids, and influencing immune response, which helps reduce immune resistance and immune-related adverse effects. The formidable challenge in

identifying specific microbiota profiles of patients can be tailored and bioactive compounds impacting immunotherapy that should be achieved with minimal side effects. Current applications are primarily focused on combination with immune checkpoint inhibitors (ICIs), while other forms of immunotherapy remain underexplored. Variations in microbiota due to demographic factors may offer strategic opportunities for FMT exploration across different countries.

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Conflict of Interest

Authors declare no conflict of interest

Acknowledgment

None declared

Authors contribution

AD and FFA conceived the conceptualization; AD wrote the initial draft of the manuscript; AD and RIA performed data curation and visualization; TK and FFA reviewed the manuscript. All authors revised the manuscript for important intellectual content.

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