Review

Gut Microbiota a Novel Source of Biomarkers for Immunotherapy in Non-small Cell Lung Cancer (NSCLC)

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Posted Date: 15 March 2024

doi: 10.20944/preprints202403.0902.v1

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Review

Gut Microbiota a Novel Source of Biomarkers for Immunotherapy in Non-Small Cell Lung Cancer (NSCLC)

Teresa Del Giudice [1,*], Nicoletta Staropoli [2], Pierfrancesco Tassone [2], Pierosandro Tagliaferri [2] and Vito Barbieri [1]

[1] Department of Hematology-Oncology, Azienda Ospedaliera Renato Dulbecco, Catanzaro; teresadelgiudice87@gmail.com, vitobarbieri@yahoo.it
[2] Department of Experimental and Clinical Medicine, Magna Graecia University, Catanzaro, Italy; nicolettastaropoli@gmail.com, tassone@unicz.it, tagliaferri@unicz.it
* Correspondence: teresadelgiudice87@gmail.com

Simple Summary: Lung cancer is the most frequent cause of cancer-related death. Unfortunately, only 30% of patients treated with immunotherapy gain benefit; it is, therefore, important to increase the number of patients who can receive benefit from immunotherapy. Biomarkers can help clinicians to reach this target and the gut microbiota is a potential excellent source of predictive factors. All conditions that modify the gut microbiota may influence cancer onset and progression, its prognosis and response to immunotherapy with a relevant impact in the clinical practice.

Abstract: Despite the recent availability of immune checkpoint inhibitors, not all patients affected by Non-Small Cell Lung Cancer (NSCLC) benefit from immunotherapy. The reason for this variability relies to a variety of factors which may allow the identification of novel biomarkers. Presently, a variety of biomarkers are under investigation, including the PD1/PDL1 axis, the tumor mutational burden, and the Microbiota. This latter is made by all bacteria and other microorganisms hosted in our body. The gut microbiota is the most represented and has been involved in different physiological and pathological events, including cancer. In this light, it appears that all conditions modifying gut microbiota can influence cancer, its treatment, and its treatment-related toxicities. The aim of this review is to analyze all conditions influencing the gut microbiota and, therefore, affecting response to immunotherapy, iRAEs and their management in NSCLC patients. The investigation of the landscape of these biological events can allow novel insights for optimal management of NSCLC immunotherapy.

Keywords: NSCLC; lung cancer; gut microbiota; immunotherapy; immunecheckpoint inhibitors

1. Introduction

Lung cancer (LC) is one of the most common causes of cancer-related deaths worldwide. In the past years, new therapeutic approaches have been discovered and presently from 15 to 30% of Non-Small Cell Lung Cancer (NSCLC) patients survive. These patients, in the absence of driver mutations, are treated with immune checkpoint inhibitors (ICIs) alone or in combination, or with chemotherapy. Among ICIs, monoclonal antibodies (mabs) against CTLA4 (cytotoxic T lymphocyte-associated protein 4), and against PD1 (programmed death type 1), or its ligand PDL1 (programmed death ligand type 1) can be used. Ipilimumab, Nivolumab, Pembrolizumab, Cemiplimab, Durvalumab and Atezolizumab are at the present the ICIs used in clinical practice [1]. Not all NSCLC patients benefit of the immunotherapy at the beginning of the treatment, while others progress after initial response to the treatment. At present, a suitable predictive marker for immunotherapy in not available: PDL1 as part of PD1/PDL1 axis seems to have a role in predicting response to immunotherapy but its expression is inducible and editable by different factors and for this reason its role is challenging, even if it is still the unique predictive biomarker for patients' selection by regulatory agencies [2].

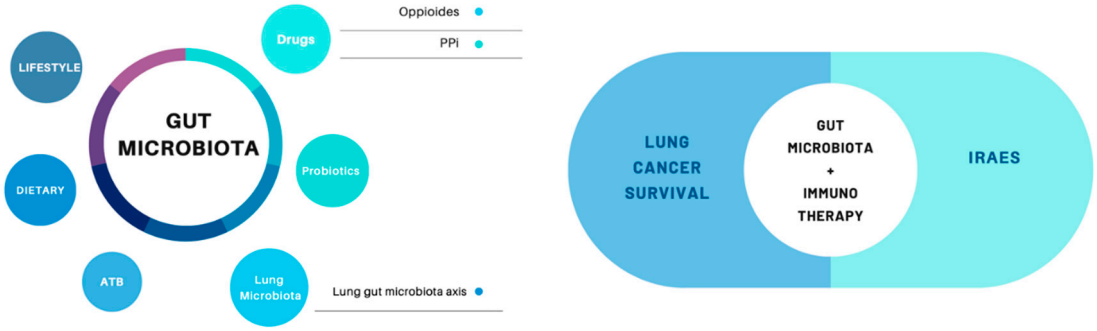
 doi:10.20944/preprints202403.0902.v1

Biomarker identification still represents an open challenge to identify immunotherapy predictors of response. Example of different biomarkers are: neutrophil to lymphocytes ratio, tumor infiltrating lymphocytes, tumor mutation burden, and gut microbiota [1–3]. This latter is composed by all commensal microorganisms present in our gastrointestinal tract including bacteria, fungi, viruses, and protozoans, while the term microbiome refers to the total genetic material possessed by the microbiota . Alterations on composition of this microbiota correlate with some disease like inflammatory intestinal or metabolic disease. In the last decades the gut microbiota emerged as a crucial player in immunosurveillance and both in cancer onset and progression. In particular, it is possible to hypothesize a gut-lung axis: this theory could explain the correlation between a gut microbiota and an active immune response in LC. The advent of Next Generation Sequencing (NGS) allowed extensive investigation on gut microbiome and correlated it with cancer onset and response to immunotherapy [4].

The correlation between gut microbiota and immune response in LC could be a potential biomarker in immunity against cancer and particular in NSCLC patients. Is important to understand how basal microbiota diversity among different patients could affect prognosis and understand how the gut microbiota could be modified and how this might change immune-response and therefore impact on survival of NSCLC patients [5,6]. If this correlation is true, the changes on gut microbiota can potentially improve immunotherapy response, reduce immunotherapy-related adverse events (IRAE) and prolong survival on immunotherapy treatment [6].

At this time, is important to discover factors which might influence gut microbiota and particularly antibiotics and/or other agents used for different diseases (proton pump inhibitor, antidiabetics as insulin) in cancer patient and specifically in NSCLC patients [7].

The gut microbiota will be candidate, for its role in cancer development and immunity, as a new cancer modulator; the objective of this review is to explain, from the current literature, the role of microbiota gut in LC and how its modulation can improve cancer immunotherapy and IRAEs management [6]. Figure 1.



(a) **(b)**

Figure 1. (a) Factors involved in microbiota modulation [1–3]. **(b)** The Gut Microbiota's relevance in LC [6–8].

2. Gut Microbiota and NSCLC

2.1. Gut – Lung – Microbiota Axis

Lung Microbiota has not been investigated as gut microbiota but its role in different respiratory diseases appears clear [9]. Compared to gut microbiota, the lung microbiota is smaller but not less important: gut microbiota, lung microbiota and other sites in human host, in which there are Bacteroides and other elements, are defined as microbial communities [3]. Lung microbiota is composed by *Staphylococcus*, *Streptococcus*, and *Lactobacillus* followed by *Proteobacteria* and *Actinobacteria*, a microbiota composition similar, in healthy patients, to gut microbiota; the lung

 doi:10.20944/preprints202403.0902.v1

microbiota undergoes to change during inflammation and interacts with metabolites and other pathogens from external and internal to the host [2].

The correlation between lung and gut microbiota could depend by similarity in mucosa microenvironment characterized by same interactions between microbiota and immune system in Mucosal Immune System (MIS). MIS is the most important link between two microbial systems and underlies the participation of the immune system and peptide and proteins secretion as IgA and metabolites production [3].

The microbiota lung homeostasis is controlled not only within lung but also by interactions with other organs and gut microbiota particularly. Gut microbiota and lung microbiota are linked in many ways: lymphatic and blood circulation system through gut microbiota could induce many respiratory diseases as asthma, respiratory infection and others. The Gut Lung Microbiota Axis is a unique complex bidirectionally linked that maintains an homeostat: alteration of this condition can leads to cancer development, tissue damage and infections susceptibility [10].

2.2. Gut Microbiota Composition, Anti-Tumor Activity and Antibiotics

The Gut Microbiota composition seems to be more heterogeneous among different individuals due to different diet, genetic heritage, lifestyle, medical exposition and other factors. It is clear that its composition correlates with many diseases, including autoimmune disease, inflammatory disease, and cancer [11]. Microbiota composition impacts on disease pathogenesis, disease prognosis and response to therapy. All these depend on other factors: for example, it is described that Helicobacter Infections is strongly related with gastric adenocarcinoma but it is protective for Barret Esophagus development [3,12]. Some data demonstrate that a microbiota enriched of some bacterias such as Akkermansia muciniphila and Ruminococcacae correlates with a favorable outcome in melanoma and NSCLC patients than head and neck patients, in which the same gut microbiota composition does not modify survival [12]. The presence of Phascolarbacterium is linked to a prolonged Progression Free Survival (PFS) in NSCLC patients on treatment; while a microbiota enriched of Dialister bacteria occurs in NSCLC patient with worse prognosis [7]. Patients with with heterogenous gut microbiota composition at baseline have better prognosis taht poor heterogeneous microbiota patientis [13]. It appears clear how all conditions modulating the gut microbiota with reducing variability or eliminating good bacteria, can have a negative impact in prognosis or treatment efficacy for LC patients, suggesting how relevant it is learning how to modulate them [14].

Recently some authors demonstrated, through Mendelian Randomization, a potentially correlation among gut microbiota phyla and lung carcinoma subtypes [15]. Three groups of protective microbiota for the development of NSCLC and nine microbiota groups as risk factors have been identified. However, only one protective intestinal microbiota for the development of small cell lung cancer (SCLC) and six groups of intestinal microbiota potentially causing SCLC have been identified. The same author have just identified some gut microbiota phyla predisposing to lung adenocarcinoma or squamous lung carcinoma. These findings, according with information from retrospective trial, confirm the correlation between microbiota and lung cancer development also linked to other conditions [15].

LC patients are sometimes treated with antibiotics. There is evidence that exposure to antibiotics in first days of life can modify microbiota characteristic, making children susceptible for future inflammatory and autoimmune disease as compared to non-exposed children. This observation is due to modification of the gut microbiota composition for months and sometimes for year [16]. It is not surprising therefore that antibiotic exposition correlates with cancer onset and progression or immunotherapy efficacy [17–19].

Potentially, antibiotics can, through microbiota modulation and changing composition, reduce and alter immunotherapeutic activity in LC patients [20]. Numerous data exist in the literature to support how antibiotics, through gut microbiota modulation, could have negative impact to Immune checkpoint activity and chemotherapy activity in cancer and NSCLC patients and there is evidence that, despite microbiota alterations during antibiotic therapy, no changes in immunotherapy efficacy occurs thanks to the ability of microbiota to return at baseline conditions. The use of antibiotics during

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immunotherapy in cancer patients could be correlated with primary or secondary immune resistance and, considering that 15-30% of NSCLC patients are treated with antibiotics in clinical practice, the problem is relevant [18].

Studies in mice models demonstrate that the anti CTLA4 efficacy in cancer patients depends on gut microbiota composition: a gut microbiota enrich of Bacteroides fragilis or Bacteroides Thetaiotaomicron through polysaccharides products and Th1 response inducing dendritic cell maturation, is related to an improvement in the effectiveness of anti-CTLA4 therapy and of this effectiveness is restored by diet or oral supplementation of this bacterium. At the same time Bifidobacterioides improve anti PD1 efficacy in melanoma affected mice, while mice with a different gut microbiota but undergoing fecal microbiota transplation bifidobacterioides based, become anti PD1 responders thanks to an increased T cell anti-tumor response [6].

From 2017 several retrospective studies about antibiotic effect on lung cancer patients treated with immunotherapy have been reported. The data from these trials are not completely consistent and the antibiotic role remains unclear. Particularly, some studies demonstrate a correlation between antibiotic exposure and worse prognosis and reduced immunotherapy efficacy, while other studies did not demonstrate such correlation that could be associated to several factors, some to the host microbiota and host characteristics and others to the type of study. (Table 1).

Table 1. Retrospective studies from 2017 to 2023 including lung cancer patients treated with immunotherapy and exposed to antibiotics.

Year	Study	Patients	Treatment	ANTIBIOTICS typologies	ANTIBIOTICS exposistion timing	Reference
2017	Antibiotic Use Does Not Appear to Influence Response to Nivolumab	74	Anti PD1, Nivolumab	Fluoroquinolones	3 Months Before ICIs starting	[21]
2018	Negative association of antibiotics on clinical activity of immune checkpoint inhibitors in patients with advanced renal cell and non-small-cell lung cancer	239	Anti PD1, Anti CTLA4 Monotherapy or Combination	Fluoroquinolones Betalactams	30 days before immunotherapy starting	[22]
2018	Impact of prior antibiotic use on the efficacy of nivolumab for non-small cell lung cancer	90	Anti PD1, Nivolumab	Not specified	30 days before immunotherapy starting	[18]
2018	Impact of antibiotic treatment on immune-checkpoint blockade efficacy in advanced non-squamous non-	30	Anti PD1, Nivolumab, Pembrolizumab	Not specified	30 days before and after immunotherapy starting	[23]

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	small cell lung cancer					
2019	Antibiotics are associated with attenuated efficacy of anti-PD-1/PD-L1 therapies in Chinese patients with advanced non-small cell lung cancer	109	Anti PD1, Anti PDL1	Not specified	Not Specified	[24]
2019	The effect of antibiotics on the clinical outcomes of patients with solid cancers undergoing immune checkpoint inhibitor treatment: a retrospective study	131	Anti PD1, Anti PDL1, Anti CTLA4 Monotherapy or Combination	Fluoroquinolones, Betalactms Cephalosporins	60 days before immunotgerapy starting	[19]
2019	Association between Antibiotic-Immunotherapy Exposure Ratio and outcome in metastatic Non-Small Cell Lung Cancer	157	Anti PD1, Anti PDL1, Anti CTLA4 Monotherapy or Combination	Not Specified	Before and During Immunotherapy	[25]
2020	Association of prior fluoroquinolone treatment with survival outcomes of immune checkpoint inhibitors in Asia	340	Anti PD1, Anti PD1, Anti CTLA4 Monotherapy or Combination	Fluoroquinolones	30 days before immunotherapy starting	[26]
2020	Impact of intestinal dysbiosis-related drugs on the efficacy of immune checkpoint inhibitors in clinical practice	120	Anti PD1, Anti CTLA4 Monotherapy or Combination	Not Specified	Not Specified	[27]
2020	Impact of Concomitant Medication Administered at the Time of Initiation of	224	Anti PD1, Nivolumab	Not Specified	Not Specified	[28]

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	Nivolumab Therapy on Outcome in Non-small Cell Lung Cancer					
2020	Efficacy of chemotherapy and atezolizumab in patients with non-small-cell lung cancer receiving antibiotics and proton pump inhibitors: pooled post hoc analyses of the OAK and POPLAR trials	757	Anti PDL1, Atezolizumab	Fluoroquinolones, Carbapanems, Macrolides, Glycopeptides	30 days before and after immunotherapy starting	[29]
2020	Cumulative Antibiotic Use Significantly Decreases Efficacy of Checkpoint Inhibitors in Patients with Advanced Cancer	64	Anti PD1	Not Specified	15 days before and 45 after starting immunotherapy	[30]
2020	Comparative analysis of antibiotic exposure association with clinical outcomes of chemotherapy versus immunotherapy across three tumour types	140	Anti PD1	Vancomicyn, Nitrofurantoin, Rifampin, Rifaximin, Tobramicyn,	30 days befor and after immunotherapy stating	[31]
2021	Broad-Spectrum Antibiotic Regimen Affects Survival in Patients Receiving Nivolumab for Non-Small Cell Lung Cancer	140	Anti PD1, Nivolumab	Not Specified	Not Specified	[32]
2021	Antibiotic-exposed patients with non-small-cell lung cancer preserve efficacy outcomes following first-	302	Chemotherapy Immunotherapy	Not Specified	7 days before and after immunotherapy starting	[33]

 doi:10.20944/preprints202403.0902.v1

	line chemo-immunotherapy					
2021	Impact of Antibiotics and Proton Pump Inhibitors on Efficacy and Tolerance of Anti-PD-1 Immune Checkpoint Inhibitors	65	Anti PD1, Anti CTLA4 Monotherapy or Combination	Not Specified	60 days before immunotherapy starting	[34]
2021	Differential influence of antibiotic therapy and other medications on oncological outcomes of patients with non-small cell lung cancer treated with first-line pembrolizumab versus cytotoxic chemotherapy	950	Anti PD1, Pembrolizumab	Piperacillin-Tazobactam, Clindamycin, Metronidazole, Meropenem	30 days before immunotherapy starting	[35]
2021	Antibiotic Usage Reduced Overall Survival by over 70% in Non-small Cell Lung Cancer Patients	69	Anti PD1	Not Specified	21 days before immunotherapy starting	[36]
2022	Efficacy of Atezolizumab in Patients With Advanced NSCLC Receiving Concomitant Antibiotic or Proton Pump Inhibitor Treatment: Pooled Analysis of Five Randomized Control Trials	2723	Anti PDL1, Atezolizumab	Not Specified	30 days before immunotherapy starting	[37]
2022	Impact of the use of antibiotics on the clinical response to immune checkpoint inhibitors in patients with	140	Anti PD1, Anti PD1, Anti CTLA4 Monotherapy or Combination	Fluoroquinolones, Betalactms	2 months before and after immunotherapy starting	[17]

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	non-small cell lung cancer					
2022	Effect of prior antibiotic or chemotherapy treatment on immunotherapy response in non-small cell lung cancer	256	Anti PD1, Anti PDL1, Anti CTLA4 Monotherapy or Combination	Fluoroquinolones, Cefazolin, Azithromicin	60 days before and after immunotherapy starting	[38]
2022	Different classes of antibiotics exhibit disparate negative impacts on the therapeutic efficacy of immune checkpoint inhibitors in advanced non-small cell lung cancer patients	148	Anti PD1, Anti PDL1, Chemotherapy	Fluoroquinolones, Betalactms	60 days before and after immunotherapy starting	[39]
2023	Prognostic Associations of Concomitant Antibiotic Use in Patients with Advanced NSCLC Treated with Atezolizumab: Sensitivity Analysis of a Pooled Investigation of Five Randomised Control Trials	2724	Anti PDL1, Atezolizumab, Alone or in combination with chemotherapy	Not Specified	Not Specified	[40]
2023	Antibiotic Treatment is an Independent Poor Risk Factor in NSCLC But Not in Melanoma Patients Who had Received Anti-PD-1/L1 Monotherapy	199	Anti PD1, Anti PDL1	Not Specified	3 months before and 1 months after immunotherapy starting	[41]

A retrospective analysis of 70 NSCLC patients treated with ICI investigated the gut microbiota diversity in patients with OS (Overall Survival) > 12 months and < 12 months. The gut microbiota of long survivors was enriched of *Lachnospiraceae*, a member of the *Clostridiale*, with increased circulating CD4 and CD8 T cell and CD8 T cell infiltrating tumor. The study confirmed that a diversified microbiota correlates with a better prognosis and that the use of antibiotics also reduces the diversity of the gut microbiota [42].

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The antibiotic exposure from 60 days before starting immunotherapy and 30 days after last immunotherapy correlated with a poor prognosis and immunotherapy resistance [14]. Scarce information on antibiotics type, antibiotics route and duration of therapy are available. Greater information would help us to use microbiota modulation to improve immunotherapy in cancer. As just mentioned, the microbiota has the ability to return to homeostasis after the antibiotic damage: different time frame of reconstitution of baseline status might explain the unclear data on prognosis and exposure to antibiotics in different patients with lung cancer [9,11,16].

There is evidence about relevance of gut microbiota on efficacy and toxicity of chemotherapy and the intestinal microbiota, the maximum effectiveness of chemotherapy in treated cancer is mediated by a good balance between the intestinal microbiota and the immune system [3]. The relevance of gut microbiota in chemotherapy management and efficacy represents today an important issue considering NSCLC treatment based not only on ICIs monotherapy but on chemo-immunotherapy association too [43,44].

It is clear that the microbiota have a role in cancer from onset to therapy response, but the knowledge of conditions implicated in the change of the intestinal microbiota that should be avoided if not necessary, such as the use of antibiotics, remains to be clarified [11,12,20].

2.3. Gut Microbiota and Probiotics Use

Since modulation of the gut microbiota can modify the effectiveness of immunotherapy in cancer patients, finding a way to remodulate the microbiota and restore it to improve the effectiveness of immunotherapy could be an option for our patients. Oral probiotic supplements have been associated with improved efficacy of immunotherapy for cancer patients [45,46]. Probiotics are a bacterial strain not altering antibiotic resistance, they reach the colon and the entire intestine where they carry out their metabolism. Probiotics may be safe in animals, resistant to acids and able to colonize the intestine [3]. Probiotics can modulate the gut microbiota by a) modifying humoral, cellular and innate immunity, b) improving NK (Natural Killer) immune activity, c) macrophages and neutrophils activation, d) IgA secretion, and e) inflammation cytokines inhibition. Moreover, probiotics can modulate chemotherapy toxicity and iRAES development. To date, the more used probiotics are composed by Bifidobacterium spp and Lactobacillus spp; their role appears marginal in NSCLC patients treated with immunotherapy because they are not specifically chosen for this reason. It could be very interesting the discovery of probiotics able to modulate immune response on immunotherapy and able to prevent and improve iRAES and chemotherapy toxicities [3] or in limiting the unfavorable effect of antibiotics [47].

With the recent advent of Next Generation Sequencing (NGS), new species of probiotics have been identified and called next-generation probiotics (NGPs), which are presently under evaluation in the context of specific diseases. NPGs are able to modulate gut microbiota for improving immunotherapy and control iRAEs. *Eubacterium limosum*, *E. hirae*, *Enterococcus faecium*, *Collinsella aerofaciens*, and *Burkholderia cepacia* appear to have promising efficacy in this setting [3].

A recent metanalysis underlyined the role of the probiotics and their effect on survival of NSCLC patients treated with immunotherapy. This study demonstrated a positive correlation between probiotics exposure and OS and PFS. There was no correlation with ORR but it can be demonstrated by the types of studies and sample size [46].

Probiotics use can improve immunotherapy efficacy through modulation of inflammation. The data about this correlation are limited by study design, cancer types, sample size and duration of oral probiotics implementation [46]. Certainly the use of probiotics increases the heterogeneity of the intestinal microbiota which is the basis of a better prognosis, a better response and less iRAE in cancer patients [8]. It's important to understand if a single bacterial species can modulate the entire microbiota or if it is necessary the presence of different bacterial species at the same time. It is also important to understand the single species amount are needed for a beneficial effect into a well-balanced gut microflora [13].

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2.4. Gut Microbiota and iRAes

In the era of immunotherapy, in which cancer patients underwent to ICI treatment for long period, the toxicity become most relevant. Few data evidenced correlations between gut microbiota composition and IRAes: some bacterial strain seems to be protective for iRAEs, while other strain could increase iRAES risk [48].

In a retrospective analysis, a link between gut microbiota, antibiotic exposure and iRAEs was investigated: it was not observed a correlation between antibiotics use and iRAEs but a gut microbiota enriched of Akkermansia Mucinipihila corelated with less iRAEs [42].

Microbiota diversity correlates with the development of iRAEs during immunotherapy. As demonstrated for the survival and prognosis, it appears that patients with a low diversity of gut microbiota exhibit skin iRAes more than patients with gut microbiota enriched in many bacterial types [1,8].

The presence, in gut microbiota composition of mouse models and cancer patients, of Bacterioides and others microbe implicated in vitamin B production seems to be protective against colitis development during immunotherapy; detecting some type of bacterioides in gut microbiota can help us to predict colitis presentation during immunotherapy while bacterial supplementation as *Bacteroidales* and *Burkholderiales* can improve colitis particularly in cancer patient treated with antibiotics during immunotherapy [48].

The gut microbiota composition could be implicated in skin iRAEs during immunotherapy in cancer. This theory is supported by modulatory effects on skin mediated by gut microbiota through immunity regulation and metabolites products. There is evidence of dermatitis' improvement with oral Lactobacillales e Bifidobacteriales use. Oral use of Bifidobacteriodes in human reduce inflammatory markers as peptide C and TNF alfa with improvement of psoriasis [8]. It is necessary to gain more information and data about gut microbiota and iRAEs for improving immunotherapy management[8].

2.5. Other Conditions Modifying Gut Microbiota

In addition to the role of antibiotics and probiotics, in the modulation of the intestinal microbiota for the improvement of the efficacy of ICIs in LC patients, there are other molecules potentially implicated and recently studied with more hypotheses and few relevant points that need to be confirmed by other studies.

One of the retrospective work existing in literature, about gut microbiota modulation mediated by drugs, describes of 132 lung patients treated with immunotherapy presents shorter PFS and OS if exposed to opioid through impairment of T cell function, upregulating Treg cells modulating gut microbiota; the opioid exposition does not correlate with different iRAEs incidence. This exploratory data could be important considering the high percentage of cancer and LC patients exposed to opioid drugs for pain management and this research area needs to be focused. It could be very important to acquire data to confirm these results, considering that pain is one of the most important causes of quality of life reduction and that this could be correlated to a worse response to immunotherapy. This could mean, as suggested in a small study, that worse PFS and OS are not related to opioid exposure but to poor Performance Status (PS) [49].

In 2021 was presented on of the first restrospective paper about correlation between PPi (Proton Pump Inhibitors) exposition and LC patients survival treated with immunotherapy. Lung cancer patients treated with ICIs and exposed to PPi have a 28% increased risk of death and shorter survival compared to unexposed patients; this relationship is not observed in subgroups patients exposed to PPi but treated with chemotherapy only. The PPi/survival association is consistent considering sample size and data are confirmed when exposition window changes. The possible cause of this negative relationship can be linked to PPi mediated acid reduction which alters the intestinal microbiota [50]. Recently, another author, published a metanalysis regarding PPi exposition and survival in cancer patients treated with immunotherapy that confirm negative relationship; also, in this case relationship could be modified by poor quality of life in cancer patients exposed to PPi [51]. In both works presented there are many limitations, one of that the presence of retrospective

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trials and along the PPi exposition (dose, time, PPi use). However other retrospective study and metanalyses did not cofirm the relationship between PPi and Immunotherapy efficacy [50,51].

3. Future Directions

Despite the recent huge impact of cancer immunotherapy, only 30% of lung cancer patients gain benefit. This condition depends on different factors intrinsic and extrinsic to LC patients [10]. For these reasons, it is relevant to identify biomarkers to select responder patients, improve immunotherapy efficacy, and manage iRAEs. Among well known biomarkers, such as PDL1 and PD1-PDL1 axis, TMB (Tumor mutational burden), and others, the microbiota appears to be potentially the most relevant predictor of immunotherapy efficacy. There are many factors that can influence and modulate the microbiota and all of them could play an important role in cancer development as well as in efficacy and toxicity of immunotherapy [48,52].

The intestinal microbiota plays a role in anti-tumor immunotherapy and, therefore, all conditions that modify the intestinal microbiota reducing its diversity might potentially modify the efficacy and also the occurrence of iRAEs. We have relevant information about the microbiota-immunotherapy relationship, but we have no clear cut information that presently allows us to improve our approach to cancer therapy management [9,48] . It is not only important to know the composition of the microbiota but also the interplay between all players of the intestinal microbiota and the microenvironment that surrounds it, taking into account that all elements are regulated by different intrinsic and extrinsic factors, including the genetic host features, diet, lifestyle, age, concomitant medication [10]. This is why, microbiota, cannot be considered a weapon to be used but to a biological entity whose relevance is still underestimated

What we at the present know about microbiota and its relationship with cancer immunotherapy is derived from retrospective analyses or meta-analyses based on retrospective studies. All derived information could be defined hypothesis generating and not clear-cut findings which can be derived only by prospective and randomized trials. For example, we know that exposure to antibiotics exposition can adversely modulate gut microbiota thus reducing efficacy of immunotherapy [25]. However, we don't know which class of antibiotics are implicated, the timing of exposure or differences in routes of administration; at the same time, we know that not all patients immunotherapy-treated have a worse prognosis related to exposure and many sources of biases can be identified. The same difficult occurs in understanding gut microbiota-immunotherapy efficacy relationship with opioids and PPis, whose role has been always investigated retrospectively. Table 1.

Therefore the more relevant question is: what is next step for translating microbiota knowledge into LC management?

Again, a major role is played by the study design retrospective *versus* prospective trials, pivotal trials with adequate stratification for administered drugs.

Considering the huge world represented by the microbiota, it is clear as the current methodologies are not completely applicable and new methodologies might be considered in the next future. The machine learning (ML) just used in cancer immunotherapy for predicting iRAES developing in cancer patient during immunotherapy [53], could help us to identify the role of exposure to many drugs, through microbiota modulation. Instead of as for iRAEs predicting, microbiota modifying conditions depends on various elements not simple to manage in clinical practice: for this reason, the AI (artificial intelligence) and ML could help us in our objective. There are several reports about ML using and microbiota. From data on microbiota existing by omics based methods (metagenomics, meta transcriptomics, and metabolomics), ML can predictand find new information non theoretically inferred and help us to increase efficacy and reduce iRAEs [54,55].

Among AI and ML, that could be of help in the future on this topic, it needs to be highlighted that microbiota and microbiome not are the same in all populations, in all persons and in all cancers. That we today know about LC microbiota and its correlation with immunotherapy efficacy is different from head and neck microbiota or SCLC microbiota or bladder microbiota. It's important to make clear that the relevance of microbiota in LC does not necessarily translate in other cancers which grow in a different tumor microenvironment.

 doi:10.20944/preprints202403.0902.v1

4. Conclusion

To date, the intestinal microbiota appears to be an important biomarker of immunotherapy in LC. However, taking into account the complexity of the whole scenario, it is necessary to make a great effort to gain more functional information. It is also necessary that data available are confirmed by more robust trials or in alternative way trough new metrologies as AI. While waiting for all this information, what we can tell about microbiota, cancer prevention, and immunotherapy is that it is important to limit the use of antibiotics, PPi, and opioids, which might be used if necessary, and that probiotics use could improve some conditions as colitis management and other iRAEs. More adequate probiotics might be identified with NGS and NPS. Healthy diet and better lifestyle might be in any case the main stream proposal to our patients.

Author Contributions: T.D and V.B. designed the paper. T.D. wrote the paper, constructed image and table. P.T and P.T. revised the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding

Conflicts of Interest: The authors declare no conflict of interest.

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