Association between Antibiotic-Immunotherapy Exposure Ratio and outcome in metastatic Non Small Cell Lung Cancer

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Abstract

Objectives Immunotherapy (IO) is effective for metastatic Non Small Cell Lung Cancer (NSCLC). Gut microbiota has an impact on immunity and its imbalance due to antibiotics may impair the efficacy of IO. We investigated this topic in a case series of NSCLC patients treated with IO.

Materials and Methods Data about all patients with metastatic NSCLC treated with IO between 04/2013 and 01/2018 were collected. Patients were stratified according to antibiotic use during the Early IO Period (EIOP), and according to the Antibiotic-Immunotherapy Exposure Ratio (AIER) defined as "days of antibiotic/days of IO" during the Whole IO Period (WIOP). Survival was estimated with Kaplan-Meier method. Log-rank test was used to compare curves. Multivariate analyses were performed with Cox model.

Results We analyzed 157 patients. Forty-six patients received antibiotics during the WIOP, 27 during the EIOP. No differences in either Progression-Free Survival (PFS) or Overall Survival (OS) were observed according to antibiotic use during the EIOP ($p=0.1772$ and $p=0.2492$, respectively). Considering the WIOP, median AIER was 4.2%. The patients with a higher AIER had worse PFS ($p<0.0001$) and OS ($p=0.0004$) than the others. These results maintained significance after correction for line of IO ($p=0.0018$ for PFS) and performance status ($p<0.0001$ for PFS, $p=0.0052$ for OS).

Conclusion Although no difference in outcome could be observed according to antibiotic use in the EIOP, a detrimental effect became evident for patients with a higher AIER in the WIOP. If its relevance was confirmed, AIER may become an innovative variable to estimate the impact of antibiotics on the efficacy of IO.

Keywords

antibiotic; immunotherapy; microbiota; Non Small Cell Lung Cancer.

Introduction

Metastatic Non Small Cell Lung Cancer (NSCLC) is the leading cause of cancer-related death worldwide [1].

Only a limited subgroup of patients harbors genetic alterations amenable of targeted treatments (i.e. *EGFR*, *ALK*, *ROS-1*, *MET*). For the remaining ones, the only option available until few years ago was platinum-based chemotherapy [2].

In the last years, different clinical trials proved the superior efficacy of immunotherapy (IO) over standard chemotherapy for metastatic NSCLC [3-8]. This led first to the approval of anti-PD1 and anti-PD-L1 agents in second and more advanced lines of therapy [3-7], then in first line setting [8]. Many other compounds with similar or slightly different mechanism of action are currently under investigation, with promising results [9-15]. These studies will probably lead to an expansion of the indications for IO, likely extending also to non-metastatic stages of disease and to combinations with other immunotherapeutic or cytotoxic agents [9-15]. Despite such promising premises, only 25-30% of patients appear to derive a durable benefit from IO and the event of primary resistance, up to its worst expression arising as hyper-progression , is well known, [16] strengthening the importance of predictive factors of response to therapy in order to explain this variability [17-22].

Emerging evidence suggests that factors not directly tumor-associated contribute to response to cancer therapy. Bacteria inhabiting the gut, collectively named as gut microbiota, maintain host physiology and health by exerting fundamental functions, spanning from metabolic to immunomodulatory properties [23].

It was recently demonstrated that gut microbiota constitutes one of the environmental factors affecting response to chemotherapeutic and immunotherapeutic drugs through its ability to regulate the immune response. Indeed, works in mouse models clearly showed the cause-effect relationship between the composition of gut microbiota and the efficacy of both chemotherapy and IO [24-27]. Accordingly, antibiotic-induced dysbiosis in tumor-bearing mice has been associated with failure of IO with anti-CTLA4 and anti-PD1 antibodies [26-28]. Moreover, fecal microbiota transplantation of mice with stool of responders and non-responders to IO transfers the capability to respond or not to IO [26,28,29]. In addition to these experimental findings, analyses in human cohorts of metastatic patients with different malignancies showed

that the use of broad-spectrum antibiotics known to severely reduce the bacterial diversity and the function of intestinal flora, around the beginning of IO, has a detrimental effect on patient response and Progression-Free Survival (PFS) [28,29]. In particular, Derosa et al. in a retrospective cohort of patients with metastatic renal cell carcinoma and NSCLC treated with IO, showed that the administration of antibiotics within one month before the beginning of treatment has a detrimental effect on Response Rate (RR) and PFS [29]. Routy et al. confirmed this evidence in a large cohort of patients with different malignancies (NSCLC, renal and urothelial carcinomas), showing that cases receiving antibiotics between 2 months before and 1 month after the first administration of IO had worse PFS and OS than non-treated counterparts. The same researchers performed a molecular characterization of microbiota through shotgun sequencing of stool DNA, finding that the clinical response to Immune Checkpoint Inhibitors (ICIs) is correlated with the abundance of *Akkermansia muciniphila*. These data were subsequently confirmed in mice models, in which the transplant of a fecal microbiota rich in *Akkermansia* and *Alistipes* induced enhanced response to ICIs potentiating T-mediated response [28]. Another work by Gopalakrishnan et al. prospectively studied a population of patients with metastatic melanoma treated with IO. The patients were classified as responders provided that they achieved at least disease stability for 6 months, or as non-responders, provided that they showed progressive disease within the first 6 months. The researchers identified significant differences in the composition of bacterial flora between the two subgroups, with a predominance of *Clostridiales, Fecalibacterium* and *Ruminococcaceae* in the stool specimens obtained from responders, and a prevalence of *Bacteroidales*, *Escherichia* and *Anaerotruncus* in the stool specimens obtained from non-responders. Again, these results were confirmed and replicated in germ-free mice, which achieved a better response to IO after fecal transplant with stool specimens of responding patients [30]. A fourth work by Matson et al. analyzed a similar population of melanoma patients treated with IO and evidenced that cases showing an objective tumor response had basal stool samples enriched in *Bifidobacterium longum, Collinsella aerofaciens* and *Enterococcus faecium.* Germ-free mice transplanted with these specimens showed improved response to anti-PD-L1, with increase in T-cell infiltrates in tumor masses [31].

We aimed at investigating the association between the use of antibiotics and response to IO in a population of patients with metastatic NSCLC treated with IO at our Institution. The impact of antibiotics on outcome was investigated considering not only the simple antibiotic use in the Early IO Period (EIOP), but also the

cumulative exposure to antibiotics in relation to the Whole IO Period (WIOP). For this purpose, we coined a new variable called Antibiotic-Immunotherapy Exposure Ratio (AIER), defined as "days of antibiotic/days of IO" during the WIOP.

Materials and methods

Patients

We analyzed data about all consecutive patients with metastatic NSCLC treated with ICIs at Istituto Nazionale dei Tumori, Milan, Italy, between April 2013 and January 2018.

All the patients signed a written informed consent declaring their agreement to the use of personal data for research purposes at some time of their disease history.

Clinical data were retrospectively collected from institutional database and included age, gender, performance status (PS) according to Eastern Cooperative Oncology Group (ECOG) [32], smoking habits, histology, tumor molecular characterization whenever performed, line of IO, ICI prescribed, response to IO according to Response Evaluation Criteria for Solid Tumors (RECIST) [33], number of metastatic sites at the beginning of IO, and details of antibiotic use (including specific drug, route of administration, dosage, and duration).

For the purpose of the analysis concerning the EIOP, antibiotic use was considered relevant when it happened between 1 month before and 3 months after the beginning of IO. Patients receiving antibiotics outside this time frame were considered as non-antibiotic treated. The AIER was defined as the rate "days of antibiotic therapy/days of IO" during the WIOP. At first, the AIER was considered as a continuous variable for statistical analyses. Then, the median value of AIER was used as cut-off, and patients with an AIER lower than the cut-off were considered in the control group as those never receiving antibiotics.

All ICIs were included, irrespective of mechanism of action. Cases receiving only the first dose of drug and then discontinuing for toxicity, worsening of general conditions or death were excluded from the analysis. Patients receiving at least two doses of ICI and then discontinuing treatment for unequivocal clinical progression were included in the analyses and accounted as progressors at first evaluation.

Statistical analysis

Descriptive statistics were used to analyze and report clinical variables.

All patients were followed until death, loss at follow-up or time of data lock, which was set on the 1st March 2018. PFS was calculated as the time from the beginning of IO to disease progression, or death for any cause. Disease response was assessed using RECIST, at the last version applicable at the time of IO administration (ranging from 2013 to 2018). Overall survival (OS) was calculated as the time from the beginning of IO to death for any cause. Alive patients were right-censored at the time of last contact. PFS and OS were estimated with Kaplan-Meier method. Duration of follow-up was calculated with reverse Kaplan-Meier method. Differences between survival curves were analyzed with log-rank test.

Fisher's exact test was used to compare discrete qualitative variables. Differences between continuous ordinal variables were tested with Mann-Whitney non-parametric U test. Univariate analyses were performed according to sex (male *versus* female), age (<70 years *versus* ≥70 years), smoking status (current or former smoker *versus* never smoker), tumor histology (squamous NSCLC *versus* non-squamous NSCLC), ICI (anti-PD1 *versus* anti-PD-L1 *versus* anti-CTLA4 and combinations), line of IO (first *versus* second *versus* third or more), basal ECOG PS (0 *versus* 1 or 2), and number of metastatic sites at the beginning of IO (0 or 1 *versus* 2 or more). χ square test was used for univariate analyses. Cox proportional hazard model was applied for multivariate analyses, which were calculated for the significant variables at univariate test. All analyses were two-sided and values of $p<0.05$ were considered statistically significant.

Statistical analyses were performed with SAS (version 9.4, SAS Institute, Cary, NC, USA).

Results

Patient and IO treatment characteristics

A total of 157 patients with NSCLC were identified. The population included 94 men and 63 women. Median age was 66.7 years (range: 30.6-86.5 years). One hundred thirty-seven patients (87.2%) were current or former smokers.

Non-squamous NSCLC accounted for 121 cases (77.1%) and included mostly adenocarcinomas, but also a limited number of sarcomatoid, adeno-squamous, intestinal-like and not otherwise specified lung cancers. PD-L1 expression was high in 44 patients (28.0%), negative in 46 patients (29.3%), low in 3 patients (1.9%); data about PD-L1 were unknown in 64 patients (40.8%). Only 7 tumors (4.5%) harbored an *EGFR* mutation;

EGFR was unknown in 27 patients (17.2%). *ALK* rearrangement was found in one patient (0.6%); missing data were 29 (18.4%). Neither cases of *ROS-1* rearrangement nor *BRAF* mutations were found.

PS ECOG at the beginning of IO was 0 in 47.8% of cases, 1 in 45.2%, 2 in 7.0%.

Most of the patients (86.0%) had 2 or more sites of metastatic disease at the time of IO initiation. The most common sites of distant disease were lymph nodes (133 cases), lung (78 cases), bones (67 cases), liver and/or adrenal glands (55 cases), brain (32 cases).

Patient characteristics are summarized in Table 1.

The majority of patients (98 of 157, 62.4%) received an anti-PD1 (nivolumab in 88 cases, pembrolizumab in 10 cases); 33.1% of patients received an anti-PD-L1 (durvalumab in 31 cases, atezolizumab in 16 cases, avelumab in 4 cases, MSB0011359C in 1 case); 1 patient received an anti-CTLA4 (tremelimumab); 6 patients received a combined IO (durvalumab + tremelimumab).

IO was administered as first line treatment in 25 cases (15.9%), as second line treatment in 66 cases (42.0%), as third line treatment in 44 cases (28.0%), as a more advanced line of therapy in 22 cases (14.1%).

Disease stability was obtained in 52 patients (33.1%), partial response in 34 (21.7%); only 1 complete response was documented (0.6%), while the remaining patients (70, 44.6%) underwent disease progression.

Median time to best response was 1.9 months (range: 0.1-17.5 months).

Eighty-two patients (52.2%) discontinued IO due to disease progression, 30 (19.1%) for physician's decision as a consequence of clinical deterioration, 10 (6.4%) after regular conclusion of planned treatment program, one (0.6%) for patient's withdrawal of consent. IO was still ongoing at the time of database lock in 34 cases (21.7%).

Median duration of IO was 4.2 months (range: 0.2-91.0 months).

Antibiotic treatment characteristics

A total number of 46 patients (29.3%) received at least one administration of antibiotic through the WIOP.

Twenty-seven patients (17.2%) received antibiotics during the EIOP. In 7 cases (25.9%), more than one antibiotic was subsequently administered. All patients received single agent antibiotic therapy.

Considering all the 46 patients receiving antibiotics, the reason for prescription was a respiratory tract infection in almost all cases. Four patients (8.7%) received an antibiotic for a reactivation of diverticular bowel disease, one for colitis, and one for urinary tract infection; all these cases were also treated with a different antibiotic for pneumonia.

Accordingly to the etiology, the most commonly prescribed antibiotic was levofloxacin (30 cases, 65.2%), followed by amoxicillin/clavulanate (10 cases, 21.7%), claritromicin (5 cases, 10.9%), ceftriaxon (4 cases, 8.6%), rifaximin (4 cases, 8.6%), ciprofloxacin (3 cases, 6.5%), and azitromicin (3 cases, 6.5%).

Antibiotics were administered by oral route in 44 cases (95.7%), by intra-muscular route in 3 cases (6.5%), and by intra-venous route in 2 cases (4.4%).

Median duration of a single cycle of antibiotics was 6 days (range: 2-17 days). Considering the cumulative duration of antibiotic treatment, thus summing the length of single cycles, the median value was 7.0 days (range: 5.0-33.0 days).

Considering the WIOP, the median AIER was 4.2% (range: 0.6-42.9%). Twenty-three patients (14.7% of the global population) had an AIER higher than the median one. The median duration of antibiotic treatment was significantly different between the 2 subgroups defined by this cutoff (9 *versus* 6 days, p=0.0087). All other main clinical and pathological variables were balanced between the 2 subgroups (Table 2).

Outcome results

Median follow-up was 28.6 months.

Antibiotic administration in the EIOP was not associated with reduced RR (11.1% *versus* 24.6%, p=0.2018) and Disease Control Rate (DCR) (51.9% *versus* 56.2%, p=0.8319).

After stratifying the global population according to the AIER in the WIOP, patients with a higher AIER than the median (4.2%) had a RR of 8.7%, while the RR of the control group was 26.6%. Corresponding DCR were 47.8% and 56.0%, respectively. These differences were not statistically significant (p=0.1082 and p=0.5030, respectively).

Median PFS of the global population was 3.0 months (95% confidence interval (CI) 2.3-3.8 months). Median OS of the global population was 11.3 months (95% CI 8.8-15.2 months).

At univariate analyses, no impact on PFS could be evidenced for smoking status, ICI mechanism of action, gender, and age. A significant detrimental effect on PFS was observed for basal PS ≥1 and for second or more advanced line of IO. Similarly, no significant differences in median OS were seen when stratifying patients according to smoking status, ICI mechanism of action, gender , age, and line of IO. The only variable negatively impacting on OS was basal PS (p<0.0001) (Table 3).

Regarding the effects of antibiotics, the use of one or more anti-microbic agents in the EIOP did not influence either PFS (3.3 months for non-treated patients *versus* 2.2 months for treated patients; p=0.1772) or OS (11.9 months for non-treated patients *versus* 5.9 months for treated patients; p=0.2492), although a numeric trend towards a better prognosis was seen for cases not receiving antibiotics (Table 3 and Figure 1).

When considering a different time cutoff of 2 instead of 3 months after the first administration of ICI, the results did not change (median PFS 3.35 for non-treated patients *versus* 2.2 for treated patients; p=0.0992; median OS 11.9 months for non-treated patients *versus* 5.6 months for treated patients; p=0.2147).

In the WIOP, a higher AIER appeared to have a detrimental effect on PFS and OS when considered as a continuous variable (hazard ratio (HR) 1.053, p=0.0029 for PFS; HR 1.069, p <0.0001 for OS).

Performing the evaluation on AIER with the cutoff value of 4.2%, a significant difference between the two subgroups thus defined became evident in terms of both PFS (3.5 months for patients with AIER <4.2% *versus* 1.9 months for patients with AIER ≥4.2%, p<0.0001) and OS (13.2 months for patients with AIER <4.2% *versus* 5.1 months for patients with AIER ≥4.2%, p=0.0004) (Table 3 and Figure 2).

At multivariate analyses, the impact of AIER on PFS retained significance after correction for the effects of basal PS (HR 1.053, p=0.0052) and line of IO (HR 1.059, p=0.0018). Similarly, multivariate analyses confirmed that the detrimental effect of high AIER on OS was independent from that of PS (HR 1.064, p=0.0002) (Table 4).

Discussion

The role of immune system in maintaining active surveillance against malignancies is known since decades [34,35]. Pre-clinical and clinical data have demonstrated that immune-compromised hosts have a higher incidence of cancers, which often show an aggressive behavior. Moreover, these subjects tend to have a poorer response to treatments and a worse prognosis than immune-competent hosts [36].

The concept of immune activation against tumors has dramatically increased its relevance since the introduction of ICIs in clinical practice. Indeed, the rationale for the use of these agents relies on their stimulation of systemic immunity against cancer, leading to an immune-mediated killing of malignant cells. Recent years have shown a growing broadening of indications for IO, which has entered clinical practice in the treatment of various malignancies [37].

In the field of lung cancer, different agents have been approved or are in advanced phase of study for locally advanced and metastatic disease. Many trials showed a benefit for ICIs over traditional cytotoxic agents in terms of survival in different lines of therapy [3-14]. Although the general validity of this observation, it is well known from everyday practice that only a limited number of patients derive a real benefit from IO [13,14,17,19-22]. While about one-third of cases show a disease response and may expect a quite long survival, the remaining two-thirds never respond to ICIs and maintain a dismal prognosis [13,14,17,19-22].

Given the relevance of this topic, many studies have focused on variables potentially able to predict the response to IO. Data obtained on different agents and in different settings have supported a role for PD-L1 expression, tumor mutation burden and microsatellite instability [13,14,17,38-40]. Nonetheless, it is likely that the majority of factors accounting for such a variability still remains unknown. In this field, research has recently been developed about gut microbiota and its imbalances due to antibiotics.

In this work, we performed a single Institution retrospective analysis of a cohort of patients with metastatic NSCLC treated with IO. At first, we considered significant for stratification purposes the administration of antibiotics in the EIOP. We could not identify any differences in RR, PFS and OS between antibiotic treated and non-treated patients, although a numerical tendency in favor of non-treated cases was evidenced. Afterwards, when we evaluated the impact of the AIER on PFS and OS in the WIOP, this continuous variable showed to have a significant detrimental effect on outcome. Given this result, we performed a second analysis using the median value of the AIER as a cut-off for patient stratification. When we stratified the population according to the AIER, a significant advantage emerged for patients with an AIER inferior to the median one of 4.2%.

The AIER is a completely new empiric variable, that we proposed in order to explore whether the length of antibiotic therapy in relation to that of IO could be more influent that the timing of antibiotic administration.

To our knowledge, this is the first time that a variable influencing the duration of treatment and not its relation to the beginning of IO shows a correlation to outcome. The potential rationale behind this observation may lay in the physiological capability of intestinal flora to regain homeostasis after an alteration. In other words, each antibiotic administration induces an imbalance in bacterial species populating the intestinal tract. After the end of the antibiotic cycle, the commensal bacteria species progressively return to their primitive composition, and the alteration induced by treatment is overcome. If the process is limited in time, in particular if IO is carried on meanwhile, it is likely that the effects of antibiotics on intestinal flora, and consequently on anti-tumor immune response, is negligible. On the contrary, if a patient receives multiple or prolonged antibiotic cycles, the repeated hits on gut microbiota may interfere with bacterial reconstitution, leading to a deeper impact on systemic immunity. The significant relation between AIER and outcome, irrespective of the effects of PS and IO line, and in absence of a clear role for the simple antibiotic use at the beginning of IO, supports the potential role of AIER as a new independent determinant.

As regards the lack of association between antibiotic use in the EIOP and outcome, this result differs from most recent research data showing a significant negative impact of early antibiotics on PFS. Only hypotheses can be made to explain this discrepancy, maybe due to population characteristics and small sample size. In any case, a numerical trend towards superior PFS and OS for patients not receiving antibiotics was identified, in line with literature data. Nonetheless, it has to be evidenced that also a previous retrospective work on 74 NSCLC cases treated with nivolumab did not find differences in RR and PFS according to antibiotic use in the 3 months before the beginning of the ICI [41]. This underlines the lack of definitive evidences in the field of microbiota and IO, in particular for NSCLC, and the consequent need of further research.

This study has some limitations that have to be underlined. First, it is a retrospective analysis of a single Institution population, with a small number of patients. This might limit the possibility of generalizing its results. Second, as this is a retrospective work on an unselected population, we did not perform either biologic analyses on stool samples, or translational correlations on animal models. This prevents from identifying a biologic rationale and confirmation for our observations. Indeed, in absence of a translational correlative, it cannot be excluded that patients receiving repeated courses of antibiotics have a worse

prognosis because of frequent infections, and not for the use of antibiotics itself. In the end, AIER is a merely empiric variable, based on a theoretical rationale, which will require additional studies to prove its scientific basis. Moreover, the cutoff chosen for stratification derives from the analysis of the population itself, being defined as the median value of the observed cases. This cutoff may be different when analyzing other case series, and the results may change consequently.

Despite these limitations, the present work presents some interesting points. For the first time it proposes the AIER as a new variable that may condition the impact of antibiotic use on the efficacy of IO. Although the selection of a specific cut-off may be questionable, we conducted our analysis in the attempt of testing the potential applications of this new variable. Indeed, a definite cut-off is essential for any tool aiming to be useful in clinical practice, whereas continuous variables are barely applicable. AIER has been tested on an unselected, intentionally heterogeneous population, which reflects the characteristics of patients in clinical practice. The variability in treatments, disease extension and patient PS, and the prolonged time span of data collection, instead of being a bias, may constitute a strong point of the analysis, depicting a realistic population of outpatient cases with NSCLC.

In conclusion, this work suggests that the recently emerged concept that antibiotics impair the efficacy of IO may be considered from a different point of view. In particular, the most relevant factor modulating this effect may be the AIER in relation to the duration of IO, instead of the kind of antibiotic prescribed or the time between the antibiotic use and the beginning of IO. Given the limited number of patients and the retrospective nature of the analysis, this hypothesis deserves confirmation and further investigation in larger case series. Furthermore, a translational and biological correlation with stool analyses and animal models may contribute to give a strong scientific evidence to our observation, which still remains a hypothesis-generating finding. However, if this relevance was confirmed, the AIER may become a new variable to help predicting the response to IO of NSCLC patients. Finally, it suggests that clinicians should carefully weigh the risk/benefit ratio of prescribing antibiotics, in particular for repeated or long courses, to patients with metastatic NSCLC.

Conflict of interest statement

CP declares travel accommodations and honoraria with MSD International GmbH, BMS, Eli Lilly. DS declares travel accommodations and honoraria with AstraZeneca, MSD International GmbH, BMS. FdB provided consultation, attended advisory boards and/or provided lectures for the following organizations, from whom received honoraria or education grants: Amgen, AstraZeneca, Boehringer-Ingelheim, BMS, Eli Lilly, F. Hoffmann-La Roche, Ignyta, Merck Sharp and Dohme, Merck Serono, Novartis, Pfizer. MCG declares personal financial interests with the following organizations: AstraZeneca, MSD International GmbH, BMS, Boehringer Ingelheim Italia S.p.A, Celgene, Eli Lilly, Ignyta, Incyte, Inivata, MedImmune, Novartis, Pfizer, Roche, Takeda; she also declares Institutional financial interests with the following organizations: Eli Lilly, MSD, Pfizer (MISP), AstraZeneca, MSD International GmbH, BMS, Boehringer Ingelheim Italia S.p.A, Celgene, Ignyta, Incyte, Inivata, MedImmune, Novartis, Pfizer, Roche, Takeda, Tiziana, Foundation Medicine; at the end, she has received research funding from the following organizations: AIRC, AIFA, Italian Moh, TRANSCAN. GLR declares travel accommodations and honoraria with AstraZeneca, MSD International GmbH, BMS, Eli Lilly. All other authors have no relevant conflicts of interest to disclose.

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Figures

1.1 Kaplan-Meier curves for PFS and OS according to antibiotic use in the EIOP

PFS = Progression Free Survival

Atb = Antibiotics

EIOP = Early Immunotherapy Period

OS = Overall Survival

2.1 Kaplan-Meier curves for PFS and OS according to AIER during the WIOP

PFS = Progression Free Survival

AIER = Antibiotic/Immunotherapy Exposure Ratio

OS = Overall Survival