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The impact of intestinal microflora on the effectiveness of immunotherapy with antibodies against immune checkpoints — case report and literature review

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ABSTRACT

Inhibitors of immune checkpoints (anti-PD-1 or anti-PD-L1 monoclonal antibodies) are effective in non-small cell lung cancer treatment, significantly extending the overall survival of some patients. However, there are no predictive factors, which could allow precise qualification of cancer patients to immunotherapy. The best evaluated in this regard is the expression of PD-L1 molecule on tumour cells, the occurrence of which is associated with higher response rate and prolonged time to progression in patients undergoing immunotherapy. Some recent reports indicate that the composition of the patient's intestinal microflora, the presence of inflammation, and antibiotic therapy used before or during immunotherapy may affect the effectiveness of anti-PD-1 or anti-PD-L1 antibodies. Disturbance of the body's natural balance, e.g. due to the use of antibiotics, may reduce the effectiveness of immunotherapy. This may be due to a lack of stimulation of the immune system by antigens from bacteria found naturally in the gut. On the other hand, supplementing the microflora with the necessary ingredients can improve the effectiveness of immunotherapy. The future goal is to develop so-called "immunotherapeutic probiotics", the use of which could enhance the effect of cancer immunotherapy.

Key words: microbiome, intestinal microflora, immunotherapy, anti-PD-1 and anti-PD-L1 antibodies, probiotics

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Introduction

Cancer immunotherapy refers to a wide range of methods to modify the functioning of the immune system in cancer patients, aiming to enhance or stimulate the anti-cancer effect. Currently, the highest clinical efficacy in the treatment of various types of tumours is demonstrated by monoclonal antibodies directed against immune checkpoint (ICP) molecules. The two most important ICP molecules for immunotherapy include: PD-1 (programmed cell death protein 1), which occurs mainly on T- and B-cells, and a PD-

-L1 molecule (programmed death-ligand 1), occurring on non-specific immune cells, i.e. macrophages or dendritic cells, normal body cells, and cancer cells. The interaction of PD-1 molecule with PD-L1 suppresses the activity of PD-1-positive lymphocytes. The presence of PD-L1 molecule on the surface of tumour cells and its interaction with PD-1 on the surface of lymphocytes leads to inactivity of the latter cells. For these reasons, blocking the pathway of suppressing lymphocyte activity by blocking PD-1 or PD-L1 molecules with specific monoclonal antibodies restores antitumoral activity of lymphocytes [1–3].

The use of immune checkpoint inhibitors influenced the efficacy of treatment of patients with non-small cell lung cancer (NSCLC), extending their survival. One of the best evaluated predictors of response to anti-PD-1 or anti-PD-L1 antibody therapy is the expression of PD-L1 molecule on the surface of tumour cells and tumour-infiltrating immune cells. However, there is still no answer to the question of why some patients do not respond to anti-PD-1 or anti-PD-L1 treatment, despite positive PD-L1 expression on cancer cells. In addition, it is not clear why the response to immunotherapy can be observed in patients without PD-L1 expression on tumour cells. Primary resistance to immune checkpoints inhibitors, observed in about 60–70% of patients, may result from low tumour mutation burden (TMB), and hence low antigenicity of tumour cells. Other reasons for lack of response to immune checkpoints inhibitors include defective presentation of tumour antigens by antigen presenting cells, local immunosuppression caused by factors produced by cancer cells, or functional depletion of T-cells in the tumour microenvironment [1–3].

Many recent reports indicate that the antitumour activity of immune checkpoint inhibitors may be affected by intestinal microflora, which modulates the innate and acquired immune response. It is well-known that there are approximately 500–1000 unique bacterial strains in the human large intestine. They are called intestinal microflora when we analyse the content of individual bacterial strains, or as a microbiome when we analyse the genetic “content” of these bacteria. Although many details remain unknown, available studies in mice and humans suggest that this complex bacterial community is essential for many aspects of health, including physiology, disease resistance, and digestion [4, 5].

Previous studies evaluating the effectiveness of drugs directed against immunological checkpoints have completely ignored patient’s microflora. When looking for predictors of response to treatment with molecular-targeted drugs, chemotherapy, and immunotherapy, the importance of microbiome was not suspected. The interactions between microbiome, cancer process, and immune system are not fully understood. A lot of data point to the effect of bacterial flora on oncogenesis, tumour progression, and response to immunotherapy. The disturbance of natural balance of a patient’s bacterial flora, e.g. using antibiotic therapy and chemotherapy, can significantly impact anticancer treatment effectiveness [6, 7]. The question arises of whether patients undergoing antibiotic therapy should therefore be considered as ineligible to immunotherapy. Data regarding microbiome may be a predictor of response to therapy, on the other hand, supplementing the patient’s microflora with bacterial strains with proven positive effects on the body may improve the effectiveness of immunotherapy.

Case report

A 66-year-old woman, chronic cigarette smoker, with medical history of breast cancer in 2009 (after surgery and radiotherapy) and bladder cancer in 2013 (after transurethral tumour resection) was admitted to our department in 2016 for diagnosis of an entirely asymptomatic tissue mass in the right lung detected during chest X-ray examination.

After the tomographic assessment, invasive diagnostics were implemented. Pathomorphological examination of the material obtained during bronchofiberoscopy revealed the presence of adenocarcinoma cells. Molecular studies were performed that did not confirm the presence of mutations in *EGFR* gene and rearrangement of *ALK* gene. Due to stage IV cancer at diagnosis and an inability to use surgical treatment, the patient was qualified for chemotherapy, that was initiated in October 2016. The patient received a total of four cycles of platinum-based systemic treatment. Initially, cisplatin was used, and then, due to side effects, chemotherapy was modified by the use of carboplatin in combination with vinorelbine. The treatment was completed in December 2016 due to disease progression found during computed tomography examination. In February 2017, chest radiotherapy was used and the tumour area was irradiated with a total dose of 20 Gy.

Due to the patient’s deteriorating general condition, weight loss (8 kg from the beginning of treatment), and available treatment with anti-PD-1 antibody, nivolumab, within an expanded access program, the decision was made to start immunotherapy. In February 2017, the qualification for treatment began. A computed tomography was performed in which measurable changes were visualised (Fig. 1) Unfortunately, at the time of admission, the patient’s performance status was determined as moderate (PS = 2). The patient had fever up to 38°C. Initial CT examination showed the presence of pneumothorax at the top of right lung, and effusion in the right pleural cavity with a layer thickness of up to approx. 30 mm. Elevated levels of inflammation markers [C-reactive protein (CRP) 54.1 mg/L] were reported in laboratory tests. After surgical consultation the decision to abandon the pleural cavity drainage was made. Due to increased inflammation parameters and episodes of increased body temperature, detailed microbiological diagnostics were carried out. However, no unequivocal confirmation of fever origin was obtained. Therefore, after oncological consultation, having in mind an improvement of performance status (PS = 1) and reduction of fever (body temperature did not exceed 38°C but remained elevated), it was decided to introduce a treatment with nivolumab in a dose of 3 mg/kg body weight (bw) every two weeks with concomitant prophylactic antibiotic administration

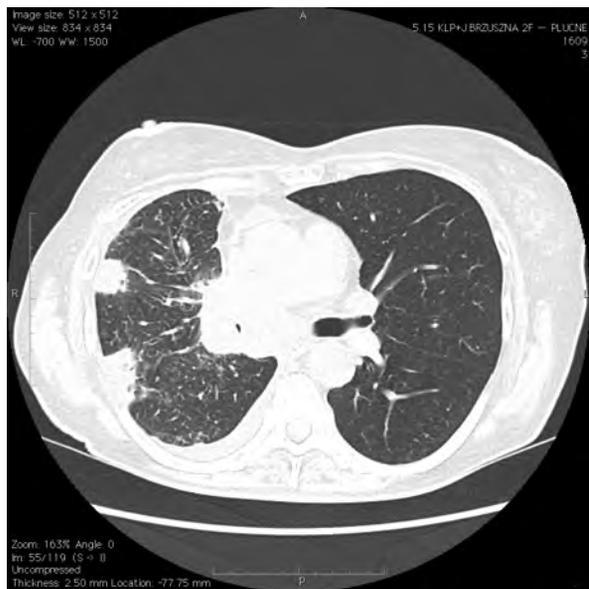


Figure 1. Computed tomography scan at the time of qualification for immunotherapy

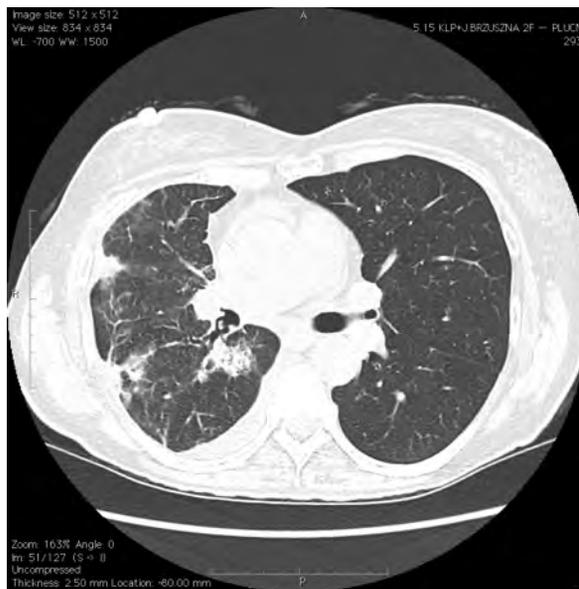


Figure 2. Computed tomography scan showing partial remission of NSCLC. Visible beginning of the process of lung tissue consolidation

(intravenous Tarcefadol 3 × 1 g). The patient received the first infusion of nivolumab with good tolerability, and was then discharged home with the recommendation to take clarithromycin 500 mg orally two times a day for five days. The patient, during the use of antibiotic therapy, took probiotic forms of bacteria (*Bifidobacterium sp.* and *Lactobacillus sp.*).

Control laboratory tests performed before the second administration of nivolumab showed a decrease of CRP level to 32.0 mg/L; the patient recovered from the fever. The subsequent doses of nivolumab were administered with good tolerability and a visible clinical effect. There was a gradual improvement in appetite, stopping the decline, and then an increase in body weight and a significant improvement in the patient's well-being. During the fifth infusion of nivolumab the CRP level was 15.0 mg/L and most of the symptoms disappeared. Chest computed tomography performed after six doses of the drug showed partial regression of the disease (Fig. 2).

In the follow-up computed tomography examination after the 14th drug administration, the progression of lesions in the upper right lung were noted as areas of pulmonary consolidation with air bronchogram (Fig. 3). In view of the clinical response and absence of other therapeutic options, it was decided to continue immunotherapy. A good immunotherapy effect was maintained. The patient's condition remained stable, and the observed lesions in the lung did not progress and even partially reduced in subsequent studies, which led to the recognition of the observed consolidation as a pseudoprogression during immunotherapy (Fig. 4).

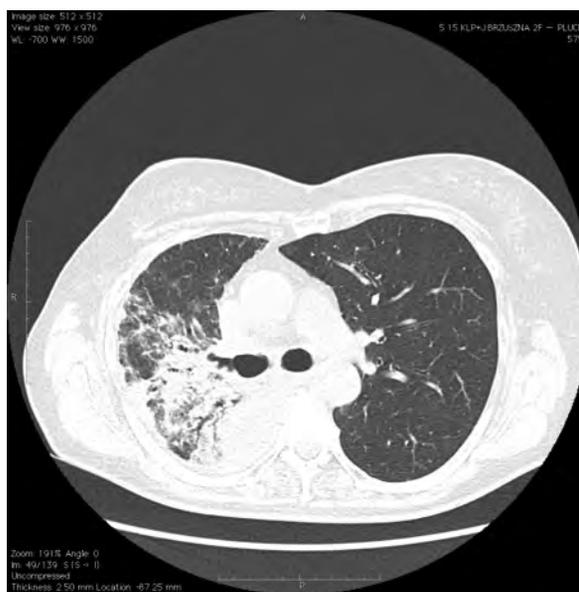


Figure 3. Increased lesions in the upper lobe of right lung, which form areas of pulmonary tissue consolidation with air bronchogram visible in computed tomography scan

Subsequent computed tomography examination performed in March 2018 showed stabilisation of chest lesions. However, the study revealed asymptomatic peripheral pulmonary embolism, which again caused the postponement of nivolumab administration and use of appropriate treatment of embolism. The patient currently is receiving the 26th dose of the drug. She is in good general condition, without cancer

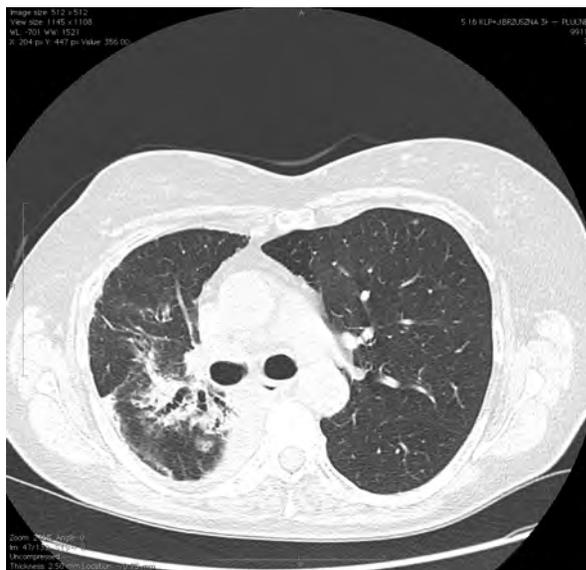


Figure 4. Yielding consolidation of lung tissue lesions during the continuation of immunotherapy

symptoms, and continuing treatment with anti-PD-1 antibody.

Review of the literature

The first studies describing the effect of selected intestinal bacteria strains on immunotherapy efficacy appeared in 2015 [8]. Sivan et al. compared melanoma growth in two strains of mice with distinct commensal bacterial flora and observed differences in spontaneous anti-tumour immunity in these animals [8]. They showed that oral administration of selected intestinal bacteria (including mainly supplementation of *Bifidobacterium sp.*) together with simultaneous injections of anti-PD-L1 antibodies reduced tumour mass as compared to mice not receiving bacterial supplementation during immunotherapy. In cancer tissue samples, the increased activity of dendritic cells was observed, leading to enhanced stimulation and accumulation of CD8-positive T-cells in tumour microenvironment in animals supplemented with intestinal bacteria. The authors suggest that manipulation of intestinal microflora of an immunised organism may modulate the response to immunotherapy. Studies by Sivan et al. indicated the increase of anti-tumour immunity *in vivo* by commensal *Bifidobacterium sp.* Moreover, this immune activity seems to increase regardless of presentation of cancer antigens. The authors suggest that *Bifidobacterium* colonises a specific compartment in the intestine, which allows interaction with host cells, which are in turn key to modulating dendritic cell function and the release of

other agents (e.g. IL-12) activating remaining cellular elements of the immune system [8].

In a study published in the *Journal of Thoracic Oncology* in 2017, Thompson et al. retrospectively evaluated the efficacy of anti-PD-1 treatment in patients with metastatic NSCLC, who had previously received antibiotic therapy [9]. In the studied group ($n = 74$), 55% were men, and adenocarcinoma was diagnosed in 57% of patients. In total 15% of respondents had metastases to the central nervous system, while 38% underwent radiotherapy; 24% of patients received antibiotics (mainly fluoroquinolones) before immunotherapy, due to infection of the respiratory system. Treatment with nivolumab was used in 95% of patients. Patients exposed to antibiotic therapy up to six weeks before anti-PD-1 antibody treatment had shorter progression-free survival (PFS) [hazard ratio (HR) = 2.5, $p = 0.02$] and shorter overall survival (OS) (HR = 3.5, $p = 0.004$) than patients who had not received prior antibiotic therapy. The authors point to the negative effect of antibiotics on intestinal flora, which may adversely affect the immunotherapy's effectiveness [9]. However, it remains unanswered by the authors whether the patients receiving antibiotics used probiotic supplementation at the same time, which could improve the efficacy of treatment with anti-PD-1 inhibitors.

Routy et al. showed in a study published in the journal *Science* in January 2018 how important intestinal microbiome could be for the efficacy of immunotherapy using immune checkpoints inhibitors [10]. The authors conducted an experiment in which faecal microflora were taken from patients with kidney or lung cancer, who responded to anti-PD-1 antibody therapy ("responders"), and from patients who did not respond to this therapy ("non-responders"), which was then transplanted to "germ-free" (free of microbes) mice with cancer and mice previously treated with antibiotics. The anti-tumour response to anti-PD-1 therapy was observed in mouse strains after intestinal microflora transplantation from patients responding to treatment. Contrary to this, response to anti-PD-1 therapy was observed in animals after transplantation of intestinal microflora from patients with no response to anti-PD-1 therapy. Identification of microorganisms from faeces samples of non-responders to immune checkpoints inhibitors showed a lower number of *Akkermansia muciniphila*, *Bifidobacterium sp.* and *Faecalibacterium sp.* in comparison to faeces of patients responding to immunotherapy. Response to immunotherapy was improved in mice after oral supplementation with *Akkermansia muciniphila*, despite earlier transplantation of intestinal flora from patients not responding to the treatment. The infiltration of tumour tissue with helper lymphocytes expressing receptors for chemokines (CCR9 and CXCR3) was also increased in these animals [10].

The authors indicated the need to examine the composition of the patient's intestinal microflora as a predictive factor for immunotherapy with anti-PD-1 antibodies, and in the case of abnormalities or earlier treatment with antibiotics, they suggested the need for supplementation with probiotics [10]. The activity of lymphocytes, and production of pro-inflammatory cytokines and chemokines by non-specific response-related cells is over-stimulated during chronic inflammation; however, it in turn enhances the expression of immune checkpoint molecules on body cells.

An important relationship between antibiotic therapy and response to treatment with immune checkpoint inhibitors was also presented by Derosa et al. [11]. A cohort of 80 patients with renal cell carcinoma included 67 patients treated with anti-PD-1 or anti-PD-L1 inhibitor monotherapy, 10 patients receiving combined anti-PD-1 and anti-CTLA-4 treatment, and three patients treated with atezolizumab (anti-PD-L1 antibody) and bevacizumab. With respect to risk groups according to the IMDC (International Metastatic Renal Cell Carcinoma Database Consortium), 21% of patients were in a favourable-risk group, 57% of patients were in an intermediate-risk group, and 22% of patients were in a poor-risk group with a low chance of responding to the treatment. Twenty percent of patients received antibiotic therapy (β -lactamases and fluoroquinolones) during the first month before immunotherapy. Multifactorial analysis regarding age, gender, risk group according to IMDC, antibiotic therapy, and tumour mutational burden (TMB) in relation to immunotherapy effectiveness showed that patients receiving antibiotic therapy have shorter PFS after immunotherapy compared to patients not treated with antibiotics (2.3 months vs. 8.1 months, respectively; $p < 0.001$) regardless of stratifying prognostic factor. The objective response rate (ORR) to immunotherapy was also significantly ($p < 0.002$) lower in the group of patients receiving antibiotics before immunotherapy [11].

The case report presented in this paper, of a patient receiving antibiotic therapy and immunotherapy with the anti-PD-1 antibody, indicates the possibility of obtaining a clinical response to immunotherapy despite the use of simultaneous antibiotic therapy. It seems that the coexistence of chronic inflammation and probiotic supplementation during antibiotic treatment were of great importance for the response to nivolumab. Chronic inflammation may have contributed to long-term stimulation of the immune system, which further enhanced the anti-tumour cell-mediated immune response. Moreover, antibiotic therapy was carried out several times in the course of persistent sub-febrile states, but this did not affect the effectiveness of anti-PD-1 treatment. It seems that another situation occurs in patients with acute infection, leading to the use of antibiotics. In this case, the

natural body reaction is to suppress the immune system activity when the infection is controlled, in order to prevent the development of autoimmune reactions. This, in turn, induces the expression of immune checkpoint molecules, including PD-L1 on various cells. Therefore, in patients after acute infection and antibiotic therapy, the effect of immunotherapy may be negligible [12].

The key question to answer is how colonisation of the intestines with certain microbial species can enhance the response to immunotherapy with anti-PD-1 or anti-PD-L1 antibodies. One explanation may be that the antigen mimicry between microbial and tumour antigens might increase the activation of immune response [13]. In this case, it seems that each patient would require another species of bacteria that could mimic the neo-antigen profile different to his/her cancer. In addition, it is possible to develop cross-reactions between the antigens of the patient's microbiome and the common antigens for the cancer [13].

It should also be remembered that constant circulation of immune cells and immunomodulators between intestinal lymphoid tissue, peripheral circulation, and lymph nodes may also non-specifically "enhance" immunity by increased production of pro-inflammatory cytokines by antigen presenting cells (e.g. dendritic cells). Additionally, in about 10–15% of patients treated with anti-PD-1 antibodies it can also induce autoimmune phenomena. As shown in clinical trials, patients experiencing autoimmunity show a better response to the treatment with immune checkpoint inhibitors than patients with no autoimmune phenomena [13, 14]. On the other hand, these patients are at risk of serious complications of immunotherapy associated with autoimmunity reactions such as thyroiditis, intestinal mucositis, and pneumonitis.

The expression of the PD-L1 molecule on tumour cells is undoubtedly one of the more thoroughly investigated predictive factors of response and prolonging PFS in patients undergoing immunotherapy with anti-PD-1 and anti-PD-L1 antibodies. Considering the influence of microbiome on anti-PD-1 and anti-PD-L1 treatment efficacy, it is necessary to determine whether the presence of selected bacterial species in the intestinal microflora may serve as a single predictor of immunotherapy or whether it should be combined with the PD-L1 expression and TMB. The question arises of whether a microbiome should be routinely examined in cancer patients before starting treatment with immune checkpoints inhibitors.

Despite numerous reports on the negative impact of antibiotics taken before and during treatment with immune checkpoints inhibitors, first of all it is necessary to understand if there is a connection between the duration of use, group of antibiotics, the indication for use of antibiotics and the effectiveness of immunotherapy.

In addition, it should also be remembered that the presence of inflammation in the body may be associated with increased expression of immune checkpoint molecules, including the activation of a pathway to suppress the activity of the immune system through the interaction of PD-1 and PD-L1. On the other hand, the presence of chronic inflammation causes permanent activation of the immune system, which may increase its ability to conduct an anti-cancer response (e.g. it has been proven that tumours in tuberculosis patients often progress very slowly, and BCG vaccine is still used in adjuvant treatment of bladder cancer). These hypotheses need to be proven in clinical trials that could elicit new predictors of response to immunotherapy present in the patient's microflora. Such research is also aimed at developing so-called "immunotherapeutic probiotics", the use of which could strengthen the effect of immunotherapy of cancers [13].

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