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ONCO-RHEUMATOLOGY: (SIDE)EFFECTS OF ONCOLOGICAL AND RHEUMATOLOGICAL TARGETED THERAPIES

by Dr. Enikő Sebestyén

Supervisor: Dr. Éva Szekanecz

UNIVERSITY OF DEBRECEN

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By: Dr. Enikő Sebestyén, MD

Supervisor: Dr. Éva Szekanecz, PhD

Doctoral School of Clinical Medicine, University of Debrecen

Head of the Defense Committee:	Prof. Dr. Attila Bácsi, DSc
Reviewers:	Dr. Gabriella Emri, PhD Dr. Zsófia Kardos, PhD
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Introduction and Literature Review

Immuno-oncological aspects

The immune system plays a key role in maintaining the body's homeostatic balance and in the pathogenesis of both oncological and autoimmune diseases. In the case of oncological diseases, the immune response is attenuated, in autoimmune diseases, it is pathologically aggravated. In both cases, there is a disruption in the immune system's functioning, resulting in an imbalance in the body's natural balance. The molecular level actors and therapeutic challenges found in the pathomechanism of the two disease groups overlap in many aspects: while in one the immune response is enhanced, in the other it is attenuated - in both cases, however, a balance would be needed.

Nowadays, one of the most significant breakthroughs in the field of medicine was the emergence of immunotherapy. The continuous development of these treatments has enabled targeting of both autoimmune and oncological diseases by modulating the immune system's activity. However, oncological immunotherapies often cause autoimmune side effects (so-called immune-related adverse events (irAEs), which need to be treated with immunosuppressive agents, if necessary. Managing these side effects is a challenge in clinical practice.

Mechanisms of the antitumor immune response

The immune system encounters pathological cells at the site of cancer formation, where antigen-presenting cells (APCs) process the proteins released from the tumour cells (tumour antigens). Protein antigens then reach the lymph nodes, where they are presented to T cells via MHC-II (major histocompatibility complex) molecules.

When the B7-1 molecule on the surface of antigen-presenting cells (APCs) binds to the CD28 antigen on T cells, a positive costimulatory signal is triggered, leading to T-lymphocyte activation. Conversely, when B7-2 or programmed death-ligand 1 (PD-L1) on the surface of

APCs binds to the cytotoxic T-lymphocyte antigen 4 (CTLA-4) or programmed cell death protein 1 (PD-1) receptor on T cells, an inhibitory signal (coinhibition) is generated, which results in T-lymphocyte anergy and weakens the antitumor immune response. After clonal expansion of the activated T cells, they enter the primary tumour and metastases through the lymphatic system or the bloodstream, where they destroy the recognized cancer cells. The PD-L1 ligand of the tumour cell binds to the PD-1 receptor of the T cell, causing an inhibitory (coinhibitory) effect that prevents the T cell from killing the tumour cell and prevents tumour growth. Immune checkpoint inhibitors (ICIs) block CTLA-4 or PD-1-mediated inhibition, thereby restoring antitumour immunity.

The role of immune checkpoints

The essence of influencing immune checkpoints is to stimulate antitumour immunity, which is crucial for the success of oncotherapy. However, this approach can also result in the development of autoimmune processes. Therefore, a successful treatment from an oncological perspective using these therapies may be associated with the development of autoimmune diseases. In a quiescent state, the costimulatory and coinhibitory processes are in balance, so neither tumour formation nor autoimmune disease develops. If positive costimulatory signals activate the immune system, it may promote antitumour defence, but it may also increase the risk of autoimmune reactions. In contrast, coinhibitory signals suppress the immune response, which can potentially lead to cancer progression. Antibodies and coinhibitory molecules that inhibit coinhibition can be used therapeutically in antitumour treatments, but they may increase the risk of autoimmune reactions, irAEs.

PD-1 and PD-L1 molecules

The PD-1 protein is a glycosylated transmembrane receptor, the extracellular part of which contains a variable region. The intracellular part carries immunoreceptor motifs called ITIM and ITSM, which are important in regulating signal transduction. The PD-L1 protein, on the other hand, has a longer extracellular immunoglobulin-like part and a shorter intracellular part.

Cells that produce PD-L1 – such as T and B lymphocytes, macrophages, dendritic cells, and other cell types – are not attacked by the immune system, thus reducing the immune response. CD4+ and CD8+ T cells activated in the lymph nodes express PD-1 and produce the cytokine interferon-γ (IFN-γ), which activates antitumor macrophages. This process, on the one hand, increases the expression of PD-L1 on tumor cells, which inhibits cytotoxic T lymphocytes expressing the PD-1 receptor. However, it also increases the presentation of tumour antigens.

Treatments that block the interaction between PD-1 and PD-L1 block the inhibition of T-cell-mediated immune activation, thus allowing effector T lymphocytes to attack tumour cells.

Based on literature data, immunohistochemical (IHC) determination of PD-L1 expression can help in the selection of therapy, but it does not always accurately predict the therapeutic response. In some malignancies, PD-L1 IHC testing is required for drug use, while in other cases, PD-L1-negative patients can also benefit from therapy.

Clinical use of immune checkpoint inhibitors and their side effects

The use of ICIs has radically changed the treatment of cancer. They are increasingly used in both first- and later-line treatment, so that almost all cancer types now have at least one ICI among the therapeutic options. Unlike side effects caused by conventional chemotherapy, irAEs most often have autoimmune or autoinflammatory origin and can occur as single toxicities or in combination, affecting almost all organ systems. Unlike side effects occurring with chemotherapy, irAEs usually have a later onset, a longer duration, and their effective management depends on their timely recognition and intervention. In patients receiving such therapy, it is imperative to accurately record symptoms before and during treatment, including necessary imaging and laboratory tests, to ensure appropriate patient follow-up. Accurate differential diagnosis is also of paramount importance when an undesirable complication is detected. irAEs typically occur within the first three months after the start of ICI therapy. These include endocrine (thyroid, pituitary disorders, diabetes mellitus), gastrointestinal (colitis), respiratory (pneumonitis), musculoskeletal (arthritis, manifest autoimmune rheumatological diseases), dermatological (rash, itching), neurological (polyneuropathy, aseptic meningitis, demyelination, Guillain-Barré syndrome) symptoms, as well as less common renal (nephritis), hepatobiliary (hepatitis, cholangitis) and ophthalmological (uveitis, keratitis, retinopathy, dacryoadenitis) involvement. Among the general symptoms, the most common complaint is fatigue, which occurs in 16-37% of cases. Interestingly, the occurrence of side effects and their

severity are associated with better overall therapeutic efficacy. In combination treatments, based on previous research, the risk of side effects can be considered additive, with 95% of these treatments experiencing a complication related to the therapy, 55% of which were Grade 3-4 in severity.

The irAEs associated with anti-PD-1 antibody treatment are generally reversible and well-tolerated, but in severe cases, they can even be life-threatening. In patients who have previously received ICI treatment and have not experienced side effects during treatment, irAEs may occur even after therapy. Based on literature data, in not all cases, but in patients suffering from certain autoimmune diseases, the risk of developing side effects caused by ICI therapy is increased. In rheumatic diseases, this risk is relatively low.

Treatment of immune-mediated side effects

From a clinical perspective, the treatment of irAEs is mainly determined by the affected organ and the severity of the side effect (Grade), and not by the type of ICI used. The severity of adverse events is assessed according to the Common Terminology Criteria for Adverse Events (CTCAE) system. In mild cases (Grade 1) – except for cardiovascular and neurological adverse events – only symptomatic treatment (NSAIDs, corticosteroids [CS]) is required, and ICI treatment can be continued. In moderate (Grade 2) irAEs, oral CS treatment is required, with close observation. Grade 3 and Grade 4 irAEs may occur in 20-25% of patients receiving anti-PD-1 treatment, and these are most often respiratory and gastrointestinal side effects. In severe (Grade 3) irAEs, temporary discontinuation of ICI therapy and parenteral CS may be considered. Therapy can be resumed when symptoms improve to Grade 1. In case of Grade 4 side effects, treatment should be permanently discontinued, and high-dose parenteral CS should be administered, and synthetic or biological immunosuppressive agents may also be initiated. The treatment of irAEs requires a multidisciplinary approach: cooperation between different medical specialties and specialists is necessary.

Tumour-associated antigens as potential biomarkers in rheumatoid arthritis

Monitoring tumour marker levels has been a fundamental method for evaluating the effectiveness of oncological therapies and disease follow-up. Most tumour markers studied in clinical practice are tumour-associated antigens (TAA), which may play an important role not

only in cancers, but also in autoimmune diseases, such as the pathophysiology of rheumatoid arthritis (RA). The appearance of TAAs in autoimmune diseases and the quantitative changes in their serum levels may indicate a dysregulation of the immune system, on the one hand, it may be related to processes involving chronic inflammation and the response of diseases to the influence of the immune system.

TAAs can be expressed on the surface of inflammatory cells in addition to tumour cells. Furthermore, some of them also serve as cell adhesion molecules (CAMs). These TAAs may participate, among others, in the maintenance of inflammation in RA and other inflammatory rheumatological diseases. In the case of detachment of TAAs from the cell surface, they can be detected in the serum of RA patients as well as in cancer patients.

Among the TAAs, members of the carcinoembryonic antigen (CEA) family, also known as CD66a-e molecules, belong to the immunoglobulin superfamily of CAMs. Our previous studies, as well as the results of other research groups, have also shown the presence of CEA antigens in the RA synovium. Furthermore, higher serum CEA levels have been measured in several cases in RA patients, and serum levels of this TAA have been associated with RA-associated interstitial lung disease (RA-ILD).

In addition to the CEA family, other TAAs, e.g., CA15-3, which is often elevated in breast cancer and is also known as an alternative epitope of MUC1, and KL-6, have also been associated with inflammatory diseases, including RA-ILD.

The epitope of CA19-9 is the carbohydrate antigen sialyl-Lewis a (sLe(a)), a ligand for E-selectin. Based on literature data, it is known that CA19-9 levels are elevated in various malignancies affecting the gastrointestinal system. In some studies, elevated CA19-9 levels have been observed in the serum of patients with RA. Furthermore, changes in serum CA19-9 levels can also be associated with the development of RA-ILD.

Elevated serum levels of CA125, also known as MUC16, can be detected in patients with ovarian cancer. In the latter disease, it can help in the diagnosis, assessment of progression, and prediction of relapses. However, in RA patients, elevated serum levels of CA125 may be associated with inflammatory (ILD) and fibrotic changes in the lung parenchyma and have been closely associated with blood levels of rheumatoid factor (RF).

CA72-4 is a non-specific marker of various malignancies, such as gastrointestinal, ovarian, breast, and lung cancer. Relatively little information is available on the role of CA72-4 in RA and RA-ILD. However, it is known from literature that CA72-4 levels may be significantly elevated in gout in some cases.

Human epididymal protein 4 (HE4) is a biomarker for epithelial ovarian cancer, lung cancer, and some other malignancies. In addition to cancer, HE4 has been associated with various non-cancer lung diseases, including RA-ILD and cystic fibrosis. In RA-ILD, HE4 levels are associated with disease severity, and elevated levels indicate a poor prognosis.

Finally, tissue polypeptide antigen (TPA) is known as a mixture of cytokeratins 8, 18, and 19. TPA is a specific marker for bladder cancer, but in combination with other TAAs, it is also an indicator of proliferation in most solid malignancies. The literature on the possible relationship between RA and TPA is limited, and in our previous studies, we did not find elevated TPA levels in RA.

A few years ago, our group was among the first to investigate the production of certain TAAs in RA. We assessed serum CEA, CA19-9, CA125, CA15-3, and CA72-4 levels in RA patients and healthy controls. Significantly, more RA patients showed abnormally high levels of CA125, CA19-9, and CA15-3 compared to controls. In addition, serum levels of CA125 and CA19-9 were higher in the sample of RA patients compared to controls. Furthermore, CEA levels in RA showed a close correlation with serum RF concentrations.

Cardiovascular comorbidities and JAK inhibitors in rheumatoid arthritis

Since the mechanism of action and side effect profile of antitumour ICIs are closely related to immunological and rheumatological processes, these fields will play an increasingly prominent role in the future. Based on these considerations, we also aimed to investigate the role of TAAs – as potential biomarkers – in RA. During my research work, we examined the effect of tofacitinib treatment, a Janus kinase (JAK) inhibitor used in RA therapy, on TAA levels.

JAKs are tyrosine kinase intracellular enzymes that play an important role in several cytokine signalling pathways and in the synovial inflammation underlying RA. Four JAK inhibitors, including tofacitinib, have been approved for the treatment of RA. Ultrasound-based imaging techniques are used in everyday practice to detect vascular lesions in patients with RA. Impaired endothelium-dependent flow-mediated vasodilation (FMD), increased carotid intima-media thickness (ccIMT), and increased carotid-femoral pulse wave velocity (cfPWV) are indicative of brachial artery endothelial dysfunction, carotid atherosclerosis, and increased arterial stiffness, respectively. Anti-inflammatory therapies, including JAK inhibitors, may attenuate arterial inflammation and improve vascular damage in patients with RA.

Objectives

During my research, we investigated the incidence of various irAEs among patients with solid malignancies receiving nivolumab or pembrolizumab treatment at the University of Debrecen Clinical Centre between 2017 and 2021. We analysed the frequency of side effects, compared their occurrence in the nivolumab and pembrolizumab groups, and examined the factors that may play a role in the development of adverse events in each patient group (study 1).

Like oncological immunotherapies, the treatment of autoimmune diseases also requires intensive immunomodulation, and their pathomechanism is also the subject of active research worldwide. TAAs can be elevated in the serum of patients with both malignant and autoimmune diseases (RA, RA-ILD); this common feature may also indicate common elements of pathomechanism or therapeutic options. However, the role of TAAs in autoimmune processes and the effect of targeted therapies on TAA production in the treatment of RA are only partially known. We aimed to incorporate the effects of immunomodulation in autoimmune patients treated for oncological diseases, with a particular focus on immune responses induced by immunotherapies and targeted therapies, as well as their systemic consequences. We examined changes in seven different TAA levels in RA patients treated with tofacitinib (Study 2).

Furthermore, we investigated the possible relationship of TAA production with disease activity, inflammatory markers, cytokines, angiogenic growth factors, adhesion molecules, and pathological changes in the vascular system, which were determined based on ccIMT, FMD, and cfPWV assessed by ultrasound. Our results may contribute to a better understanding of the mechanisms of action of immunomodulation in the treatment of both oncological and autoimmune diseases, with a particular focus on immune responses induced by immunotherapies and targeted therapies, as well as their systemic consequences.

Patients and methods

Study 1. Retrospective analysis of cancer patients treated with pembrolizumab or nivolumab

Between June 2017 and May 2021, 207 patients received pembrolizumab or nivolumab therapy at the Oncology and Pulmonology Departments of the University of Debrecen Clinical Centre. Among the patients, 138 were male and 69 were female. 157 patients received nivolumab, and 50 patients received pembrolizumab therapy. The mean age was 64.6 ± 8.2 years. During immunotherapy, patients did not receive additional chemotherapy or radiotherapy. All patients were followed up until December 31, 2021.

During data collection, all patient records were reviewed in detail retrospectively, and the necessary data were recorded in an Excel spreadsheet. Statistical analysis was performed using SPSS version 26 (IBM, Armonk, NY, USA). Data were expressed as mean ± standard deviation for continuous variables and as percentages for categorical variables. The distribution of continuous variables was examined using the Kolmogorov–Smirnov test. Since the data distribution was not normal, nonparametric tests were used. Comparisons of continuous variables were performed using the Mann–Whitney test, while comparisons of nominal variables were performed using the chi-square (χ^2) or Fisher's exact test, depending on the applicability. Correlation between two continuous variables was determined using the Spearman rank correlation test. Binary logistic regression analysis was used to evaluate prognostic factors for irAEs.

Receiver Operating Characteristic (ROC) curves showed the sensitivity and specificity of the given test for each possible threshold value. A $p<0.05$ value was considered statistically significant.

Study 2. Analysis of TAAs in patients with rheumatoid arthritis treated with tofacitinib

This study enrolled 30 patients with active RA. Inclusion criteria included a precise diagnosis of RA according to the 2010 European League Against Rheumatism (EULAR) and American College of Rheumatology (ACR) RA classification criteria, moderate or high disease activity (DAS28 > 3.2) at baseline, and a clinical indication for targeted therapy. Some patients (n=16) were naïve to targeted therapy, while others (n=14) received tofacitinib after discontinuation of a biologic therapy and an appropriate washout period. Exclusion criteria included the presence of inflammatory diseases other than RA, acute or recent infection, common contraindications to JAK inhibition, untreated cardiovascular disease or hypertension, chronic renal or hepatic insufficiency, and malignancy diagnosed within 10 years prior to the study. All patients were receiving tofacitinib 5 mg or 10 mg twice daily in combination with methotrexate (MTX) (n = 23) or leflunomide (n = 7). Patients had been receiving MTX and leflunomide at stable doses for at least 1 year prior to the initiation of the present study. Although most patients had previously received CS, none had received them for at least 3 months prior to the study or during the study. Clinical assessments were performed at baseline and at 6 and 12 months. 26 patients completed the one-year treatment period and were therefore included in the final data analysis. As the first step of the study, a detailed medical history was taken from the patients. Additional clinical and laboratory examinations, as well as physical examinations, were performed at baseline and at 3, 6, and 12 months after the start of tofacitinib treatment.

Tumor-associated antigens and other biomarkers

Concentrations of serum TAAs, including CEA (normal value: <3.4 μg/L), CA15-3 (normal value: <25 kIU/L), CA19-9 (normal value: <34 kIU/L), CA125 (normal value: <35 kIU/L), CA72-4 (normal value: <6.9 kIU/L), HE4 (normal value: <70 pmol/L), and TPA (normal value: <75 U/L), as well as tumor necrosis factor-α (TNF-α; normal value: <136.3 pg/mL), interleukin-6 (IL-6; normal value: <3.7 pg/mL), IL-8/CXCL8 (normal value: <120.1 pg/mL), vascular endothelial growth factor (VEGF; normal value: <204.2 pg/ml) and platelet-endothelial cell adhesion molecule-1 (PECAM-1; normal value: <26.9 ng/ml) were determined by flow cytometry using a bead-based multiplex assay (Human Angiogenesis Panel 1, 10-plex, LEGENDplex, BioLegend, San Diego, CA, USA). Data were analysed using LEGEND plex software. Normal values were determined based on standard human serum samples.

Statistical analysis

Statistical analysis was performed using SPSS version 26.0 software (IBM, Armonk, NY, USA). Data were expressed as mean ± standard deviation for continuous variables and as percentages for categorical variables. The distribution of continuous variables was assessed using the Kolmogorov-Smirnov test. Paired two-sample t-test and the Wilcoxon test were used to analyse continuous variables. Comparisons of nominal variables between groups were performed using the chi-square test or Fisher's exact test, as applicable. Correlations were determined using Pearson's correlation analysis. Univariate and multivariate linear regression analyses were performed using a stepwise method to explore independent associations, with TAA levels as the dependent variable and all other parameters as independent variables. The standardized regression coefficient β was used to characterize the strength and direction of the linear relationship between the variables studied. Repeated measures analysis of variance (RM-ANOVA) within a general linear model (GLM) was used to determine the additional effects of different biomarkers on changes in TAA levels over a 12-month period (dependent variable). Analysis of variance (two-way RM-ANOVA) was used to examine the associations between changes in TAA levels over one year and changes in other parameters studied over the same period. In the RM-ANOVA and two-way RM-ANOVA analyses, the partial η^2 value was used to characterize the effect size, where 0.01 indicated a small effect, 0.06 a medium effect, and 0.14 a significant effect. 96,97 Values of $p<0.05$ were considered statistically significant.

Results and Discussion

Study 1. Retrospective analysis of cancer patients treated with pembrolizumab or nivolumab

Of the 207 patients included in this study, 157 received nivolumab and 50 received pembrolizumab ($p<0.01$). The male/female ratio in the nivolumab arm was 107/50. The mean age of the patients included was 62.3 ± 10.1 years. A total of 157 patients were included in the study, of whom 4 received first-line treatment, 123 received second-line treatment, and 15 received third-line or later treatment. The mean duration of immunotherapy was 2.13±0.90 years, and patients received an average of 18.9±19.3 cycles of immunotherapy, of whom 97 received at least nine cycles of therapy. At the end of the study period, 25% of patients (n = 40) were still undergoing active treatment, and 75% (n = 117) had completed their therapy. The most common reason for discontinuation or modification of therapy was disease progression, which occurred in 74% (n = 86) of cases. Additional reasons were death in 19 cases (16%), complete remission in 5 cases (4%), therapy-induced complications in 3 cases (2%), patient request or unknown cause in 2 cases (2%) each. The most common malignancy among those receiving nivolumab was lung cancer (n=95), followed by renal cancer (n=34), tonsillopharyngeal malignancies (n=14), oesophageal cancer (n=4), and oral cancer (n=4).

The male/female ratio in the pembrolizumab group was 31/19. The mean age of the patients at the start of treatment was 65,2±11,3 years, while at the start of therapy, this value was 63,4±11,2 years. In the cohort of 50 patients, 25 received pembrolizumab-based immunotherapy as first-line treatment, 21 as second-line, and four as third- or additional-line treatment. The mean treatment duration was 1,86 ± 0,86 years. At the end of the study period, 30% of the patients (15 patients) were still receiving active immunotherapy, while the majority (35 patients, 70%) had their treatment interrupted for some reason. The most common reason for interruption or modification of therapy was disease progression, which occurred in 19 cases, i.e., in 54% of the patients. This was followed by death (10 cases, 29%), achievement of complete remission (1 case, 3%), treatment-related complication (3 cases, 8%), discontinuation at the patient's request (1 case, 3%), and unknown cause (1 case, 3%). Patients received an

average of 13.9 ± 12.2 treatment cycles, and a total of 28 patients received at least nine cycles of treatment.

In the final phase of our studies, we compared the patient groups treated with pembrolizumab and nivolumab. Three times as many patients in the sample received nivolumab treatment as those who received pembrolizumab. A significant statistical difference was also observed in the analysis of the linearity of the treatments: 88% of patients treated with nivolumab received second-line immunotherapy, while in the case of patients receiving pembrolizumab, the proportion of first-line use was 50% and second-line use was 42% ($p<0.01$). There were no significant differences between the two groups in terms of other parameters examined, including gender, age at the start of treatment, duration of treatment, number of therapy cycles, number of patients receiving at least nine cycles, and whether treatment was active or discontinued, along with the reason for discontinuation.

Descriptive statistical characteristics of immunotherapy-related adverse events

A total of 103 immunotherapy-induced adverse events were experienced in 66 patients (32%) out of the total (n=207). Of the patients with at least one adverse event, 55% (n=36) experienced one, 35% (n=23) experienced two, and 10% (n=7) experienced three adverse events. The most common immune-mediated adverse events (irAEs) were thyroid (33 cases; 50% of patients with irAEs), dermatological (25 cases; 38%), pneumonitis occurred in 14 cases (21%), and gastrointestinal involvement in 13 cases (20%). In addition, nephropathy (7 cases; 11%), hepatopathy (6 cases; 9%), conjunctivitis (2 cases; 3%), pancreatitis (1 case; 1.5%), polyneuropathy (1 case; 1.5%), and polyarthritis (1 case; 1.5%) also occurred.

Of the 157 patients treated with nivolumab, 45 (29% of nivolumab-treated patients) experienced adverse events (68 in total): 26 (58% of nivolumab-treated patients) experienced one adverse event, 15 (33%) experienced two adverse events, and 4 (9%) experienced three or more adverse events. The most common irAEs in this group were thyroid involvement (23 cases, 30% of nivolumab-treated patients with irAEs), dermatological lesions (17 cases; 38%), gastrointestinal symptoms (11 cases; 24%), and pneumonitis (9 cases; 20%). In addition, hepatopathy (3 cases, 7%), nephropathy (2 cases, 4%), conjunctivitis (2 cases, 4%), and polyarthritis (1 case, 2%) were also observed.

In the subgroup of patients treated with pembrolizumab, 21 of 50 patients (42%) experienced a total of 35 immune-related adverse events (irAEs). Of these, 10 patients (48% of pembrolizumab-treated patients with adverse events) developed one type of irAE, eight patients (38%) two types, and three patients (14%) three types. The most common immune-mediated adverse events (irAEs) in this group were: thyroid involvement (10 cases; 48% of pembrolizumab-treated patients with complications), dermatological lesions (8 cases; 38%), nephropathy (5 cases; 24%), and pneumonitis (5 cases; 24%). In addition, hepatopathy (3 cases; 14%), gastrointestinal toxicity (2 cases; 10%), pancreatitis (1 case; 5%), and polyneuropathy (1 case; 5%) were also observed. In patients receiving anti-PD-1 therapy, irAEs occurred after a mean of 10.0±10.4 treatment cycles.

Regarding the severity of irAEs, the rates of Grade 1, Grade 2, and Grade 3 adverse events were 60%-35%-5% for all anti-PD-1-treated patients, 50%-46%-4% for nivolumab, and 80%-14%-6% for pembrolizumab. Most irAEs were well controlled with NSAIDs, KS, or other immunosuppressive agents. Only six irAE events (3% of all patients) led to treatment discontinuation, of which 3 patients were in the nivolumab group and 3 patients in the pembrolizumab group. Comparing patients treated with nivolumab and pembrolizumab, we found no significant difference in the proportion of patients with immune-mediated complications ($p=0.078$) or in the relative number of different types of irAEs ($p=0.566$). When examining specific adverse events, we found that renal involvement was significantly more common in the pembrolizumab group ($p=0.010$). In addition, there were no other differences between the two groups regarding the comparison of specific adverse events. Furthermore, adverse events occurred significantly ($p=0.034$) later in the nivolumab group compared to the pembrolizumab group. While nivolumab-associated irAEs were approximately equally graded at Grade 1 and 2, pembrolizumab treatment resulted in 80% of mild Grade 1 irAEs ($p=0.027$).

Factors associated with the development of immune-mediated adverse events

When comparing the groups of patients who developed an adverse event (n=66) and those who did not (n=141), we found that patients who developed at least one adverse event had received significantly more treatment cycles (21.8±18.7 versus 15.8±17.4; $p=0.002$) and were also younger at the start of treatment (60.7±10.8 versus 63.4±10.1 years; $p=0.042$). The number of irAEs correlated with the number of treatment cycles in each patient ($R=0.227$; $p=0.001$). When performing a simple Spearman correlation analysis, the development of

immune-mediated adverse events showed a positive, significant correlation with the length of progression-free survival (PFS) (R=0.264; p<0.001), the number of ICI treatment cycles used (R=0.273; p<0.001), and current (ongoing) ICI therapy (R=0.183; p=0.008). The number of irAEs also showed a significant correlation with the length of PFS (R=0.263; p<0.001), the number of ICI treatment cycles (R=0.276; p<0.001), and the current ICI treatment (R=0.193; p=0.005). Finally, it can be said that the number of ICI cycles used before the development of the first irAE also showed a strong correlation with the length of PFS (R=0.603; p<0.001).

When comparing nivolumab and pembrolizumab treatments, we also examined the frequency of irAEs. To identify possible prognostic factors influencing the development of adverse events, we performed a binary logistic regression analysis. In the univariate binary logistic regression, there was no statistically significant difference in the frequency of irAEs occurring during pembrolizumab versus nivolumab treatment (OR: 1.878 [95% CI: 0.980–3.599]; p=0.058). However, in the Forward LR analysis, the difference in the frequency of adverse events associated with the two agents proved to be statistically significant, with an OR of 2.169 (95% CI: 1.089–4.321; p=0.028). Furthermore, the binary logistic regression revealed that using nine or more treatment cycles as a threshold was associated with an increased risk of immunotherapy-related complications, with an OR of 3.328 (p=0.004). Regarding specific adverse events, binary comparisons showed that patients who developed thyroid involvement received more cycles of treatment compared to the group that did not develop any adverse events (23±18.8 versus 16.8±17.7; p=0.04). Patients who developed pneumonitis as an adverse event also received more cycles of therapy (23.1±12.0 versus 17.3±18.3; p=0.022) and their treatment duration was longer (2.5±1.2 versus 2.0±0.9 years; p=0.032) compared to patients who did not experience pneumonitis. We found no significant association between skin involvement, gastrointestinal side effects, and other specific side effects and other factors studied.

Study 2. Analysis of TAAs in rheumatoid arthritis patients treated with tofacitinib

Clinical and vascular characteristics of patients

The results of some studies conducted on the patient group have been previously published in the co-authored researchers' own publications. During our study, two patients from

the 2×5 mg tofacitinib therapy group and two patients from the 2×10 mg tofacitinib therapy group dropped out. Thus, a total of 26 patients, out of the initial 30, completed the study, and their data were analysed. The characteristics of these 26 patients are presented in Table 4, which was previously presented.

Several publications have shown that one year of tofacitinib treatment is highly effective in the treatment of RA. In their study, tofacitinib therapy significantly improved DAS28, CRP, and HAQ values at both 6 and 12 months. Regarding vascular pathophysiological parameters, there was no detectable change in FMD and cfPWV values, while ccIMT time showed an increase. In our research, we used only clinical, disease activity, and vascular ultrasound data from previous publications to compare these with TAA results.

In the RA patient group studied, serum CA15-3 levels significantly decreased from baseline (19.4±6.1 kIU/L) after both 6 months (18.4±5.5 kIU/L; p=0.049) and 12 months (18.3±6.3 kIU/L; p=0.031) of tofacitinib therapy. HE4 levels similarly significantly decreased from baseline (68.7±26.6 pmol/L) after both 6 months (57.6±22.9 pmol/L; p=0.001) and 12 months (61.1±24.5 pmol/L; p=0.014) of tofacitinib therapy.

However, CA19-9 levels, in contrast, showed a significant increase compared to baseline (9.1±5.8 kIU/L) after both 6 (11.1±8.4 kIU/L; p=0.014) and 12 months (11.4±9.2 kIU/L; p=0.008) of therapy. CEA levels increased transiently compared to baseline (2.82±2.06 µg/L) after 6 months (3.46±2.21 µg/L; p=0.029), but this increase was no longer significant after 12 months (3.05±1.97 µg/L; p=0.124). No significant changes were observed in the levels of CA125 (12.1±3.9 kIU/L; 11.5±3.7 kIU/L; 11.7±4.7 kIU/L;), CA 72-4 (2.30±3.46 kIU/L; 1.90±2.42 kIU/L; 2.15±2.60 kIU/L;) and TPA (37.7±24.0 U/L; 31.6±16.4 U/L; 38.2±33.2 U/L;) compared to baseline values after 6 and 12 months of therapy.

Furthermore, during our measurements, we determined the number of patients whose serum TAA levels exceeded the upper limit of the normal range at various time points; these data are presented in Table 6. The number of patients with elevated serum CA15-3 levels clearly decreased at both months 6 (n = 3) and 12 (n = 3) compared to baseline (n = 7). In contrast, the number of patients with CEA levels above the upper limit of normal increased significantly at months 6 (n = 13) and 12 (n = 11) compared to baseline (n = 8). No changes were observed in CA19-9, CA125, CA72-4, HE4, and TPA levels.

Relationships of TAA levels with other parameters in RA

In univariate regression analysis, in cases where TAA levels were included as dependent variables, CA15-3 and HE4 values showed different but statistically significant positive relationships with DAS28, CRP, RF, TNF-α, IL-6, IL-8, and PECAM-1 levels ($p<0.05$). More limited relationships were observed for the other TAAs. CEA showed a relationship with TNF-α and RF levels, while CA19-9 was only associated with RF levels. CA125 was linked to PECAM-1, CA72-4, and IL-6, as well as TPA levels, and this association was statistically significant ($p <0.05$).

The temporal changes in TAA levels and their determinants were examined using a generalized linear model-based repeated measures analysis of variance (GLM RM-ANOVA). The analysis considered the effect of tofacitinib treatment and compared the baseline values to those at the 6- and 12-month follow-up points. The change in CEA levels over 12 months was a function of the disease activity measured at baseline ($p = 0.037$). Furthermore, the change in CA19-9 levels over 12 months was a function of the RF and TNF-α levels measured at baseline ($p<0.05$). The change in CA72-4 levels over the long term was determined by the DAS28, IL-6, and PECAM-1 levels measured at baseline ($p<0.05$). In the two-way RM-ANOVA test, we found varying degrees of statistical correlation between the change in TAA levels and the change in other parameters during the study period. Changes in CEA, CA15-3, CA19-9, CA72-4, and HE4 levels over 1 year showed varying degrees of statistical correlation with changes in DAS28, HAQ, CRP, ESR, IL-6, and VEGF levels during the study period ($p < 0.05$). Finally, in the RM-ANOVA analysis, we found that baseline CA125 values were associated with changes in ccIMT within one year ($p = 0.046$).

Discussion

Study 1. Retrospective analysis of cancer patients treated with pembrolizumab or nivolumab

The advent of immunotherapy (ICI) has represented a significant breakthrough in the treatment of many cancers. The mechanism of action of these therapeutic modalities is based on the activation of the immune system, which can also lead to the appearance of irAEs, both during and after treatment ("late onset" irAEs). Due to the relative novelty of immunotherapy, limited data are available from the Central and Eastern European region, including Hungary, on the possible side effects of immunotherapy and their management. Our research aimed to be the first in Hungary to summarize and analyse the experiences gained at the Clinical Centre of the University of Debrecen, based on data from a relatively large group of patients – 207 patients treated with PD-1 inhibitors (nivolumab or pembrolizumab).

Patients were followed up for a long time with a mean number of treatment cycles of 16.6±13.7 for pembrolizumab and 18.9±19.3 for nivolumab. One-third of patients developed at least one irAE, which occurred after an average of 10 treatment cycles. These adverse events can occur during or after treatment. However, monitoring late adverse events is a significant challenge, partly due to the limitations of available diagnostic and monitoring tools, and partly because an even more extended follow-up period is likely to be required to assess them reliably.

Half of the patients affected by an adverse event experienced one, a third experienced two, and only 10% experienced three different adverse events. The most common adverse events were thyroid dysfunction, skin symptoms, pneumonitis, and gastrointestinal symptoms, which are consistent with literature data. Myocarditis or pronounced neurotoxicity did not occur, and polyneuropathy was observed in one case.

The majority of irAEs – approximately 60% – were mild (Grade 1). These events were typically managed quickly and effectively using domestic and international protocols, and in most cases did not require interruption of therapy. Based on international clinical trials, the rate of therapy interruption for PD-1 inhibitors ranges from 3% to 8%, and side effects occur less frequently with PD-1 inhibitors compared to CTLA-4 inhibitors.

When comparing the two PD-1 inhibitors studied, nivolumab and pembrolizumab, we also observed a difference in the rate of side effects: twice as many immune-mediated side effects were observed among patients receiving pembrolizumab as among those receiving nivolumab. However, in terms of the severity of adverse events, nivolumab had a lower incidence of mild (Grade 1) events but a higher incidence of moderate (Grade 2) adverse events compared to pembrolizumab. That is, although adverse events associated with pembrolizumab occur more frequently, they are typically milder in clinical severity. In contrast, adverse events associated with nivolumab may occur less frequently but potentially be more severe.

Most systematic reviews and metaanalyses suggest that nivolumab and pembrolizumab have similar safety and tolerability profiles. In our study, irAEs occurred slightly more frequently with pembrolizumab than with nivolumab (42% and 29%, respectively). However, they were milder in severity, with 80% of adverse events with pembrolizumab being Grade 1. There may be several factors behind this. From a clinical perspective, we saw a difference in treatment use, where pembrolizumab was often used in an earlier therapeutic line than nivolumab, and the therapeutic indications between the two antibodies differed significantly. It is not yet clear how the underlying cancer type influences the development, severity, and outcome of irAEs, and our data do not provide further support in this regard due to the heterogeneity of the treatment environment (e.g., tumour type, staging, therapeutic line). However, our studies supported the existing literature data indicating a relationship between the development of side effects and the effectiveness of immunotherapy.

We showed that in the event of the development of at least one side effect, patients received more cycles of therapy, meaning that the tumour probably responded well to the treatment. Furthermore, the number of irAEs correlated and showed a significant relationship with the number of treatment cycles and the length of progression-free survival. The number of treatment cycles used before the development of the first irAE also showed a close correlation with the length of PFS. That is, it is advantageous from the point of view of cancer treatment if an immune-mediated side effect develops, but it should develop as late as possible.

In further research, it was described that autoimmune disease that already existed before treatment, especially if active, is a risk factor for the development of side effects in these patients. In addition, treatment-related factors, such as the type of ICI used (anti-PD-1 or anti-CTLA-4) or the ICIs used in combination, as well as intrinsic, individual characteristics, such as tumour and genetic heterogeneity, cancer type, tumour microenvironment, and the microbiome, can all influence the development of irAEs.

There are several recommendations regarding the management and possible prevention of side effects. In the patient group we studied, 60% of irAEs were mild (Grade 1) and were well controlled. Most side effects occurred relatively quickly, on average, after 10 treatment cycles. Very few patients needed to stop ICI therapy (6 patients). In most cases, “rechallenge” after discontinuation of ICI proved to be safe and did not lead to recurrence of irAEs. The strength of our study is that this may be the most extensive study to date in Central and Eastern Europe to analyse irAEs with ICI, with the largest number of patients. Of course, there are also limitations to the study, such as the fact that the patients came from a single centre and that the issue was examined exclusively from a clinical perspective.

Study 2. Analysis of TAAs in rheumatoid arthritis patients treated with tofacitinib

Our second study is the first to investigate the effects of the JAK inhibitor tofacitinib on serum levels of seven different TAAs, particularly in the context of the vascular comorbidity associated with RA.

TAAs are widely known in the field of oncology, but some studies have also suggested their role in inflammatory processes. On the other hand, little information is available on the role of TAAs in RA. CEA and MUC1 can be detected in the synovium of patients with RA. Additionally, levels of CEA, CA15-3, CA19-9, and CA125 may be elevated in RA. Elevated levels of certain TAAs have been associated with the development of RA-ILD.

According to a previous study conducted at the University of Debrecen, elevated CA15-3 levels were more common in patients with RA than in healthy controls. However, the associations between these TAAs and other clinical parameters associated with RA, as well as the possible effects of antirheumatic therapies on these antigens, have not been systematically investigated. In our study, we examined TAA levels during tofacitinib therapy and measured a significant decrease in CA15-3 and HE4 levels after the initiation of therapy (at 6 and 12 months). In addition, twice as many RA patients had CA15-3 levels above the normal range at baseline as after 6 and 12 months of therapy. Currently, there is no further information available on the role of CA15-3 and HE4 in RA, only on their role in the development of RA-ILD.

CA15-3 is an epitope of MUC1, and MUC1 expression is known to be increased in RA synovium compared to controls. Additionally, MUC1 has been previously linked to joint destruction. Based on all of this, it can be assumed that CA15-3 - as part of MUC1 - may indeed play a role in the development of RA-associated synovitis. However, there is currently no information available on the possible role of HE4 in RA synovitis. Based on our studies, the one-year long JAK inhibitor treatment reduced disease activity and inflammation in RA, which was associated with a progressive decrease in CA15-3 and HE4 levels.

CEA levels showed a transient increase after 6 months of tofacitinib therapy but decreased after 12 months. In addition, we observed that more patients had elevated CEA levels after 6 and 12 months of tofacitinib therapy than at baseline. Previous studies have shown increased CEA expression in the synovium. These studies suggest that tofacitinib may have promoted the clearance of CEA from the synovial tissue, which was reflected in an increase in circulating CEA levels.

JAK inhibitor therapy also increased CA19-9 levels, but no patient had abnormally high CA19-9 levels during the entire study period (one year). It is not yet fully understood why tofacitinib treatment could have caused this increase in TAA levels. However, in none of the patients did CA19-9 levels exceed the upper limit of the reference range at the time points studied, which may indicate that the role of this marker in this context may be less important than that of CA15-3 or HE4. Tofacitinib therapy did not affect CA125, CA72-4, and TPA levels at the time points studied.

In the correlation analysis, CA15-3 and HE4 levels were associated with disease activity, systemic inflammatory markers, RF, specific cytokines, and PECAM-1. CEA, CA19-9, and TPA were correlated with RF, specific cytokines, and PECAM-1. Based on previous studies, CEA and CA-125 have shown a positive correlation with RF in RA. Since similar correlation analyses were not available, we could not compare our results with those of other research groups. However, based on our results, different TAAs may be indicators of disease activity, inflammatory state, and production of different proinflammatory mediators at different times. Regarding vascular pathophysiological aspects, several TAAs – CEA, CA125, HE4, and TPA – showed a positive correlation with ccIMT and cfPWV. This may indicate that these TAAs – which can also function as cell surface adhesion molecules – may also play a role in the processes of vascular changes underlying RA. We did not find any publication on the possible role of TAAs in cardiovascular diseases, only on their role in RA-ILD. ANOVA studies confirmed that changes in TAA levels between months 0 and 12 could be determined by disease

activity, CRP, We, some cytokines and PECAM-1 changes, CA15-3 and HE4, and may be reciprocally related to specific parameters of RA (CRP, We, TNF-α, IL-6, IL-8, VEGF, PECAM-1, and ccIMT and cfPWV). In the absence of similar studies, we could not compare our results with data from other studies.

One of the strengths of our study is that it is the first to analyse the effect of JAK inhibition on TAA levels and to investigate the associations between TAA and various RA-specific parameters, as well as vascular pathophysiological markers. Limitations include the absence of a control group and the relatively small sample size. In addition, although some previous studies have suggested that TAAs may be associated with RA-ILD, we were unable to investigate this issue in our current patient population due to the lack of available data.

New findings

- We observed that adverse events associated with anti-PD1 immune checkpoint inhibitors occurred in approximately one-third of the patients in our cohort. These events were predominantly mild and only rarely required discontinuation of therapy.

- We determined that differences may exist in the incidence of adverse events during nivolumab and pembrolizumab treatment. It was further observed that immune-related adverse events were more frequent but less severe with pembrolizumab, whereas they were less frequent but more severe with nivolumab.

- We found that longer duration of immunotherapy, younger age at treatment initiation, and the application of nine or more treatment cycles as a threshold were associated with an increased likelihood of developing immune-related adverse events.

- We demonstrated that the occurrence of immune-related adverse events has a positive predictive value for therapeutic efficacy.

- We established that this may be the largest study in Central and Eastern Europe analyzing the incidence of irAEs associated with nivolumab and pembrolizumab treatment.

- We observed that JAK inhibitor therapy led to a clear reduction in serum levels of the tumor markers CA15-3 and HE4.

- We observed that CA15-3 and HE4 levels showed a strong correlation with both rheumatoid arthritis activity and biomarkers of disease-associated vascular alterations.

- We observed that the serum levels of other tumor markers in patients with rheumatoid arthritis were also associated with clinical and laboratory parameters of disease activity, and that there was a relationship between serum tumor marker levels and vascular variables.

- Based on the available knowledge, this is the first study to provide a detailed analysis of the effect of tofacitinib on TAA levels, as well as to examine the relationships between TAAs and various RA-specific parameters and vascular pathophysiological markers.

Summary

The central theme of our study is the analysis of the role of immunomodulation in oncological and autoimmune diseases, as explored through two separate studies.

The first study discusses the mechanism of action and side effects of ICI therapies. The anti-PD1 type ICIs we studied – nivolumab and pembrolizumab – have brought significant clinical progress in antitumour therapy; however, the increased activation of the immune system can be associated with several unwanted immune-related adverse events (irAEs). These include skin and thyroid disorders, pneumonitis, colitis, hepatitis, and other autoimmune-related organ toxicities. During the study period, we studied 207 patients who received nivolumab or pembrolizumab therapy for solid tumours at the University of Debrecen Clinical Centre between 2017 and 2021. One third of patients experienced treatment-related adverse events, which were mainly mild (Grade 1) and did not require discontinuation of therapy except in 6 cases. In general, we did not observe any significant differences between the two drugs. Although adverse events occurred more frequently with pembrolizumab, they were less severe than those with nivolumab. Several factors may contribute to these differences, including the diversity of indications, the number of patients, and other variables. Our results also strongly suggest an association between ICI efficacy (based on PFS) and adverse events. Despite the potential limitations of our study, the data collected and analyzed in the Central and Eastern European region provide additional relevant information for practitioners regarding adverse events associated with ICI therapy. Furthermore, the study highlights that these types of side effects are not only common but also clinically relevant, as they can affect treatment success, patient quality of life, and survival. It follows that careful monitoring and targeted treatment of side effects are essential for optimizing immunotherapy treatments.

The second study investigates the impact of JAK inhibition on the levels of specific TAAs in relation to RA-specific parameters and vascular abnormalities. CA125-3 and HE4 stand out among the other TAAs studied, as we observed clear decreasing trends in their serum levels during the study, and we found a large number of correlations between these TAAs and RA, as well as markers of vascular changes in RA. Additional markers, such as CA19-9, CA125, CA72-4, and TPA, also showed associations with RA-related vascular biomarkers. The

effect of JAK inhibition on TAAs, as well as the correlations between TAAs and other parameters, suggests that TAAs may play a role in the pathogenesis of RA and RA-related vascular damage. In the future, some TAAs may even serve as therapeutic targets in the treatment of RA. However, it is also important to note that further studies are necessary to more precisely define the role of TAAs in RA and its comorbidities.

The two analyses present the complexity of immunomodulation from two different, but mutually reinforcing, sides. Activation of the immune system serves to protect against malignancies, but at the same time increases the risk of autoimmune reactions. Inhibition of the immune system, on the other hand, reduces inflammation but can potentially modify the recognition of tumor processes. The aim of the thesis is to gain a deeper understanding of the dual effect of the immune system and to help us apply treatments more effectively and individually to patients. It is also seeking answers on how to predict who is likely to experience side effects or have a good therapeutic response.

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List of publications related to the dissertation

1. **Sebestyén, E.**, Csige, D., Antal-Szalmás, P., Horváth, Á., Végh, E., Soós, B., Pethő, Z., Bodnár, N., Hamar, A. B., Bodoki, L., Kacsándi, D., Földesi, R., Kalina, E., Nagy, G., Kerekes, G., Nagy, B. J., Hódosi, K., Szamosi, S., Árkosy, P., Szűcs, G., Szekanecz, Z., Szekanecz, É.: Effects of Tofacitinib Therapy on Circulating Tumour-Associated Antigens and Their Relationship with Clinical, Laboratory and Vascular Parameters in Rheumatoid Arthritis. *Biomolecules. 15* (5), 1-15, 2025.
   DOI: http://dx.doi.org/10.3390/biom15050648
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2. **Sebestyén, E.**, Major, N., Bodoki, L., Makai, A., Balogh, I., Tóth, G., Orosz, Z., Árkosy, P., Vaskó, A., Hódosi, K., Szekanecz, Z., Szekanecz, É., Hungarian OncoRheumatology Network (HORN) initiative: Immune-related adverse events of anti-PD-1 immune checkpoint inhibitors: a single center experience. *Front Oncol. 13*, 1-9, 2023.
   DOI: http://dx.doi.org/10.3389/fonc.2023.1252215
   IF: 3.5

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List of other publications

3. Király, J., Berzi, A., El-Kareh, R., **Sebestyén, E.**, Ujvárosy, D., Emri, M., Bhattoa, H. P., Kappelmayer, J., Miller, K. E., Tóth, G.: Breaking the guidelines: how financial unawareness fuels guideline deviations and inefficient DVT diagnostics.
12 (2), 232-240, 2025.
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