SECTION 8. ONCOLOGY

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8.1 Immune-related toxicity of checkpoint inhibitors

In 2018, scientists James Ellison and Tasuku Honjo won the Nobel Prize in Medicine [277]. This event confirmed the beginning of the heyday of immunotherapy in oncology. Until now, the standard methods of treatment for malignant neoplasms were surgery, chemotherapy, and radiation therapy. However, in the last decade, monoclonal antibodies capable of inhibiting the pathological pathways of programmed cell death (PD-1/PD-L1) and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) have gained significant importance. It is believed that immune checkpoint inhibitors can provide a long relapse-free period even when the tumor resists other treatment methods [278].

Immunotherapy of advanced stages of oncological diseases is of particular importance. This treatment method significantly improves patients' overall survival [279]. However, regardless of the initial response, some patients experience disease relapse and resistance to immune checkpoint inhibitors [280]. In some cases, patients are generally insensitive to immunotherapy, even at the beginning of treatment [281]. In the USA, 43.63% of patients with advanced stages of cancer receive therapy with immune checkpoint inhibitors, but only 12.46% are sensitive to monoclonal antibodies [282].

Melanoma is one of the malignant neoplasms for which immunotherapy is used most often. Many studies have been conducted to establish the effectiveness of monotherapy with monoclonal antibodies (CTLA-4 blockers) [283] and a combination of two drugs (CTLA-4 and PD-1/PD-L1 blockers) [284]. As a result of the completion of the CheckMate-067 clinical trial with a follow-up period of patients for four years, it was concluded that the overall survival in the group of the combination of nivolumab and ipilimumab is higher compared to nivolumab or ipilimumab in monotherapy (53%, 46%, and 30%, respectively). A complete response after treatment with CTLA-4 and

PD-1/PD-L1 blockers was registered in 21% of people. In patients receiving only PD-1 or CTLA-4 blockers, a complete response was registered in 18% and 5%, respectively [285]. Therefore, different groups of immune checkpoint inhibitor drugs can potentiate each other and have a more powerful effect on T-lymphocytes. As a result, the patient should receive more benefit from immunotherapy [286].

Lung cancer has always been considered another promising area of research into the effectiveness of immunotherapy. Hellmann et al. [287] investigated that the rates of recurrence-free survival in patients with non-small cell lung cancer were 42.6% (for the nivolumab and ipilimumab group) and 13.2% (for the chemotherapy group). The advantage of immunotherapy over cytotoxic therapy was considered undeniable. Modern views of scientists are more restrained. For example, Ferrara et al. [288] evaluated the effectiveness of monotherapy and combination therapy with immune checkpoint inhibitors compared to chemotherapy in non-small cell lung cancer patients. The researchers concluded that mono- and combined immunotherapy improved relapse-free and overall survival only in PD-L1 ≥50%. The risk of side effects for both groups is practically the same.

Currently, immunotherapy is being used to treat almost all malignant neoplasms. Many clinical trials are ongoing to establish the safety and efficacy of new immune checkpoint inhibitors. Although all patients have the same inclusion criteria, some do not benefit from immunotherapy. Others can be treated for a long time and achieve a complete response to treatment. Prognostic factors on which the success of therapy depends remain unknown.

Mechanism of action of CTLA-4 and PD-1/PD-L1 pathological pathways

The immune system must protect a person from the action of various pathogens and atypical cells. T-lymphocytes play the primary role in this process. T-cell depletion is associated with poor patient survival and poor prognosis. According to Ma et al. [289], intratumoral T cells, which are dysfunctional, are induced by suppressive signals in the tumor microenvironment. Low levels of cytokines (for example, IFN-γ) and high levels of expression of the inhibitory receptors PD-1 and CTLA-4 indicate T-lymphocyte depletion. Suppressive pathological pathways block the production of

effector cytokines GZMA (cytotoxic lymphocyte granzyme A), GZMB (cytotoxic lymphocyte granzyme B), and PRF1 (perforin 1).

Although PD-1 and CTLA-4 have the same suppressive effect on T lymphocytes, there are differences in the site and time of action. The signaling mechanism of CTLA-4 acts mainly at the lymph node level and the early stage of the immune response. PD-1 suppresses T cells located in the paratumor and peripheral tissues. It happens later. In addition, PD-1 is expressed on B-lymphocytes, T-lymphocytes, and myeloid cells, while CTLA-4 is restricted to T cells only. Therefore, CTLA-4 works at the initial stages of T-lymphocyte activation in lymph nodes, and PD-1 joins during the effector phase and acts mainly in peripheral tissues [290].

The secret of the high efficiency of combined therapy with immune checkpoint inhibitors lies in the simultaneous effect on PD-1 and CTLA-4 receptors on exhausted CD8+ T-lymphocytes in different places and at different time intervals. Blocking PD-1 (or its ligand PD-L1) and CTLA-4 leads to restoring normal T-cell function. This is probably what provides an impressive therapeutic effect in patients with breast cancer [291], glioblastoma [292], and many other malignant neoplasms.

It reduces immunosuppression caused by regulatory T-lymphocytes (Treg). A blockade of the pathological PD-1 pathway is required to activate quiescent T cells. A combined effect of PD-1 and CTLA-4 results in an improved antitumor immune response.

CTLA-4 is always localized on Treg, but there is a mechanism for stimulating the appearance of these receptors. It consists of transmitting pathological signals of CD28 and T-cell receptor (TCR) and activating T cells. The CD28 and CTLA-4 receptors on the surface of T cells share two common ligands with the CD86 and CD80 receptors on the surface of antigen-presenting cells (APCs). However, CTLA-4 is more likely to interact with CD80 [293].

T lymphocytes express deficient amounts of CTLA-4. An increase in the degree of expression is observed only after the activation of T cells. After the interaction of CTLA-4 with CD86 or CD80, the signal transmission between APC and T-lymphocyte is blocked. As a result, the regulation of immune reactions is reduced, and atypical cells

can avoid the immune response. When the level of expression of CTLA-4 is significant, and its signals exceed those of CD28, the T-lymphocyte may become wholly inactive and undergo apoptosis [294]. CTLA-4 blockers disrupt the system of inhibitory signals that the T cell undergoes. After that, the activation of the immune response is observed. Figure 1 schematically shows the mechanism of action of CTLA-4 blockers.

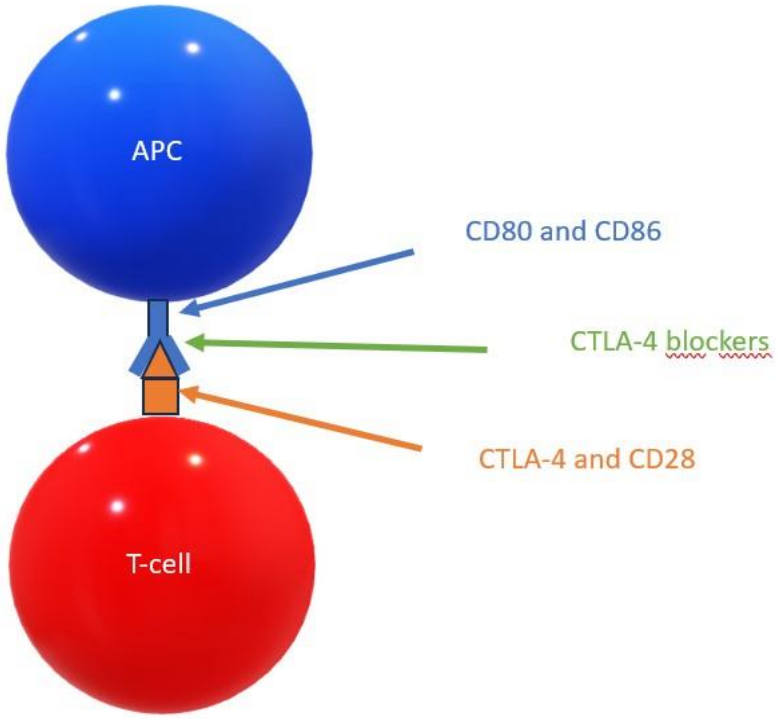

Figure 1. Mechanism of action of CTLA-4 blockers

Programmed cell death protein-1 (PD-1) is expressed much more frequently than CTLA-4. Unlike CTLA-4, this coinhibitory molecule can be detected on the surface of T-lymphocytes, B-lymphocytes, and natural killer (NK) cells. PD-1 can act 6-12 hours, while CTLA-4 is only 1 hour [295]. A variant of PD-1 is its ligand, PD-L1, which is found on the surface of tumor cells and tumor-infiltrating lymphocytes (TIL). Acting together, PD-1/PD-L1 creates the conditions for T-lymphocyte suppression and ensures tumor immune response evasion. Atypical cells can accumulate genetic mutations and use different mechanisms to control immunoediting. Their work is normalized by

blocking T-lymphocyte inhibitory signals, and activated antitumor immunity is achieved [296]. Figure 2 graphically depicts the mechanism of action of PD-1 and PD-L1 blockers.

CTLA-4 and PD-1 are the most extensively studied immune checkpoints. However, many others are under development: PD-L2 [297], VISTA [298], LAG3 [299], Siglec-15 [300], TIM3 [301] and others. All of them can be used for immunotherapy of malignant neoplasms.

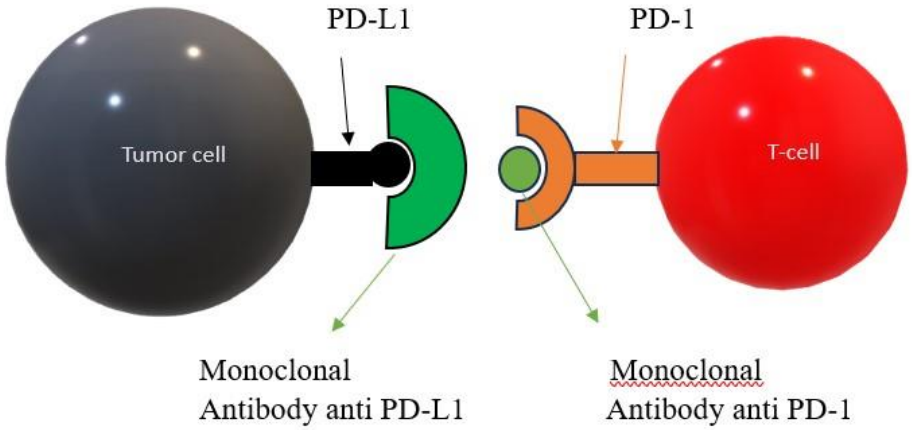

Figure 2. Mechanism of action of PD-1/PD-L1 blockers

The CTLA-4 and PD-1 signaling pathways protect healthy body cells from an excessive immune response. To distinguish between healthy and cancerous cells, particular protein molecules are located on the surface of T-lymphocytes. PD-1, mainly expressed in T-lymphocytes, looks for the PD-L1 protein in other cells and determines their health. Signaling from PD-L1 to PD-1, the atypical cell can pass itself off as expected and escape the immune response.

Immune checkpoint inhibitors block CTLA-4 and PD-1 and improve the immune response. Drugs such as nivolumab and pembrolizumab block PD-1 receptors and disrupt PD-L1 signaling. After that, malignant cells become defenseless against T-cells.

Unlike PD-1 blockers, CTLA-4 immune checkpoint inhibitors can rewire CTLA-4 receptors to enhance the anticancer immune response. In any case, the essence of the action of all immune checkpoint inhibitors is to block the "off" signal, which ensures the destruction of tumor cells by T cells [302].

Immunotherapy can be combined with chemotherapy and radiation therapy [303]. At the current stage, immune checkpoint inhibitors are used to treat cancer of the esophagus [304], breast [305], colon [306], pancreas [307], kidney [308], liver [309], lymphoma [310], stomach [311], ovaries [312], cervix [313], lungs [314] and many other malignant neoplasms.

Immune-dependent adverse events associated with the use of immune checkpoint inhibitors

The best-known drugs capable of blocking CTLA-4 and PD-1/PD-L1 receptors include pembrolizumab, nivolumab, durvalumab, avelumab, atezolizumab, cemiplimumab, dostarlimab, tremilimumab, and ipilimumab. By inhibiting down-regulated signaling pathways, they improve the immune response and activate antitumor immunity. Table 1 presents drugs according to the mechanism of action. In addition, the main localizations of malignant neoplasms for the treatment of which they are used and officially approved by the FDA (US Food and Drug Administration) are indicated.

Each of these drugs can cause immune-related adverse events (irAEs). This is due to their specific mechanism of action. For an oncologist, it is essential to detect side effects in time because such patients require the appointment of concomitant therapy, withdrawal of the drug, or targeted monitoring.

The CTCAE v5.0 classification (Common Terminology Criteria for Adverse Events, version 5) is used to assess the severity of irAEs, according to which all conditions are divided into five degrees of severity based on symptoms, signs, or laboratory findings [348]. Usually, patients with the first degree of adverse events have light symptoms or asymptomatic conditions, so they do not need to be prescribed drug therapy. Minimal, non-invasive interventions are necessary to manage conditions of the second degree of severity. Patients with the third degree of severity require

hospitalization, and patients with the fourth degree require urgent treatment to avoid severe complications and fatal consequences. According to the CTCAE v5.0 system, the fifth degree of severity means the patient's death. To control irAEs, it may be necessary to temporarily or permanently withdraw the immunotherapy drug, prescribe high doses of steroid hormones, and hospitalize the patient.

Table 1.

List of checkpoint inhibitors according to the mechanism of action.

Mechanism of action	The name of the immunotherapeutic drug	Indications
1	2	3
Monoclonal antibodies (ATs) anti-PD-1	Pembrolizumab	Lung cancer [315] Melanoma [316] Esophageal cancer [317] Stomach cancer [318] Head and neck squamous cell carcinoma [319] Endometrial cancer [320] Hepatocellular carcinoma [321] Merkel cell carcinoma [322] Urothelial carcinoma [323] Renal cell carcinoma [324] Classic variant of Hodgkin's lymphoma [325]
Monoclonal ATs anti-PD-1	Nivolumab	Lung cancer [326] Melanoma [327] Urothelial carcinoma [328] Renal cell carcinoma [329] Head and neck squamous cell carcinoma [330] Classic variant of Hodgkin's lymphoma [331]
Monoclonal ATs anti-PD-1	Cemiplimumab	Lung cancer Skin cancer
Monoclonal ATs anti-PD-1	Dostarlimab	Endometrial cancer

Continuation of Table 1.

Mechanism of action	The name of the immunotherapeutic drug	Indications
1	2	3
Monoclonal ATs anti-PD-L1	Atezolizumab	Lung cancer [332] Breast cancer [333] Urothelial carcinoma [334]
Monoclonal ATs anti PD-L1	Durvalumab	Lung cancer [335] Urothelial carcinoma [336]
Monoclonal ATs anti-PD-L1	Avelumab	Urothelial carcinoma [337] Merkel cell carcinoma [338] Renal cell carcinoma [339]
Monoclonal ATs anti-CTLA-4	Ipilimumab	Lung cancer [340] Melanoma [341] Prostate cancer [342] Ovarian cancer [343] Colorectal cancer [344] Renal cell carcinoma [345]
Monoclonal ATs anti-CTLA-4	Tremilimumab	Lung cancer [346] Hepatocellular carcinoma [347]

During treatment with immune checkpoint inhibitors, there is always a risk of irAEs from the gastrointestinal tract, liver, skin, and endocrine organs. Nausea, diarrhea, itching and rash on the skin, and thyroiditis are sometimes observed in patients taking monoclonal anti-PD-1 ATs. For CTLA-4 blockers, complications from the colon and pituitary gland are more characteristic. irAEs can occur during treatment and even after its completion. Usually, 3-6 months pass from the start of immunotherapy [349]. Table 2 contains a list of organs and systems and adverse events associated with their toxicity.

Table 2. Adverse events associated with toxicity of immune checkpoint inhibitors

Organ or organ system	Immune-related adverse events
1	2
Systemic adverse events [350]	Fever General weakness Allergic reactions of various degrees
Gastrointestinal tract [351]	Gastritis Colitis
Skin [352]	Itching Acne-like rash Eczema Bullous dermatoses Psoriasis Lichenoid reactions Vitiligo Stevens-Johnson syndrome
Respiratory system [353]	Pneumonitis Sarcoid-like granulomatous reactions Pulmonary sarcoidosis
Hepato-biliary system [354]	Hepatitis
Nervous system [355]	Neuropathy Guillain-Barre syndrome Myelopathy Encephalitis Myasthenia Meningitis
Urinary system [356]	Acute kidney injury Immune-dependent kidney disease
Endocrine system [357]	Hypothyroidism Hyperthyroidism Thyroiditis Adrenal insufficiency Diabetes Hypophysitis

Continuation of Table 2.

Organ or organ system	Immune-related adverse events
1	2
Ocular [358]	Idiopathic inflammation of the orbit Blepharitis Uveitis Choroidal neovascularization Peripheral ulcerative keratitis Swelling of the optic nerve Vogt-Koyanagi-Harada syndrome Episcleritis
Cardiovascular system [359]	Pericarditis Myocarditis Cardiofibrosis Cardiomyopathy Arrhythmias
Blood [360]	Neutropenia Aplastic anemia Immune thrombocytopenic purpura Autoimmune hemolytic anemia Cryoglobulinemia Acquired hemophilia A
Musculoskeletal system [361]	Arthralgia Myopathy Vasculitis Spondyloarthropathy Polymyalgia rheumatica Sikka syndrome

According to the meta-analysis conducted by Wang et al. [362], immune-related side effects occurred in 27% of patients receiving PD-1/PD-L1 blockers. 6.1% of patients developed grade 3 or 4 irAEs, and 0.17% died as a result of immunotherapy complications.

Gastrointestinal toxicity of immune checkpoint inhibitors is one of the most common. In most cases, it is associated with using monoclonal antibodies to CTLA-4. Thus, 40% of cases of immune-dependent adverse events are associated with damage to the stomach and/or intestines after taking CTLA-4 blockers. The combination of CTLA-4 and PD-1/PD-L1 are less toxic. Only 10% of patients develop immune-related damage to the gastrointestinal tract [363].

Immunotherapy can lead to toxic effects on any part of the digestive tract. However, CTLA-4 blockers significantly more often provoke the development of inflammation in the lower parts. Approximately every third patient has colitis. PD-1/PD-L1 blockers are safer and cause mild inflammation of the oral mucosa, esophagus, stomach, and duodenum [364].

Clinical symptoms that indicate gastrointestinal toxicity of the immunotherapy are enterocolitis. Diarrhea and abdominal pain are reported by 45% of patients taking CTLA-4 monoclonal antibodies. Less common symptoms include mucus in the stool and fever.

When the upper parts of the digestive tract are affected, the patient may lose appetite and experience nausea and abdominal pain. Sometimes, these symptoms are accompanied by vomiting. Very rarely, life-threatening conditions are observed: bleeding, dehydration, sepsis, and perforation of the stomach or intestines. Symptoms of immune toxicities usually appear 6-9 weeks after the start of treatment, that is, after receiving 2-3 doses of the drug. However, there are cases of "delayed" toxicity, where symptoms appear several months after the end of treatment [365].

The primary method for identifying changes in the mucous membrane of the gastrointestinal tract (inflammation, ulcers, erythema) is endoscopic. It is essential that a biopsy can be performed and the tissue examined during the endoscopic examination. Eosinophilic intraepithelial infiltrates, invasion of crypts, and accumulation of lymphocytes and neutrophils evidence the presence of immune-dependent inflammation. Computed tomography (CT) can be an alternative but less effective method of investigation. In some cases, CT changes precede clinical symptoms of colitis [366].

Differential diagnosis is carried out with infectious enterocolitis, nonspecific ulcerative colitis, Crohn's disease, pseudomembranous colitis, and drug-induced gastritis.

Patients with mild gastrointestinal toxicity do not need to stop immunotherapy. Symptomatic treatment is recommended. In the case of a more severe course, inhibitors of immune checkpoints are temporarily canceled, and steroids are prescribed. In case of ineffectiveness of steroids, infliximab or vedolizumab is used. If the damage to the digestive tract corresponds to the 4th degree of severity, the treatment is canceled permanently.

The respiratory system, particularly the lungs, is often under immune attack. Pneumonitis is registered in 3-6% of patients. Monoclonal ATs against CTLA-4 are less toxic to the lungs and cause inflammation in only about 1% of patients. PD-1/PD-L1 blockers affect the respiratory system in 1-5% of cases. Combined therapy with both groups of drugs increases lung toxicity, so every tenth patient develops pneumonitis [367].

Pneumonitis can be asymptomatic or have symptoms corresponding to mild, moderate, or severe severity. Fever, dry cough, shortness of breath, chest pain, and lung infiltrates are most often observed during CT, MRI, and X-ray imaging. Pneumonitis can develop at different times, but on average, a patient receives immunotherapy for 2-3 months before the first signs of toxicity appear. In the case of combined therapy, symptoms may appear earlier.

CT scan is the most appropriate method for diagnosing immune-dependent pneumonitis. Usually, radiologists describe lung infiltrates (mainly in the lower parts) as " ground-glass opacity." Additional examination methods can be bronchoscopy and spirometry. In the diagnosis process, it is necessary to rule out the infectious nature of the disease.

Pneumonitis should be differentiated from a sarcoid-like reaction. X-ray changes in the form of foci in the upper lobes of the lungs with symmetrical mediastinal lymphadenopathy are found in 5-7% of patients with melanoma after taking ipilimumab.

Another condition similar to immune-related pneumonitis is radiation pneumonitis. Such patients always have a history of radiation therapy [368].

Treatment depends on the severity of pneumonitis. In the case of the first grade without pronounced clinical symptoms, dynamic observation of the patient and repeated CT after one month is required [369]. At the appearance of clinical symptoms and the development of pulmonary toxicity in the 2nd grade, it is necessary to cancel immunotherapy and prescribe steroid hormones temporarily. Immune checkpoint inhibitors are permanently discontinued if pneumonitis is grade 3 or 4. High doses of steroids are prescribed for treatment, and in case of their ineffectiveness - immunosuppressants infliximab, tocilizumab, intravenous immunoglobulin, and hospitalization in the intensive care unit [370].

The liver is an organ of the hepatobiliary system that is also a frequent target for autoantibodies. The toxicity of immune checkpoint inhibitors increases when they are combined. In this case, immune-related hepatitis develops in about 30% of cases. With monotherapy with monoclonal ATs against CTLA-4, the rate is two times lower and is 15%.

Laboratory indicators indicate the onset of immune-dependent hepatitis: an increase in total bilirubin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST). At the same time, the beginning of the disease is mostly asymptomatic. In more severe cases, the patient complains of right abdominal pain, fever, nausea, vomiting, general weakness, and jaundice. The timing of the onset of hepatitis can be different, but most often, it happens 3-14 weeks after the start of treatment with immunotherapy [371].

The following methods are used to diagnose immune-dependent hepatitis: laboratory, ultrasound, CT, MRI, endoscopic ultrasound, and cholangiopancreatography. The disease is often associated with ascites and lymphadenopathy [372].

Differential diagnosis should be carried out with viral and alcoholic hepatitis and idiopathic autoimmune hepatitis. Treatment is similar to other irAEs. It consists of

determining the severity of hepatitis and prescribing steroid therapy or mycophenolate mofetil. Treatment with immunotherapy is canceled temporarily or permanently.

Compared to other gastrointestinal tract organs, immune checkpoint inhibitors are much less likely to affect the pancreas. The endocrine and exocrine function of the pancreas can be disturbed [373]. The combination of PD-1/PD-L1 and CTLA-4 resulted in pancreatitis in 14% of patients, whereas monotherapy with CTLA-4 blockers resulted in less than 5% of patients. The adverse event develops in the 9th to 20th week of treatment with inhibitors of immune checkpoints and is accompanied by an increase in the level of lipase and amylase in the blood.

Clinical symptoms are initially not pronounced, and there are no signs of acute pancreatitis. In favor of inflammation of the gland, laboratory indicators testify. Later, a wide range of symptoms appears: weight loss, general weakness, diarrhea, and a transient increase in blood amylase and lipase. Signs of diabetes and digestive enzyme deficiency may be observed [374].

Diagnosis of immune-dependent inflammation of the pancreas is difficult since the symptoms are similar to traditional acute pancreatitis. Ultrasound, CT, MRI, and PET-CT (positron emission tomography) are used to confirm the diagnosis. In favor of irAE, immunotherapy in the anamnesis, pain in the epigastrium, blood lipase levels more than three times higher than usual, specific results of imaging methods of research (gland lesions of the type of interstitial edematous pancreatitis) are evidenced. Sometimes, inhibitors of immune checkpoints can lead to chronic pancreatitis and atrophy of the pancreatic parenchyma. As a result, such patients develop diabetes.

Differential diagnosis of immune-dependent pancreatitis is carried out with autoimmune pancreatitis associated with immunoglobulin G4. The difference lies in the acute onset of autoimmune pancreatitis and the typical gland appearance during ultrasound and CT. The pancreas resembles a "sausage" without normal fat lobes [375].

Treatment of immune-dependent pancreatitis consists of prescribing steroids and temporary or complete withdrawal of immunotherapy.

Kidneys are rarely pathologically affected by immune checkpoint inhibitors. irAEs develop in about 5% of patients receiving combination therapy and in less than

1% after monotherapy. It takes some time (usually 3-6 months) to develop symptoms of nephrotoxicity.

The onset of the disease is acute. Signs of acute renal failure appear immediately. In some cases, renal tubular acidosis or nephrotic syndrome without renal failure is observed. Increased creatinine levels in the blood are the main sign of kidney damage.

Immune-related nephrotoxicity should be differentiated from other diseases that are accompanied by acute renal failure and occur due to a decrease in renal blood flow, obstruction of the urinary tract, or direct kidney injury.

Steroids are prescribed for the treatment of immune-related nephritis. If the creatinine level is over three times higher than usual, immunotherapy is canceled forever. The patient may require hospitalization and hemodialysis [376].

Immune-related lesions of the nervous system are difficult for clinical diagnosis. The frequency of neurological irAEs during monotherapy with PD-1 blockers is about 6%, with CTLA-4 blockers - 4%. The combination of immunotherapy drugs leads to neurological toxicity in 12% of patients [377].

Immune checkpoint inhibitors can cause toxic injury to both the peripheral and central nervous systems. Severe neurological damage occurs in less than 1% of cases but can lead to severe disorders and even death of patients.

General signs of neurotoxicity include weakness, fatigue, dizziness, and headache. Sometimes, patients have symptoms of myasthenia gravis, transverse myelitis, and Guillain-Barré syndrome. Aseptic encephalitis and meningitis are life-threatening conditions. They are rare.

Most often, on the background of taking immunotherapy, there is damage to the peripheral nervous system in the form of peripheral neuropathies, radiculitis, and, less often, Guillain-Barré syndrome. It takes about six weeks for clinical symptoms to appear. However, sometimes, the drug's neurotoxicity manifests itself several months after starting therapy with immune checkpoint inhibitors.

Differential diagnosis of immune-related toxicity to the nervous system is carried out with toxic, metabolic, and autoimmune disorders. It is necessary to exclude

the metastatic process and the presence of a malignant tumor of the central nervous system.

Treatment of immune-dependent neurotoxicity consists of prescribing steroids with temporary or permanent withdrawal of immunotherapy. Guillain–Barré syndrome management may require plasmapheresis and intravenous immunoglobulin [378].

Immune-dependent lesions of *endocrine organs* occur in 40% of patients treated with immune checkpoint inhibitors [379]. Scientists found that the frequency of registration of any degree of severity of hypothyroidism, hyperthyroidism, hypophysitis and adrenal insufficiency is 8.26, 5.48, 22.03 and 3.87%, respectively. At the same time, the immunotherapy drugs of different groups (CTLA-4 or PD-1 blockers) and the localization of the primary tumor were not considered [380].

If we list the endocrine organs in ascending order, then inhibitors of immune control points most rarely affect the beta cells of the pancreatic islets, followed by the adrenal glands, pituitary gland, and thyroid gland [381]. The degree of severity of endocrinopathies can be different. Monoclonal antibodies rarely lead to severe consequences but can worsen patients' quality of life. Sometimes, organ changes are irreversible and end in permanent dysfunction and hormonal imbalance. Most pathological conditions can be successfully treated if diagnosed in time. However, it is challenging to detect endocrinopathy clinically since the symptoms are non-specific and similar to the manifestations of the underlying disease (fatigue, general weakness). Regular monitoring of blood hormone levels is necessary for timely diagnosis.

The *thyroid gland* is susceptible to the toxic effects of immune checkpoint inhibitors, especially combined immunotherapy with PD-1/CTLA-4 blockers. Complications develop in every fifth patient. In the case of anti-PD1/anti PD-L1 monotherapy, in every tenth person, and in the case of treatment with CTLA4 blockers, in every twentieth person,

Immune-dependent damage to the thyroid gland can manifest as hypothyroidism or hyperthyroidism. The average time before the appearance of pathological abnormalities in laboratory blood tests is six weeks.

For the most part, hyperthyroidism and hypothyroidism of the first grade of severity are asymptomatic. Later, with insufficient thyroid hormones, patients complain of fatigue, sweating, weight gain, depression, and constipation. An increased heartbeat, hand tremors, general excitement, and insomnia manifest hyperthyroidism. Thyroid crises are sporadic [382].

A laboratory method is used for early diagnosis of abnormalities in the work of the thyroid gland. For this, the levels of thyroxine (T4), triiodothyronine (T3), thyroid-stimulating hormone (TSH), as well as antithyroid antibodies to thyroglobulin and peroxidase are regularly determined. The primary imaging method is ultrasound and PET-CT.

Differential diagnosis is carried out with hypothyroidism, which occurs as a result of toxic damage to the pituitary gland by inhibitors of immune control points, as well as with metastases in the thyroid gland.

Immune-related toxicity to the thyroid gland of a mild degree requires dynamic monitoring or the appointment of accompanying drug therapy (thyroid hormones in case of hypofunction or antithyroid therapy in case of hyperfunction). Treatment with immune checkpoint inhibitors is not stopped. In more severe cases and when clinical symptoms of the disease appear, the appointment of steroid hormones is recommended [383].

Hypophysitis develops in about 5% of patients taking immune checkpoint blockers. Interestingly, PD-1/PD-L1 blockers cause toxic effects in only 1% of patients, while CTLA-4 blockers cause toxic effects in 14% [384].

Hypophysitis begins with fatigue, headache, symptoms of hypogonadism, hypothyroidism, and hypocorticism. This usually occurs nine weeks after starting immunotherapy [385].

Since the symptoms of immune-dependent hypophysitis are non-specific, it is often diagnosed accidentally during CT, MRI, or PET-CT. Imaging research methods help rule out the presence of a pituitary adenoma or a metastatic lesion. If hypophysitis is suspected, blood hormone levels are measured. The diagnosis is confirmed based on

anamnestic data on therapy with immune checkpoint inhibitors, imaging results, and laboratory tests.

Differential diagnosis of immune-related hypophysitis is carried out with pituitary adenomas, lymphocytic hypophysitis, and metastatic lesions.

Hormone replacement therapy is prescribed for the treatment of patients. Immunotherapy is temporarily stopped. In some cases, it is necessary to prescribe steroids and finally withdraw immune checkpoint inhibitors [386].

Adrenal insufficiency develops in less than 5% of people receiving immunotherapy. Initially, patients complained of fatigue and hypotension. Occasionally, an adrenal crisis occurs due to hormonal insufficiency, which requires immediate treatment. It should be remembered that hormonal imbalance can be a consequence of hypopituitarism.

A laboratory method is used to diagnose immune-related toxicity to the adrenal glands. The levels of adrenocorticotropic hormone, cortisol, aldosterone, renin, and electrolytes are measured for this. Among the imaging methods, the most informative is PET-CT, which allows the detection of a bilateral increase in the size of the adrenal glands and a moderate accumulation of fluorodeoxyglucose.

Differential diagnosis of immune-related adrenalitis is carried out with metastases of malignant neoplasms in the adrenal glands.

Treatment consists of hospitalization of the patient and intravenous infusion of glucocorticoid hormones [387].

Immune-dependent toxicity to endocrine cells of the *pancreas* is a rare condition. This irAE develops in 1.5% of patients receiving combined therapy with immune checkpoint inhibitors and 1% after monotherapy with anti-PD-1/PD-L1 antibodies. The mechanism of development of diabetes is autoimmune. This fact is evidenced by the reduced expression of PD-1 on T-cells and antibodies to GAD65 (glutamate decarboxylase 65).

Hyperglycemia is not always a consequence of taking immune checkpoint inhibitors. It can be observed after taking high doses of corticosteroids. The onset of the disease is gradual. Hyperglycemia and weight loss are the main manifestations of

immune-dependent diabetes. It takes 1 to 12 months from the start of taking immune checkpoint inhibitors before laboratory signs appear.

In addition to the laboratory method, damage to the pancreas is indicated by ultrasound results. The organ acquires the shape of a "sausage" and increases in size. Immune-dependent damage to the pancreas must be differentiated from malignant neoplasms, metastatic lesions, and normal inflammation.

Blood glucose tests should be performed regularly during treatment with immune checkpoint inhibitors. This will allow timely diagnosis of an adverse event. Insulin is prescribed for the management of hyperglycemia. Immunotherapy is canceled until the indicators of sugar metabolism normalize [388].

Immune-related *cardiovascular* adverse events occur in less than 1% of patients taking immune checkpoint inhibitors. Cardiotoxicity mainly develops in patients with a history of heart disease and the case of combined therapy. It is essential to diagnose complications in time because mortality due to cardiotoxicity can reach 50%. Myocarditis is the most frequently diagnosed heart muscle injury [389].

Clinical symptoms of manifestations of myocarditis are very diverse. In some cases, the course can be fulminant and manifest as severe arrhythmias, myasthenia, and myositis. The level of troponin increases in the blood serum of such patients. Systolic dysfunction is observed in about 50% of people. The risk of death of such patients is maximum.

Cardiac arrhythmias (ventricular and atrial) and conduction disturbances often occur against the background of immune checkpoint inhibitors. Sudden death may occur in such patients. In addition, after the use of monoclonal antibodies, there is a risk of developing vasculitis and pericarditis.

Takotsubo syndrome has clinical symptoms similar to myocarditis. In favor of immune-related myocarditis, evidence of myositis, myocarditis, and the presence of immunotherapy in the anamnesis [390].

The course of diseases caused by immune-related toxic damage to the cardiovascular system is not always acute. Scientists have reported cases of "smoldering" myocarditis, which has a chronic course. A reaction to introducing

immune checkpoint inhibitors can occur from the first days of treatment. However, this can occur within a few days to 15 months after immunotherapy.

Laboratory tests and imaging methods are used to diagnose lesions of the cardiovascular system. Cardiac biomarkers and ECG changes are nonspecific but available. Echocardiography, cardiac MRI, PET-CT, and myocardial biopsy are modern and promising diagnostic methods for evaluating cardiotoxicity caused by immunotherapeutic drugs.

Myocarditis caused by the toxic effects of immune checkpoint inhibitors should be differentiated from myocarditis of other etiologies. Infectious and non-infectious factors lead to the development of this disease. In particular, some bacteria, viruses, parasites, or fungi can cause heart muscle inflammation. Non-infectious causes of myocarditis are autoimmune diseases, some medications, and radiation exposure.

A feature of treatment tactics is the final cancellation of immunotherapy at the appearance of the slightest signs of toxic damage to the heart muscle. Patients are prescribed steroids and, in complicated and refractory cases - infliximab [391].

Skin toxicity occurs in 30-60% of patients receiving treatment with immune checkpoint inhibitors. With monotherapy with PD-L1 blockers, side effects on the skin are less pronounced compared to PD-1 blockers, CTLA-4, and combined therapy (20% vs. 34-42, 44-59, and 59-72%, respectively). In addition, there is a relationship between the severity of irAEs and the immunotherapy group. Severe skin lesions occur more often after monotherapy with PD-L1 blockers (7.2% of cases) than with CTLA-4 or PD-1 (4.7% and 2.3%, respectively). Traditionally, combination therapy results in the highest incidence of grade 3 and 4 skin toxicity (14.5%) [392].

Clinical symptoms indicating an immune-dependent skin lesion can be diverse. A maculopapular rash is the most common skin adverse event. On physical examination, erythematous papules and macules with a tendency to coalesce may be seen. The rash is localized mainly on the skin of the trunk and limbs.

Sometimes, the maculopapular rash progresses and transforms into Stevens-Johnson syndrome or drug-induced hypersensitivity syndrome. The appearance of

blisters, peeling of the epidermis, mucositis development, and fever and lymphadenopathy indicate a severe degree of skin damage.

Immune checkpoint inhibitors can cause eczema, psoriasis or psoriasis-like rashes, vitiligo, bullous foci, alopecia, and pruritus. Patients with blistering, mucositis, epidermal desquamation, high fever, or enlarged lymph nodes should be closely monitored [393].

Immunocaustic skin damage develops after several weeks or months of treatment with immune checkpoint inhibitors. Each variant of the skin reaction has its time limits for the onset of the disease. For example, the most common maculopapular and psoriatic rashes occur 3-6 weeks after the start of immunotherapy. Psoriasis takes more time to develop directly - up to 12 weeks, alopecia - 12-24, vitiligo - 26 weeks. Itching of the skin can bother patients a week after the first administration of the immunotherapy drug. Scleroderma occurs very rarely. Long-term use of immune checkpoint blockers (up to 48 weeks) is usually required.

Differential diagnosis of immune-dependent skin damage involves allergic reactions, bacterial and viral infections, and fungal and autoimmune skin damage.

Ointments with corticosteroids, oral antihistamines, and moisturizing creams are used for treating a mild rash. However, patients with extensive rash and fever require systemic steroids at a dose of 0.5-1 mg/kg/day. Immunotherapy is not stopped in case of mild severity of irAE. In more severe cases, immune checkpoint inhibitors can be temporarily or permanently withdrawn [394].

Checkpoint inhibitors are successfully used for the treatment of malignant neoplasms. However, the mechanism of action of these drugs involves an effect on the immune system, so some patients experience immune-related side effects. Immunotherapeutic drugs can have a toxic effect of varying grades of severity on almost any organ and system. In some cases, the patient dies. It is worth noting that the clinical symptoms in the initial stages are nonspecific, and the diagnosis and treatment of immune-related adverse events require a multidisciplinary approach. Early diagnosis and personalized treatment are the keys to successful toxicity management.