Two Complementarity Immunotherapeutics in NSCLC

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The idea of using two different immunotherapies in cancer patients is based on the attempt to stimulate or inhibit different immune cells at different levels of their activity (e.g., in the lymph node and in the tumour). The most commonly used combination immunotherapy involves antibodies that target molecules capable of stimulation of the activity of lymphocytes and other immune cells and molecules that are able to inhibit this activity. Another combination immunotherapy method is the use of immune checkpoint inhibitors in combination with agents that modify the tumour microenvironment in a non-specific manner (e.g., pro-inflammatory cytokines, immunosuppressive cytokine inhibitors, and indoleamine 2,3-dioxygenase and adenosine inhibitors).

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1. Introduction

and/or second-line treatment in patients with various types of cancer (melanoma, non-small-cell lung cancer, renal cell carcinoma, head and neck region cancer, urothelial carcinomas, colorectal cancer, esophageal cancer, and lymphoma) In patients with non-small-cell lung cancer, pembrolizumab (anti-PD-1 antibody) and atezolizumab (anti-PD-L1 antibody) used as first-line therapy may only be appropriate for patients with PD-L1 expression on ≥50% of tumour cells (in the US, pembrolizumab can also be used in patients with a high tumour mutational burden and PD-L1 expression on ≥1% of tumour cells) Indeed, the PD-L1 expression on cancer cells is the only biomarker validated in prospective immunotherapy-based clinical trials; however, it is not an ideal one [1][2][3]. The aim of combination therapy is to create a favourable environment within the cancerous tumour and maximize the potential of the immune system to eliminate cancer cells

The idea of using two different immunotherapies in cancer patients is based on the attempt to stimulate or inhibit different immune cells at different levels of their activity (e.g., in the lymph node and in the tumour) [4][5][6][7][8]. The most commonly used combination immunotherapy involves antibodies that target molecules capable of stimulation of the activity of lymphocytes and other immune cells and molecules that are able to inhibit this activity. Another combination immunotherapy method is the use of immune checkpoint inhibitors in combination with agents that modify the tumour microenvironment in a non-specific manner (e.g., pro-inflammatory cytokines, immunosuppressive cytokine inhibitors, and indoleamine 2,3-dioxygenase and adenosine inhibitors) [4][5][6][7][8].

2. Possibilities of Combining Different Immune Checkpoint Molecules

The use of various ICIs has found the widest application in clinical practice in cancer patients without the presence of actionable mutations and based on tumour histology as well as specific clinical characteristic of patients. A summary of the most important clinical trial results from phase 2/3 using combination immunotherapies and their clinical efficacy is presented in**Table 1**[9][10][11].

Table 1. The Summary of the most important clinical trial results using combination immunotherapies. Abbreviations: ORR—overall response rate, PFS—progression free survival, HR—hazard ratio, CI—confidential interval, OS- overall survival, PD-L1—programmed death ligand 1, TC—tumor cells, ND—no data. (* PD-L1 expression examined by 22C3 monoclonal antibody; ** PD-L1 expression examined by SP263 monoclonal antibody) [9][10][11].

Clinical Trial Identifier	Phase	Predictive Factor	Stage of NSCLC	Drugs	Number of Patients	ORR (%)	Median PFS (months)	PFS (HR, 95% CI)	Median OS	OS (HR, 95% CI)
CheckMate 227 NCT02477826	3.	≥1% of PD-L1-positive TC (Part 1a)	IV	Nivolumab	396	27.5	4.2	0.82, 0.69–0.97 (nivolumab + ipilimumab vs. chemotherapy)	15.7	0.79, 0.65–0.96 (nivolumab + ipilimumab vs. chemotherapy)
				Nivolumab + ipilimumab	396	35.9	5.1	0.83, 0.71–0.97 (nivolumab + ipilimumab vs. nivolumab)	17.1	0.90, 0.76–1.07 (nivolumab + ipilimumab vs. nivolumab)
				Chemotherapy	397	30	5.6		14.9	
CheckMate 227 NCT02477826	3.	<1% of PD-L1-positive TC (Part 1b)	IV	Nivolumab + chemotherapy	177	37.9	5.6	0.75, 0.59–0.96 (nivolumab + ipilimumab vs. chemotherapy)	15.2	0.62, 0.48–0.78 (nivolumab + ipilimumab vs. chemotherapy)
				Nivolumab + ipilimumab	187	27.2	5.1	0.98, 0.77–1.24 (nivolumab + ipilimumab vs. nivolumab + chemotherapy)	17.2	0.77, 0.60–0.98 (nivolumab + ipilimumab vs. nivolumab + chemotherapy)
				Chemotherapy	186	33.1	4.7	0.73, 0.56–0.95 (nivolumab + chemotherapy vs. chemotherapy)	12.2	0.78, 0.60–1.02 (nivolumab + chemotherapy vs. chemotherapy)
CheckMate 227 NCT02477826	3.	All patients	IV	Nivolumab +	583	33.1	5.1	0.79, 0.69–0.91	17.1	0.73, 0.64–0.84

Clinical Trial Identifier	Phase	Predictive Factor	Stage of NSCLC	Drugs	Number of Patients	ORR (%)	Median PFS (months)	PFS (HR, 95% CI)	Median OS	OS (HR, 95% CI)
				ipilimumab						
				Chemotherapy	583	27.7	5.5		13.9	
CheckMate 9LA NCT03215706	3.	All patients	IV	Nivolumab + ipilimumab + 2 cycles of chemotherapy	361	38.2	6.8	0.70, 0.57–0.86	15.6	0.66, 0.55–0.80
				Chemotherapy	358	24.9	5.0		10.9	
CITYSCAPER (NCT03563716)	2.	≥1% of PD-L1-positive TC	IIIB or IV	Chemotherapy	68	21% * 23% **	3.88 * 4.11 **	0.58, 0.39–0.88 *	ND	ND
				Atezolizumab + tiragolumab	67	37% * 42% **	5.55 * 10.18 **	0.56, 0.34–0.92 **	ND	

PD-L1 expression on tumour cells and the number of somatic mutations in tumour cells (tumour mutation burden, TMB) [12][13]. It was found that, in patients with high TMB (more than 10 mutations per million base pairs) even with no PD-L1 expression on tumour cells, the use of the nivolumab and ipilimumab combination prolonged progression-free survival, compared to other treatments [12][13]. During further follow-up, prolongation of patient survival was observed in patients with PD-L1 expression on ≥1% of tumour cells using the combination of these two immunotherapies. In view of these results, the combination of nivolumab and ipilimumab for first-line therapy in NSCLC patients with high TMB was not registered and replaced by the registration of the combination of these two drugs in NSCLC patients with any PD-L1 expression on tumour cells [12][13].

It should also be mentioned at this point that the PD-L1 expression on cancer cells is the only predictive factor validated in prospective clinical trials for immunotherapy in advanced NSCLC patients [14][15]. Moreover, it has been indicated that tumours have three immunoprofiles based on the activation of the immune system: (1) “hot” tumours, which are strongly infiltrated by T lymphocytes and with many inflammatory signals; (2) “cold” tumours, which are scanted of any immune cells infiltration nor inflammatory signs; (3) tumours with immune exclusion, where immune cells are at the periphery or within the stromal tissue [16][17]. The ”hot” tumours are associated with denser PD-1-positive T lymphocyte infiltration, with pre-existing primed immune response, and are more likely to respond to the anti-PD-1 or anti-PD-L1 blockade used as monotherapy [16][17]. Therefore, the intensity of lymphocyte infiltration of tumour tissue, immunological analysis, or estimation of the gene expression profile in cancer tissue could be considered as a reliable biomarker in the prospective qualification for immunotherapy in different strategies.

Combination therapy with two different immunotherapy modalities is usually fairly well tolerated. Clinical trials did not identify a significant increase in the incidence of adverse events (AEs) in groups of patients treated with combination immunotherapy compared to monotherapy [9][10][11]. On the other hand, combination therapy with two ICIs causes a different type of side effects compared to chemotherapy. Patients receiving immunotherapy most often experience side effects related to hyperactivity of the immune system (endocrinopathies, pneumonitis, hepatotoxicity, skin reaction, and others), while patients receiving chemotherapy develop bone marrow suppression (anaemia, infections, thrombocytopenia, and febrile neutropenia)

Serious treatment-related adverse events and AEs leading to discontinuation were more common in patients treated with nivolumab plus ipilimumab than with chemotherapy (24.5% vs. 13.9% and 18.1% vs. 9.1%). The most common treatment-related adverse events (TRAEs) of any grade related to the immune system in the group that received nivolumab plus ipilimumab were skin reactions (34.0% of the patients) and endocrinopathies (23.8%). In patients with PD-L1 expression on ≥1% of tumour cells treated with nivolumab monotherapy, grade 3 or 4 TRAEs occurred in 19.4% of the patients, and TRAEs resulted in discontinuation of the therapy in 12.3% of the patients. In patients without expression of PD-L1 treated with nivolumab plus chemotherapy, serious TRAEs occurred with a frequency of 19.2%.

In the CheckMate 9LA clinical trial, serious TRAEs were reported in 30% of patients receiving combination therapy and in 18% of patients treated with chemotherapy [9]. The following causes of death were found: Six (2%) deaths due to anaemia, febrile neutropenia, pancytopenia, pulmonary sepsis, respiratory failure, and sepsis occurred in the control group [9]. The most common grade 3–4 TRAEs were neutropenia (7% of patients treated with combined therapy vs. 9% of patients receiving chemotherapy), anaemia (6% vs. 14%), diarrhoea (4% vs. 1%), and febrile neutropenia (4% vs. 3%).

In the CITYSCAPE clinical trial, grade ≥3 TRAEs occurred in 19.1% of patients treated with atezolizumab monotherapy and in 14.9% of patients receiving atezolizumab in combination with tiragolumab [11]. AEs leading to treatment withdrawal occurred in 10.3% of patients from the former group and 7.5% of patients from the latter group [11].

In conclusion, the development of certain equilibrium between the effectiveness of combination therapy and its side effects should be considered. In most cases, when the side effects of combined therapy are detected at an early stage and are not very severe, it is possible to protect the patient properly against their consequences. It can be speculated that this should bring clinicians closer to the use of combination therapy in the clinic.

The effectiveness of combination therapy with nivolumab and ipilimumab is explained by the presence of interactions of these antibodies on different immunological checkpoint molecules [6][18][19][20][21]. This is related to the fact that the PD-L1 molecule is present on tumour cells (in primary tumours and metastases), on antigen-presenting cells infiltrating the tumour and occurring in lymph nodes (also normal, which limits the development of uncontrolled inflammatory reaction), and on most normal cells (limitation of autoimmune reaction) [6][8][18][20]. According to these considerations, the synergistic effect of nivolumab and ipilimumab consists of enhancement of

the activation of T helper and cytotoxic lymphocytes by blocking one of the most potent signals inhibiting these cells (PD-1 and PD-L1 interaction) and restoring the most important, besides antigen presentation, costimulatory signal (CD28-CD80 and CD86 connections) [8][22][23][24]. Moreover, the use of ipilimumab further reduces the immunosuppressive effect of other cells of the immune system [8][25][22].

In the peripheral blood of patients treated with the combination therapy, compared to nivolumab or ipilimumab monotherapy, the percentage of T cytotoxic lymphocytes is significantly increased [26][27][28]. In addition, low expression of other negative immune checkpoints, most notably TIGIT and lymphocyte-activation gene 3 (LAG3), is observed on lymphocytes in patients responding to such treatment [26][27][28][29]. A/B, Ki-67, IL-8, and HLA-DR (Human Leukocyte Antigen—DR isotype), which indicates cytolytic and proliferative activity of T cytotoxic lymphocytes and their ability to infiltrate tumour tissue. In turn, increased expression of genes related to the capability of T lymphocytes of proliferation and production of specific cytokines (genes for Ki-67 and ICOS) is detected in patients receiving ipilimumab [26][27][28][29].

In a mouse model, tumour-infiltrating T cytotoxic lymphocytes have been divided according to their immunophenotype into 4 groups: (1) T lymphocytes with a functionally depleted cell phenotype (PD-1high, LAG3++, TIM3++), (2) terminally differentiated T lymphocytes with an activated phenotype (PD-1 However, the type of therapy has no effect on the percentages of other T cytotoxic lymphocyte subpopulations in the peripheral blood. +, CD44+, CXCR3−), and actively migrating T lymphocytes that resist apoptosis (PD-1−, CD62L+, Bcl2++) Combination therapy, compared to nivolumab or ipilimumab monotherapy, results in significantly increased infiltration of Th1 effector lymphocytes.

As noted above, patients without response to nivolumab and ipilimumab combination therapy had a significantly higher percentage of T lymphocytes with expression of these molecules. A phase I trial in which tiragolumab (anti-TIGIT antibody) was used along with atezolizumab in patients with advanced NSCLC provided particularly interesting results [30][31][32]. These encouraging results contributed to the initiation of phase II trial—CITYSCAPE and phase III trial—SKYSCRAPER-01, which used combination therapy with atezolizumab and tiragolumab compared to therapy with atezolizumab alone in advanced NSCLC patients with PD-L1 expression on tumour cells [11][33]. The CITYSCAPE trial demonstrated response in 31.3% of patients treated with the combination therapy and in 16.2% of patients receiving atezolizumab alone.

On the other hand, there are ongoing early clinical trials in which agonistic antibodies that bind to costimulatory molecules on lymphocytes have been combined with antagonistic antibodies directed against negative checkpoints (usually anti-PD-1, anti-PD-L1, or anti-CTLA-4) [34][35]. (glucocorticoid-induced TNFR-related) molecules increases lymphocyte proliferation and positively stimulates the development of immune response [36][37][38][39]. However, the use of agonist antibodies that bind to these molecules often causes serious side effects. Nevertheless, promising results have been obtained in cancer patients using a combination of classical ICIs with antibodies stimulating CD27 and CD137 activity [40].

The CD27 activation is a potent costimulatory factor in the first stages of immune response when it promotes T cell survival and memory T cell formation [41][42][43]. Chronic stimulation of CD27 by CD70 in chronic inflammation suppresses the immune response and, in the case of tumour cells expressing CD70, leads to differentiation of T lymphocytes into Treg cells [44][45]. A phase I/II clinical trial consisted in the use of varlilumab, i.e., an agonistic antibody that binds to CD27, in combination with nivolumab in patients with solid tumours [46][47]. Response to the treatment was achieved in 49% of patients, although most of them did not have PD-L1 expression on tumour cells.

3. Use of Non-Specific Immune System Stimulation and Tumour Microenvironment Modification in Immune Combination Therapies

Non-specific immunotherapy can also be associated with immune checkpoint inhibitors. Non-specific stimulation of the cytotoxic response against tumour cells can be achieved by administration of proinflammatory cytokines or by inhibition of the immunosuppressive cytokine function [48][49]. In the first case, clinical trials have been undertaken to assess combination therapy of cancer patients with anti-PD-1 and anti-PD-L1 antibodies in combination with modified cytokines IL-2 and Pegylated IL-2 with attached polyethylene glycol chains (bempegaldesleukin) has a longer half-life in the body than recombinant IL-2 (aldesleukin)

Clinical studies on the use of recombinant IL-15 have also been undertaken. However, this molecule was quickly replaced by an IL-15 superagonist (ALT-803), which consists of a modified IL-15 molecule with an introduced N72D mutation, a modified receptor for IL-15 (IL-15R), and an Fc fragment of IgG1 class antibody linking everything [50][51][52][53]. The IL-15 molecule is supposed to bind to IL-2Rβγ in order to stimulate cytotoxic T lymphocytes and NK cells. The modified IL-15R ensures specific binding of ALT-803 to IL-2Rβγ, rather than to IL-2Rαβγ, which is found on Treg cells, while the Fc fragment of the antibody prolongs the half-life of the complex and attracts NK cells [50][51][52][53][54].

Bempegaldesleukin and ALT-803 have been used in combination with nivolumab and atezolizumab in patients with various types of cancer (including hematologic) in phase IL-10 or TGF-β function to classical immunotherapy increased the risk of adverse effects in the form of autoimmune reactions [55][56][57][58]. This drug may have great potential in the treatment of cancer patients in combination with other immunotherapies (e.g., anti-CTLA-4), in first-line monotherapy, and in combination with chemotherapy. It has selective effects in PD-L1 positive tumours and has fewer side effects than other anti-TGF-β agents [58].

An unfavourable tumour microenvironment results in exclusion of immune response outside the tumour. Adenosine and ATP are present at exceptionally low concentrations in extracellular fluids. However, inflammation, ischemia, or the cancer process can lead to the release of ATP through transport channels in cell membranes, active exocytosis, and directly from damaged cells [59][60][61]. Therefore, it is not surprising that molecules that block adenosine binding to the A2a receptor and molecules that inhibit the activity of the CD39 and CD73 enzymes have been developed and used in combination with anti-PD-1 or anti-CTLA-4 antibodies in early phase clinical trials in cancer patients [59][60][61].

Another substance that causes elimination of tumour cells from the tumour area is indoleamine 2,3-dioxygenase (IDO) [62][63][64][65]. The production of IDO by tumour cells reduces tryptophan levels in the tumour. Studies on the possibility of combining IDO inhibitors (e.g., epacadostat) with classical ICIs in NSCLC and melanoma patients have been conducted for several years. However, phase III trials failed to demonstrate the effectiveness of such therapy, which resulted in the lack of registration of epacadostat in combination with pembrolizumab for the treatment of melanoma and NSCLC patients [62][63].

4. Conclusions

Standard anti-cancer therapies, such as radiotherapy or chemotherapy, destabilize tumour cell function, contribute to the release of tumour antigens and the formation of neoantigens, and affect the production of cytokines, chemokines, and other substances that stimulate immune cell activity. As a result, tumours with low immunogenicity (“cold”) could be transformed into tumours with high immunogenicity (“hot,” “inflammatory”), abundant with infiltrates of activated specific lymphocytes [16][17]. This breaks down the mechanism by which tumour cells escape from immune surveillance. The addition of immunotherapy targeting immune checkpoints to chemotherapy or chemoradiotherapy further enhances the antitumor effects of cytotoxic T lymphocytes.

On the other hand, combining two different immunotherapy methods in cancer patients may be as effective as chemoimmunotherapy or chemoradiotherapy in cancer therapy. The combination of two immunotherapy methods is based on the idea of stimulating or inhibiting different immune cells at different levels of their activity with two different immune point activators or inhibitors, or using conventional ICIs in combination with non-specific immunostimulatory agents or agents that modify the tumour microenvironment. However, patients should be very well suited to this type of treatment. At present, there are no conclusively proven predictors for combination therapies, but the selection of patients should be based on clinical factors, such as the performance status of the patients, the presence of comorbidities, and the availability to a multidisciplinary cancer centre, which is extremely important for the proper management of patients.

Attempts are underway to combine classical immunotherapy targeting immune checkpoints with treatment using modified oncolytic viruses. Already, the median survival of patients with advanced non-small-cell lung cancer has increased significantly. The development of modern personalized treatments, including immunotherapies, enables many patients to act in good functional status for 3 years and beyond. In the near future, it is expected that many patients will live with cancer just as patients with cardiovascular or infectious diseases (e.g., AIDS and hepatitis C) are currently living in near-complete comfort.

In conclusion, combination immunotherapies will be used in cancer patients, not only those with lung cancer. Therefore, is seems extremely important to understand the mechanisms of action of combined immunotherapy, firstly to understand how these therapies work in the patient’s body and, secondly, to be able to quickly recognize the side effects and properly secure the patients.

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