

Possibilities of applying a combination of targeted molecular therapies and immunotherapy in NSCLC patients

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Non-small cell lung cancer (NSCLC) advanced or metastatic with driver mutations (*EGFR*, *ALK*, *ROS1*) is treated with tyrosine kinase inhibitors (TKIs), respectively anti-*EGFR*, anti-*ALK* or anti-*ROS1*. Immunotherapy with checkpoint inhibitors (anti-PD-1 or anti-PD-L1) alone or in combination with TKIs was considered as a treatment option in several studies, but results are not promising, furthermore the toxicity profile of such a combination is potentially unacceptable. The initial findings suggest that combination therapy has failed to demonstrate clinically meaningful efficacy and there are no strong signals of its future development.

Key words: immunotherapy, lung cancer, targeted therapy, *EGFR*, *ALK*

Rationale for combination of immunotherapy and targeted therapy

Non-small cell lung cancer (NSCLC) represents 85% of diagnosed lung cancer cases. Approximately 50% of patients are diagnosed at stage IV of the disease, and their five-year survival rate is less than 10% [1].

The introduction of immunotherapy with the application of immune checkpoint inhibitors (ICIs) which target programmed death-1 receptor (PD-1), found in cytotoxic T-lymphocytes, or its ligand, PD-L1 (programmed death-1 receptor ligand 1), found, among other things, in cancer cells, has significantly changed the treatment of advanced lung cancer.

Modern methods of immunotherapy focus on the boosting of antitumor T-cell response and the bolstering of cell immunity with the ultimate destruction of the tumor. The impact of PD-1 and PD-L1 leads to the suppression of antitumor T-cell activity. The idea of using antibodies against immune checkpoint inhibitors is based on the blocking of one of these molecules, which restores cytotoxic T-cell activity [2–5].

The phenomenon of the immune checkpoint blockade (ICB) was the point of departure for the development of antibodies which target cytotoxic T-cell antigen 4 (CTLA-4) (ipilimumab and tremelimumab). Similar development was observed as regards monoclonal antibody drugs and anti-PD-L1 antibodies, which respectively block PD-1, found in T-cells (nivolumab, pembrolizumab), and PD-L1, found on both the surface of cancer cells and the immune system cells penetrating cancer tissue (durvalumab, atezolizumab, avelumab) [2].

CheckMate 057 and KEYNOTE-010 studies demonstrated a statistically significant improvement in the overall survival in NSCLC patients treated with nivolumab or pembrolizumab in comparison with patients receiving standard 2nd-line docetaxel-based chemotherapy. Those studies proved, however, that *EGFR*-mutant patients did not experience a greater benefit from using immunotherapy compared with chemotherapy. In CheckMate 057, 82 patients (14% of all) were *EGFR*-positive and 21 (4%) were *ALK*-positive. Subgroup analyses of OS revealed that patients with the *EGFR* mutation, having received or receiving an additional line of TKI, did not benefit

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from nivolumab compared with docetaxel (hazard ratio [HR] 1.18, 95% confidence interval [CI]: 0.69–2.00). In KEYNOTE-010, 86 patients (8.3%) were had the *EGFR*-mutant and 6 (0.6%) were *ALK*-positive. Patients with the *EGFR* mutation did not have prolonged OS in response to pembrolizumab compared to docetaxel. No data on OS were reported for *ALK*-positive patients. In both trials (HR 0.88, 95% CI: 0.45–1.70) [4, 5].

Unfortunately, many NSCLC patients do not benefit from immunotherapy due to their primary resistance whilst others experience disease recurrence after the initial response (secondary resistance). Adaptive resistance can also be observed when the immune system has identified cancer, but it can adapt to the immune attack and, consequently, resist it. The incidence of resistance to immunotherapy has led to the development of a new concept of combination therapy, which utilizes immunotherapy and chemotherapy, radiotherapy or targeted molecular therapies. First-line chemoimmunotherapy became the standard of care in the treatment of NSCLC. Chemotherapy increases the effectiveness of immunotherapy through the increased level of tumor antigens released, the induction of inflammation within the tumor as well as the provoked expression of various molecules found on the surface of tumor cells (e.g. calreticulin). What also became standard practice was the combination of chemotherapy and immunotherapy in the treatment of locally advanced NSCLC patients. The greatest controversy, however, was aroused by the idea of combining immunotherapy and targeted molecular therapies [6].

Undoubtedly, the dawn of targeted molecular therapies has radically changed the prognosis for NSCLC patients. Targeted molecular therapies inhibit the growth and progression of tumors by means of blocking both abnormal proteins and signaling pathways of cancer cells, which are vital to cell survival. During the last decade, considerable progress has been made in the field of identification of driver mutations, and, consequently, of drugs which can delay tumor progression, thus considerably improving the survival of patients with such mutations [7]. Three generations of epidermal growth factor receptor (*EGFR*), tyrosine kinase inhibitors (TKIs) as well as three generations of anaplastic lymphoma kinase (*ALK*) inhibitors have been developed. *ROS1*, *BRAF*, *NTRK* and *MET* kinase inhibitors have also become part of the standard treatment of NSCLC. However, the percentage of mutation-positive or gene-rearrangement-positive patients remains relatively low. For instance, the presence of *EGFR*-mutant Caucasian NSCLC patients ranges from 10 to 16% [8, 9].

Moreover, due to the emergence of tumor cell clones resistant to targeted molecular drugs, the response to this kind of therapy can be short-lived. Even therapeutic strategies developed for patients with secondary mutations, such as *EGFR* T790M, which use the latest generation of inhibitors, do not produce a durable remission. It results from the fact that for every drug, there is a different mechanism of targeted mole-

cular therapy resistance, such as secondary mutations in genes encoding cell surface receptors, gene fusions or the activation of alternative signaling pathways in tumor cells. In case all options of targeted molecular therapy have been exhausted, patients will require standard-of-care chemotherapy [10].

This is why, from the clinical standpoint, it would be worth analyzing a combination of targeted molecular therapy and immunotherapy, aiming to achieve a durable remission. It is believed that genetic alterations in specific driver genes activate the proliferation of tumor cells. It has also been demonstrated that the activation of some oncogene pathways impacts the way tumors are detected by the immune system, especially by cytotoxic T-cells. On the other hand, however, “driver” mutations usually tend to be isolated genetic alterations. It means that such tumor cells have a low count of neoantigens, encoded by mutant genes, and, as a result, they are not recognized by the immune system. That explains the reduced efficiency of immunotherapy in the treatment of non-smoking NSCLC patients, in whose case only isolated genetic alterations develop, such as *EGFR* mutations or *ALK* rearrangements. In the case of smoking patients, however, numerous genetic alterations concur and numerous neoantigens are to be found. This is why, in clinical trials, a high tumor mutation burden (TMB) is considered a positive predictive factor for immunotherapy [11].

There are views, however, that a combination of TKIs and immunotherapy in treatment-naive patients may be well-founded. The results of preclinical and clinical studies demonstrated the immunomodulatory effect of TKI therapy. The studies demonstrated that gefitinib and erlotinib promoted immune response by means of enhancing the cytotoxicity of NK cells [12].

A study by Sheng et al., on the other hand, demonstrated a significant increase in the number of NK cells as well as in the level of IFN- γ , and a decrease of IL-6 in patients' peripheral blood after 4 months of gefitinib treatment. Moreover, tumor samples collected after gefitinib treatment demonstrated a downregulation of PD-L1 expression on tumor cells following the use of this drug [13].

The level of PD-L1 expression is directly modified by *EGFR*, *ALK* and other cell receptors as well as by exposure to TKIs, which have an effect on the expression level, the activity of receptor tyrosine kinases and the following signaling cascades. The studies demonstrated that there was a much increased PD-L1 expression level in NSCLC cell lines positive for the *EGFR* mutation and *EML4-ALK* fusion gene [14–16]. There are conflicting reports regarding the effect of *EGFR*-TKIs on PD-L1 expression in *EGFR*-mutant NSCLC cell lines. According to certain reports, there is a downregulation of PD-L1 expression on tumor cells as a result of tumor cells being exposed to erlotinib or gefitinib. According to other authors, a completely reverse phenomenon takes place. To date, elevated PD-L1 expression on tumor cells has been the only recognized predictive factor for immunotherapy. The identification of PD-L1 expression

in over 50% of tumor cells allows patients to be qualified for first-line pembrolizumab therapy [13, 17].

First-line treatment of NSCLC patients

The number of clinical trials which evaluate the efficacy of combination therapy with the application of targeted molecular therapy and immunotherapy is still limited. Unfortunately, the conducted experiments to date have indicated that the benefits resulting from the use of immunotherapy in the treatment of *EGFR*-mutant or *ALK*-positive NSCLC patients are dubious [18]. In fact, clinical trials which employed immunotherapy did not demonstrate any benefit from the use of anti-PD-1 or anti-PD-L1 antibodies in the treatment of *EGFR*-mutant NSCLC patients [19, 20]. The retrospective analysis demonstrated an objective response to immunotherapy in 3.6% of the *EGFR* mutation-positive or *ALK* rearrangement-positive patients in comparison with 23.3% of the patients without these genetic alterations or individuals of an unknown profile as regards the genes under discussion [21].

There are still numerous ongoing clinical trials which investigate the efficacy of the combined immunotherapy and *EGFR* or *ALK* inhibitors in the treatment of NSCLC patients. The results of the clinical studies which have been published draw attention to the fact that there was a high percentage of adverse events as well as a frequent lack of clinical benefit from the combined therapy.

The phase I-II KEYNOTE-021 study focused on the evaluation of the efficacy of erlotinib or gefitinib in combination with pembrolizumab as first-line therapy in the treatment of *EGFR*-mutant NSCLC patients. In the group of participants enrolled to receive gefitinib, due to the significant toxicity resulting in liver damage (adverse events of the 3rd and 4th grade), the treatment was discontinued in 4 out of 7 patients. In the group of participants enrolled to receive erlotinib, however, the safety profile of the drug combination was acceptable. The patients did not require having their doses reduced and the adverse events were similar to those found in patients who received each drug as monotherapy. These findings corroborated the good safety profile of these combined drugs. The most frequent adverse events related to treatment with pembrolizumab and erlotinib were a rash (50% of the participants), dermatitis acneiform, diarrhea, hypothyroidism, and pruritus (33.3% each). The combination of pembrolizumab and erlotinib, however, did not increase the response rate in comparison with the previous trials which employed *EGFR*-TKI monotherapy [22].

The unfavorable safety profile of the application of a combination therapy based on *EGFR*-TKIs and ICIs was also the reason for the termination of a large randomized study (CAURAL) which was terminated early because of the high toxicity of the osimertinib plus durvalumab combination demonstrated in a parallel phase Ib trial (TATTON) [23]. That study CAURAL aimed to combine a third-generation *EGFR*-TKI, osimertinib, with a PD-L1 inhibitor, durvalumab, in treatment-naive *EGFR*-

-mutant patients. Aspartate transaminase concentrations of the 3rd and 4th degree were observed in blood plasma in 65% of the patients, which led to the termination of the study. The results in terms of the treatment overall response rate were not different from the previously known results of phase III studies employing osimertinib as monotherapy in treatment-naive *EGFR*-mutant NSCLC patients [24].

In another clinical study, atezolizumab (NCT02013219) was applied in combination with erlotinib in the treatment of *EGFR*-mutant NSCLC patients. 75% of the patients responded to the treatment and the safety profile proved satisfactory [25]. In a phase I trial (NCT02088112), the efficacy of durvalumab in combination with gefitinib was investigated. The participants of the study were *EGFR*-TKI-naive *EGFR*-mutant NSCLC patients. The first half of the patients received both durvalumab and gefitinib (group 1), while the other half were treated only with gefitinib for 28 days before they started the combination therapy (group 2). The employed combination therapy did not increase the response rate in comparison with gefitinib monotherapy. The objective response rate accounted for 77.8% and 80% of the patients in group 1 and group 2 respectively. The combination therapy induced serious adverse events in 55% of the patients [26].

In a phase I trial (GEFTREM), the efficacy of tremelimumab immunotherapy in combination with gefitinib was investigated in stage IV *EGFR*-mutant NSCLC patients. Stable disease was obtained in 67% of the evaluated patients, and the safety profile was in accord with the previously observed adverse events for each specific drug [27].

In the CheckMate 370 trial, a combination of nivolumab and crizotinib was applied to treat *ALK* translocation-positive NSCLC patients. 38% of them experienced serious adverse events (most frequently hepatotoxicity), which led to the discontinuation of the combination therapy, and which may have contributed to the death of two participants [28].

There are not any available results of clinical trials which evaluated the efficacy of the combined immunotherapy and targeted therapies aimed at areas other than *EGFR* or *ALK* in NSCLC patients. *ROS1* and *NTRK* rearrangements or *BRAF* and *MET* mutations occur very seldom in NSCLC patients while inhibitors of those proteins have been developed only recently. That is why there is not any data regarding the efficacy and safety of the combined therapy in the treatment of such patients.

Immunotherapy in resistance to targeted therapy

An unusually attractive concept is the idea of applying a combined therapy in the treatment of patients who progressed during the course of a targeted molecular therapy. As therapeutic possibilities to employ new-generation *EGFR*-TKIs are exhausted, new attempts have been made to overcome *EGFR*-TKI resistance by means of combining targeted molecular therapy with immunotherapy.

Phase Ib TATTON trial (NCT02143466), in which various treatment combinations were employed, has, to date, been the most advanced clinical study investigating the possibility of combining targeted molecular therapy with immunotherapy in order to overcome EGFR-TKI resistance. In that trial, *EGFR* TKI-pretreated *EGFR*-mutant advanced NSCLC patients were qualified for a combination therapy with osimertinib and one of the three following drugs: selumetinib (MEK1 and MEK2 inhibitor), savolitinib (MET inhibitor) or durvalumab (anti-PD-L1 antibody). The most frequent adverse events of any grade, which occurred in no less than 20% of all the participants were: diarrhea (75% of the cases), a rash (58% of the cases) and nausea (47% of the cases), developed by patients receiving osimertinib in combination with selumetinib; nausea (67% of the cases), a rash (56% of the cases) and vomiting (50% of the cases), developed by patients receiving osimertinib in combination with savolitinib; a rash (48% of the cases) and vomiting (43% of the cases) and diarrhea (39% of the cases) developed by patients receiving osimertinib in combination with durvalumab. Furthermore, 38% of the patients treated with osimertinib in combination with durvalumab developed interstitial lung disease, which was the reason for the discontinuation of the treatment and the termination of the study. The objective response rate accounted for 42% in the group of patients treated with osimertinib in combination with selumetinib, 44% in the group of patients treated with osimertinib in combination with savolitinib, and 43% in the group of patients treated with osimertinib in combination with durvalumab.

Even though the findings of the TATTON study demonstrated a high frequency of adverse events, which resulted

from the combination of targeted molecular therapies and immunotherapy, other studies demonstrated a much better safety profile of this type of treatment. A good example is the CheckMate 012 study, where 21 *EGFR*-mutant NSCLC patients (20 erlotinib-pretreated and 1 *EGFR*-TKI-naive) received nivolumab in combination with erlotinib in order to overcome resistance to the latter drug. The objective response rate accounted for 19%. The findings demonstrated a 24-week progression-free survival rate of 51%, and a 1-year overall survival rate of 73%. Serious adverse events (diarrhea, nephritis, an increase in liver function enzymes) occurred in 21% of the patients. The findings suggest that a combination of erlotinib and nivolumab has an acceptable safety profile and can ensure certain clinical benefits for *EGFR*-mutant NSCLC patients who developed resistance to previous *EGFR*-TKI treatment [29].

Conclusions

Currently, there is a number completed and ongoing clinical trials aiming to evaluate the combination of new-generation *EGFR* and *ALK* inhibitors and immunotherapy in selected populations of TKI-naive or *EGFR*- or *ALK*-pretreated NSCLC patients who progressed following the applied treatment (tab. I). The initial findings suggest that combination therapy has failed to demonstrate clinically meaningful efficacy and there are no strong signals of its future development; furthermore the safety profile is not always acceptable. The lack of long-term observation does not allow one to draw any definitive conclusions [30].

The ongoing attempts to combine targeted molecular therapies with immunotherapy may evolve into new thera-

Table I. Completed and active clinical trials of immune checkpoints inhibitors in combination with *EGFR/ALK* TKIs in advanced or metastatic NSCLC

| Clinical trial | Phase | ICI | TKI | Setting |
|------------------|-------|----------------------|-------------------------|---|
| KEYNOTE-021 | I | pembrolizumab | erlotinib/gefitinib | first line <i>EGFR</i> + advNSCLC |
| CAURAL | III | durvalumab | osimertinib | first line <i>EGFR</i> + advNSCLC |
| NCT02013219 | Ib | atezolizumab | erlotinib | first line <i>EGFR</i> + advNSCLC |
| NCT02088112 | I | durvalumab | gefitinib | first line <i>EGFR</i> + advNSCLC |
| GEFTREM | I | tremelimumab | gefitinib | first line <i>EGFR</i> + advNSCLC |
| CheckMate 370 | I | nivolumab | crizotinib | first line <i>ALK</i> + advNSCLC |
| TATTON | I | durvalumab | osimertinib | TKI-pretreated |
| CheckMate 012 | I | nivolumab | erlotinib | 20 erlotinib-pretreated patients, 1 TKI-naive |
| NCT01998126 | I | nivolumab/ipilimumab | erlotinib or crizotinib | first line <i>EGFR</i> + or <i>ALK</i> + advNSCLC |
| NCT02393625 | I | nivolumab | ceritinib | first or second line <i>ALK</i> + advNSCLC |
| LUX LUNG IO | II | pembrolizumab | afatinib | pretreated <i>EGFR</i> + advNSCLC |
| NCT02511184 | I | pembrolizumab | crizotinib | first line <i>ALK</i> + advNSCLC |
| Javelin Lung 101 | Ib/II | avelumab | crizotinib/lorlatinib | first or second line <i>ALK</i> + advNSCLC |
| NCT02898116 | I | durvalumab | ensartinib | first line <i>EGFR</i> + advNSCLC |

peutic strategies in the treatment of NSCLC patients. However, the application of combined targeted molecular therapy with immunotherapy in treatment-naïve patients is probably unfounded. Undoubtedly, the evaluation of the efficacy and safety of combined EGFR and ALK treatment in conjunction with immunotherapy still requires further research [32]. Perhaps the direction of further research should be changed to tumor immunophenotype profiling, and the research itself should focus on methods of modulating the immune response leading to modification of the tumor microenvironment. It appears that targeted molecular therapy can change the tumor immunophenotype from “cold” (no immune infiltration of tumor) to “hot” (significantly more immunogenic and infiltrated by the immune system). Currently, following the failure of EGFR TKI treatment of EGFR-mutant NSCLC patients, ongoing clinical trials combine immunotherapy (nivolumab) with chemotherapy and immunomodulating therapy (plinabulin-microtubule polymerization inhibitor) [22]. This creates new possibilities for the conduct of further research and sets a new course in the treatment of NSCLC.

A detailed profile of interactions between cells of the immune system and cancer cells that contain various genetic abnormalities, identification of reliable predictors in the application of immunotherapy, and expertise in the mechanisms of acquired tumor resistance to immunotherapy and targeted molecular therapies are undoubtedly research directions that will contribute to the progress in treatment of patients with NSCLC. The increasing progress of science in terms of mechanisms of targeted molecular therapy and immunotherapy will facilitate the development of new drugs and new effective strategies in the treatment of NSCLC patients.

Conflict of interest: none declared

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