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Targeting the PD-1 signaling pathway in cancer immunotherapy

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Abstract: Blocking the PD-1 signaling pathway is one of the intensively investigated technique of cancer immunotherapy. This approach consists of stimulation the patient's immune system to successfully recognize and destroy cancer cells. Anti-PD-1/PD-L1 therapy is one of the option for the therapy of relapsed and refractory malignancies. In many currently underway clinical trials various regimens of anti-PD-1 and anti-PD-L1 monoclonal antibodies are evaluated. It was proved that their application is effective in numerous cancer types treatment and some of them were already approved for clinical practice. This review summarize the latest, selected clinical trials of anti-PD-1 and anti-PD-L1 monoclonal antibodies in solid tumors.
Keywords: PD-1, PD-L1, monoclonal antibodies, cancer immunotherapy, clinical trials

1. Background

Programmed death 1 (PD-1) protein is an immunoreceptor which undergoes inducible expression on activated T and B cells, monocytes and some of dendritic cells. PD-1 belongs to CD28 molecules family transmitting the costimulatory signal during the lymphocyte activation. PD-1 is a negative immunoreceptor, it acts regulatory by transferring the braking signal into cell interior. The ligands for PD-1 are transmembrane glycoproteins PD-L1 and PD-L2. Their expression is varied, PD-L1 is found on B and T lymphocytes, monocytes and dendritic cells, its amount increases with the activation of these cells. PD-L1 molecule is also present on many types of non-hematopoetic cells, including vascular endothelial cells, pancreatic islets, neurons, astrocytes or trophoblast cells of the placenta. The PD-L2 expression is limited to dendritic cells and monocytes, however PD-L2 mRNA is also synthesized in human heart, liver, placenta and pancreatic cells. The interaction of PD-1 with its ligands is an example of the escape of tumor cells from immune surveillance by strengthening the negative signal transmitted to tumor infiltrating lymphocytes, which impairs their function. The PD-L1 widespread expression indicates the importance of this molecule in the modulation of the immune response. The presence of PD-L1 and PD-L2 was found also in many types of malignancies. High expression of these factors was correlated with more aggressive course of the disease and unfavorable prognosis for patients. Currently, many clinical trials underway to verify the effectiveness of anti-PD-1 and anti-PD-L1 antibodies and fusion proteins in cancers therapy. Conducted research allow to suppose that therapeutic use of PD-1/PD-L pathway inhibitors will increase in the future [1÷4]. The aim of this review is to summarize selected, latest clinical trials of solid tumors, that evaluate the efficiacy of anti-PD-1 and anti-PD-L1 antibodies in cancers therapy.

2. PD-1/PD-L1 blockade in clinical application

Many clinical studies have demonstrated that PD-L1 overexpression is connected with a poor prognosis in several tumor types, including esophageal cancer, gastric cancer, hepatocellular carcinoma, pancreatic cancer, renal-cell carcinoma, bladder cancer and ovarian cancer. These studies suggested that inhibition of PD-1 signaling may improve clinical outcomes of patients with these neoplasms [5].

2.1. Anti-PD-1 mAbs

The PD-1 blocking approach has unique mechanism compared to conventional therapies. In general, standard chemotherapies target certain molecule in tumor cells. Malignant cells can avoid such therapy through mutations of target molecules, which lead to rapid regression. Differently, a PD-1 blockade induces a response for a longer period because it stimulates an anti-tumor immune system to target mutated proteins, and therefore is applied in various types of cancers. What is important, PD-1 blocking exhibits significantly lower rate of high-grade toxicities than other immunotherapies or conventional therapies due to the fact that the anti-tumor immunity preferentially recognizes and combats tumor-derived antigens, not self-antigens [5÷6].

At least 500 clinical trials of PD-1 signal inhibitors were carried out so far, assessing 9 types of antibodies from 8 pharmaceutical companies in at least 20 different types of solid and hematological malignancies [5, 7].

2.1.1. Nivolumab

Nivolumab is the first PD-1 blocking antibody approved for clinical practice worldwide [8]. It is a fully humanized monoclonal antibody (mAb), directed against PD-1. Nivolumab is also known as MDX-1106, ONO4538 or BMS-936558. It was first produced using genetically modified mice possessing human immunoglobulins encoding loci. Nivolumab is IgG4 isotype, what minimizes antibody-dependent cell-mediated cytotoxicity (ADCC) and complement activity. This antibody contains a serine-to-proline substitution at 228 position, which significantly reduces ADCC effect against activated T cells. Clinical trials with nivolumab have started in 2006 in the United States. The phase 1 large study of nivolumab (NCT00730639) reported cumulative response rates of 28% for melanoma, 27% for renal carcinoma and 18% for non-small-cell lung cancer (NSCLC). Grade 3 or 4 drug-related adverse events were reported in 14% of patients. Importantly, nivolumab has exhibited durable clinical effectiveness as a single agent and significantly fewer side effects than ipilimumab – mAb against another immune checkpoint CTLA-4. A clinical trial of anti-PD-L1 mAb demonstrated relatively low response rates in comparison with anti-PD-1 mAb (NCT00729664) [5, 9÷10].

In a phase 1 trial of nivolumab (NCT00730639) in 17 patients with kidney cancer, objective responses were seen in 24% and 31% of patients at 1 mg/kg and 10 mg/kg dose, respectively. Stabilization of the disease was seen in other 27% of patients. Five patients had a durable response remaining more than 1 year [9, 11].

Nivolumab was also assessed in a phase 1, dose-escalation trial in 17 patients with castrate-resistant prostate cancer (CRPC) (NCT00730639). However, no objective responses were seen; thus, nivolumab was not examined in phase 2 or 3 studies in prostate cancer [9, 11].

In ongoing phase 1/2 study nivolumab is being evaluated in advanced hepatocellular carcinoma (HCC) patients who can not be treated with sorafenib (NCT01658878). It was observed that responses were independent of the PD-L1 expression on tumor cells. Based on promising preliminary data from this trial, a phase 3 study is currently ongoing com-paring nivolumab versus sorafenib in patients with advanced HCC (NCT02576509) [12].

In the first-in-human phase 1 study of nivolumab (NCT00441337) in treatment-refractory solid cancers, only one patient in the trial group reached a durable CR. It was demonstrated, that this was a patient with microsatellite instability and high T cell infiltrated colorectal cancer (MSI-Hi CRC). The rest of 19 CRC patients did not exhibit any tumor response, it was checked that all of them had microsatellite-stable (MSS) disease [12÷13].

In randomized, phase 3 study, nivolumab was compared to everolimus (immuno-suppressant) in previously treated patients with advanced renal cell carcinoma (NCT01668784). The median overall survival (OS) was 25 months with nivolumab in comparison with 19.6 with everolimus. The median progression free survival (PFS) was similar and amounted to 4.6 months with nivolumab and 4.4 months with everolimus. The overall response rate (ORR) was statistically higher with nivolumab and reached 25% versus 5% with everolimus. Interestingly, the use of nivolumab did not improve PFS, although ORR and OS were significantly superior with nivolumab in comparison with everolimus [11, 14].

In a phase 3 study, which compared nivolumab to docetaxel (the plant alkaloid chemotherapeutic) in patients with advanced squamous-cell NSCLC (NCT01642004), the response rate (RR) with nivolumab was 20% versus 9% with docetaxel. The ORR after 1 year was 42% with nivolumab versus 24% with docetaxel. The frequency of occurrence grade 3 or 4 treatment-related adverse events was greatly lower in nivo-lumab group (7%) compared to docetaxel group (55%) [5, 15].

In a phase 2 clinical study of nivolumab (UMIN000005714) in 20 patients with platinum-resistant recurrent ovarian cancer, the objective RR was 20%, including two cases of complete response (CR). For all patients the RR was 15% and the durable CR (DCR) was 45%. The median progression-free survival (PFS) and OS were 3.5 and 20 months,

respectively. After completing 1-year nivolumab treatment, two patients who reached CR survived over 2 years without any antitumor treatment. The phase 2 trial currently underways, comparing nivolumab versus standard 2nd-line chemotherapy in ovarian cancer (JapicCTI-153004) [5, 16].

Immunotherapeutic strategy of targeting PD-1/PD-L1 is also investigated in the treatment of pancreatic cancer. In ongoing phase 2 study the combination of pancreatic cancer vaccine GVAX/CRS-207 with or without nivolumab is comparing in previously treated patients with metastatic pancreatic cancer (NCT02243371). An another pilot study is evaluating nivolumab combined with dendritic cell vaccine in patients with metastatic pancreatic cancer. In the cohort of 7 patients treated until data cutoff, 2 of them had PR so far [12, 17].

In ongoing phase 1/2 study, also combination of anti-CTLA-4 and anti-PD-1 mAbs together is being explored (NCT01928394). This trial compares the activity of nivo-lumab alone or together with ipilimumab, in resistant to chemotherapy, advanced solid cancers. The three treatment arms involve nivolumab 3 mg/kg, nivolumab 3 mg/kg with ipilimumab 1 mg/kg, and nivolumab 1 mg/kg with ipilimumab 3 mg/kg. The early results of the gastric cancer cohort revealed an ORR of 14, 10, and 26% in treatment arms, respectively. Nivolumab was assessed in combination with ipilimumab also in another phase 2 study (NCT02060188). In this trial patients with metastatic CRC were enrolled into two cohorts, nivolumab with or without ipilimumab. The preliminary results show that the combination of this two agents is associated with favorable clinical activity, particularly in the MMR-deficient subgroup, where 4-month PFS was 80% and 5-month OS was 100% [12, 18].

The U.S. Food and Drug Administration (FDA) approved nivolumab for patients with unresectable or metastatic melanoma in 2014, metastatic NSCLC in 2015, classical Hodgkin's lymphoma, advanced RCC and for recurrent/metastatic squamos cell carcinoma of the head and neck in 2016. In 2015 FDA approved also combined therapy nivolumab with ipilimumab for unresectable or metastatic melanoma. This combined therapy is now being clinically applied to numerous cancer types, including NSCLC, RCC and ovarian cancer. Many combination therapies with nivolumab and ipilimumab or VEGF tyrosine kinase inhibitors currently underway [5, 8, 11].

2.1.2. Pembrolizumab

Pembrolizumab (MK-3475) is another anti-PD-1 antibody. Pembrolizumab is a highly selective, high-affinity, humanized monoclonal IgG4 antibody that prevents PD-1 binding to its ligands. In several countries pembrolizumab is approved for the treatment of advanced melanoma, NSCLC, and in second-line treatment of head and neck squamous cell carcinoma. Clinical studies have demonstrated encouraging efficacy of pembrolizumab in many advanced cancers, such as gastric and urothelial cancer [8].

One of the first clinical studies of pembrolizumab in PD-L1-positive, previously treated, advanced solid tumors was the multi-cohort, phase 1b trial (NCT01848834). The tumors were classified as PD-L1-positive if this marker was detected on ≥1% of cancer cells or any positive stainig was found in the stroma. In gastric cancer cohort 39 patients were enrolled with PD-L1-positive relapsed or metastatic stomach adenocarcinoma or gastroesophageal junction (GEJ). The ORR was 22% in 36 evaluable patients with advanced gastric cancer. In this trial, no association between response and the level of PD-L1 expression on cancer cells was observed. In another similar phase 1b study pembrolizumab was evaluated as a single agent in PD-L1-positive advanced solid malignancies (NCT02054806). To the esophageal carcinoma cohort 23 patients were enrolled with esophagus squamous cell carcinoma (SqCC) or adenocarcinoma, or GEJ, who had progressed with standard therapies. In this group of heavily pretreated patients the ORR primary endpoint was established at 30%. During data cutoff, four out of seven responses were still continuing and the median duration of response had not been achieved. This promising clinical activity was the basis for phase 2 and 3 trials of anti-PD-L1 regimens in gastric, esophageal and GEJ malignancies. Currently, there is phase 2 multi-cohort study in progress for patients with relapsed or metastatic gastric or GEJ adenocarcinomas (NCT02335411). The three cohorts in this trial comprise pembrolizumab with fluoropyrimidine and cisplatin in previously untreated patients, pembrolizumab as a single

agent in previously treated patients and pembrolizumab monotherapy in treatment-naive patients. Another ongoing phase 2 study (NCT02559687) is assessing pembrolizumab monotherapy in previously treated patients with esophagus advanced adenocarcinoma or SqCC either with GEJ. Many phase 3 trials of anti-PD-L1 therapy in gastric and esophageal cancers are currently ongoing. One of them (NCT02370498) compares pembrolizumab versus paclitaxel in metastatic or unresectable gastric or GEJ adenocarcinoma as a second line treatment. Another study (NCT02494583) is a three-arms study, which compares pembrolizumab monotherapy versus 5-fluorouracil with cisplatin, versus this three agents together in PD-L1-positive advanced gastric or GEJ adenocarcinoma as a first-line treatment. Another large, phase 3 study (NCT02564263) will compare pembrolizumab towards paclitaxel, docetaxel or irinotecan as a single-agent chemotherapy in previously treated advanced SqCC or adenocarcinoma of the esophagus or GEJ patients [12, 19÷21].

In phase 1b trial pembrolizumab was evaluated in patients with metastatic, relapsed urogenital tract cancers (NCT01848834). For this study 33 bladder cancer patients with PD-L1 expression in ≥1% of tumor cells or in stroma were enrolled. The ORR was 24%, with 10% of CR and 14% of partial responses (PR) to pembrolizumab. The 1-year PFS was 19%. Obtained results allow to conclude that pembrolizumab exhibits significant antitumor activity in PD-L1-positive bladder cancer patients. The registra-tion phase 3 trial of pembrolizumab compared to selected regimen (docetaxel, paclitaxel or vinflunine) in advanced or metastatic bladder cancer has finished recently and results are expected (NCT02256436). Several combination therapies with pembrolizumab and cytotoxic agents or targeted therapies as first-line and rescue therapies are presently being verified in clinical studies [11, 22].

Pembrolizumab was also evaluated in single-arm, phase 2 study in patients with CRPC after progression on enzalutamide (an androgen receptor antagonist) (NCT02312557). In 3 out of 10 patients a rapid PSA response was noted and 2 subjects had a partial response. In a retrospective study, PD-L1 expression on testicular germ cell tumors (TGCT) was evaluated. Using immunohistochemistry, PD-L1 expression was noted in 73% of seminomas and in 64% of non-seminomas. In normal testicular tissue no PD-L1 expression was observed at all. In another study it was confirmed that PD-L1 expression in TGCTs was higher than in normal testicular tissue. The highest level of PD-L1 protein was detected in choriocarcinoma, a little less in embryonal carcinoma, teratoma, yolk sac tumor and seminoma. Low-PD-L1 expression was associated with a better PFS. Also OS of patients with low-PD-L1 expression was improved compared to subjects with high-PD-L1 expression. Currently, there is a phase 2 ongoing study, in which the role of pembrolizumab is assessed in patients with relapsed or metastatic, cisplatin resistant germ cell cancer (NCT02499952) [11, 23].

In a phase 2 study the activity of pembrolizumab monotherapy was evaluated in patients with already treated, progressive metastatic cancers, with and without mismatch repair (MMR) deficiency (NCT01876511). The patients were divided into three cohorts: MMR-deficient CRC, MMR-proficient CRC and MMR-deficient non-colorectal cancers. Subjects with MMR-deficient CRCs were found to have favorable responses to pembrolizumab. The ORR and durable CR were 50% and 89% for MMR-deficient CRCs and 0% and 16% for MMR-proficient CRCs, respectively. The median PFS and median OS was not achieved in the MMR-deficient CRC, otherwise than 2.4 months and 6 months in the MMR-proficient CRC cohort, respectively. Based on the obtained results it may be concluded that MMR-deficient CRC patients benefit from anti-PD-L1 treatment. This data was the reason for ongoing phase 2 and 3 studies of pembrolizumab in MMR-deficient advanced CRC (NCT02460198 and NCT02563002) [12, 24].

An ongoing phase 2 study is assessing the effect of combination pembrolizumab with radiotherapy or tumor ablation in MMR-proficient metastatic CRC patients (NCT02437071). Out of 11 subjects in the pembrolizumab plus radiotherapy group, one patient achieved PR in the non-irradiated tumor. No responses were noted in in the eight patients from the pembrolizumab plus ablation group. There is also an ongoing pilot study evaluating the combination of 5-azacitidine (DNA methyltransferases inhibitor) or romidepsin (histone deacetylases inhibitor) or both with

pembrolizumab in patients with MMR-proficient advanced CRC (NCT02512172) [12, 25].

In open-label phase 2 study acalabrutinib (Bruton's tyrosine kinase inhibitor) was assessed in combination with or without pembrolizumab in metastatic pancreatic cancer patients (NCT02362048) and it was noted that this regimen has a favorable clinical activity. In the combination arm, in 23 evaluable patients, 3 of them achieved PR and 5 had the stabilization of the disease [12, 26].

2.2. Anti-PD-L1 mAbs

2.2.1. Atezolizumab

Atezolizumab (MPDL3280A) is an engineered, humanized IgG1 monoclonal anti-PD-L1 antibody which inhibits its interaction with receptors PD-1 and B7-1. Atezolizumab has been approved in USA for the treatment of locally advanced or metastatic urothelial carcinoma and metastatic NSCLC [8].

Atezolizumab was evaluated in phase 1 trial in patients with urothelial bladder cancer. Neoplasms were differentiated based on PD-L1 expression defined as ≥5% of tumor-infiltrating immune cells or tumor cells in immunohistochemistry (IHC) staining. For 67 assessed patients, ORR was 43% in PD-L1-positive group and 11% in PD-L1-negative group. 7% of patients in PD-L1-positive cohort reached a CR including several patients with durable responses. On the basis of these results, atezolizumab was granted by the FDA a breakthrough status in bladder cancer. Long-term results reported that the median OS was 28.9 months and median PFS was 5.6 months. Altogether, atezolizumab was well tolerated and an increased baseline effector T cell to regulatory T cell ratio was connected with better response [11, 27].

In a large, phase 2 study atezolizumab was examined in patients with platinum resistant locally advanced or metastatic urothelial carcinoma (NCT02108652). PD-L1 expression on tumor-infiltrating immune cells (IC) was evaluated by IHC and classified according to staining intensity. ≥5% of staining was stratified as IC2/3, ≥1-4% as IC1 and <1% of staining as IC0. Patients in IC2/3 had a median OS of 11.4 months, in IC1 6.7 months and in IC0 6.5 months. In all patients median PFS was 2.1 months and ORR was 15%. The ORR was significantly higher for all patients in comparison with historical control, where ORR was 10%. Obtained results reveal that responders had an elevated median mutational load compared to non-responders. Differently than in lung cancer, smoking was not connected with higher mutational load and did not foresee response to atezolizumab. Higher response rates were observed in the luminal II subtype of bladder cancer, which is associated with the inherence of activated T cells in cancer site. In view all of this, atezolizumab was approved by the FDA for the use in platinum-resistant advanced or metastatic urothelial carcinoma patients. At present, a large phase 3 trial comparing atezolizumab to chemotherapy in urothelial bladder cancer progressed during or following a platinum-based regimen is underway (NCT02302807) [11, 28].

Atezolizumab was evaluated in phase 1 study in 70 patients with metastatic RCC (NCT01375842). Median OS of patients with clear-cell RCC (62 subjects) was 28.9 months, median PFS was 5.6 months, and ORR was 15%. Response to atezolizumab was associated with decrease in circulating plasma markers and acute phase proteins, the increase of baseline effector T cell to regulatory T cell gene expression ratio was also reported [11, 27].

It was demonstrated that the inhibition of MAPK/ERK kinase (MEK) is associated with enhanced anti-PD-L1 activity. Thus, in phase 1b trial the combination of atezolizumab with cobimetinib – MEK inhibitor, was evaluated in patients with advanced solid cancers (NCT1988896). The early results of 23 CRC patients who have been administered this combination revealed that 4 subjects, including 3 MMR-proficient patients, reached a PR and another 5 had SD. These results contributed to the design of phase 3 study which is analysing cobimetinib plus atezolizumab combination in patients with MMR-proficient advanced CRCs [12, 29].

2.2.2. Durvalumab

Durvalumab (MED14736) is another anti-PD-L1 antibody. It was assessed in a phase 1/2 study in patients with advanced urothelial cancer (NCT01693562). For this trial, 61 patients with advanced or metastatic transitional cell carcinoma of the bladder were enrolled. The ORR was 31% in 42 assessed patients. PD-L1 positivity was established if ≥25% of tumor or immune cells expressed PD-L1. With this qualification of PD-L1 positivity, the ORR was 46% for the PD-L1-positive group and 0% for PD-L1-negative group [11, 30].

In ongoing phase 2 study durvalumab is evaluated in combination with or without tremelimumab (anti-CTLA-4 mAb) in patients with previously treated metastatic pancreatic cancer (NCT02558894) [12].

2.2.3. Avelumab

Avelumab (MSB0010718C) is a fully human anti-PD-L1 monoclonal antibody of IgG1 isotype. It has been approved recently by FDA for the treatment of metastatic Merkel cell carcinoma [8, 31].

Avelumab was evaluated in phase 1b trial in advanced gastric or GEJ malignancies as a first-line maintenance or second-line treatment (NCT01772004). The preliminary ORR was 9%, including 2 CR and 6 PR in the first-line maintenance arm and 10%, including 6 PR in the second-line therapy arm. The disease control rate (DCR) was 57% and 29% and median PFS was 12 and 6 weeks in this two treatment arms, respectively. Because of favorable activity of avelumab, two large phase 3 clinical trials of this mAb in gastric cancer have been started (NCT02625623 and NCT02625610) [12, 32].

3. Conclusions

Immunotherapy has enriched the treatment options accessible for patients with different types of malignancies. The application of PD-1 or PD-L1 inhibitors induced durable responses in many patients, who had limited treatment alternatives before. A number of checkpoint inhibitors trials are currently ongoing, which are aimed at development a new treatment regimens that will provide better outcomes for cancer patients.

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