# IMMUNOTHERAPY FOR MELANOMA – THE NEWEST THERAPEUTIC POSSIBILITIES TARGETING CANCER IMMUNITY CYCLE AND TUMOR MICROENVIRONMENT

## IMMUNOTERAPIA CZERNIAKA – NAJNOWSZE MOŻLIWOŚCI TERAPEUTYCZNE UKIERUNKOWANE NA CYKL IMMUNOLOGICZNY I MIKROŚRODOWISKO NOWOTWORU

Aleksander KIELBIK[1], Piotr WAWRYKA[1], Agnieszka CHWIŁKOWSKA[2]

[1]Faculty of Medicine, Wroclaw Medical University, Wroclaw, Poland
[2]Department of Molecular and Cellular Biology, Wroclaw Medical University, Wroclaw, Poland

*Summary:* Though years of studies for cancer biology and immunology, melanoma, in its late stage, still remains lethal for patients and cause a problem to physicians. Large diversity, rapid grow, and neglection of early signs and symptoms often leads to late diagnosis. Once melanoma has been detected in its early stage, it can be cured by surgical resection, but with further progression to metastatic stage, the therapy become more complex. Searching for the safe systemic therapy resulted in developmnet of cutting edge solutions such as check-point inhibitors, dendritic cells based vaccines and adoptive T-cell transfer. Since melanoma serves as a 'model' tumour for research, most of the newest immune terapeutics found an application in malanomas treatment. Present invastigations explore the mechanisms of tumor resistance to immunotherapy such us, avoiding detection (MHC I downregulation), production of inhibitory microenvironment (IDO, Adenosin, PD-L1 up-regulation) and accumulation of immunosuppressive lymphocytes and myeloid cells. The future task is to determine which of these mechanisms are important and how suppress them. Inhibition of immunosuppressive elements of tumor microenvironment may by unenviably use as an adjuvant to the standard therapy. To summarize, regarding all recent successes, immunotherapy might be the most promising treatment for cancer patients.

*Keywords:* melanoma, immunotherapy, tumor microenvironment, medical oncology

*Streszczenie:* Pomimo lat badań nad biologią i immunologią nowotworu, czerniak, w zaawansowanym stadium, pozostaje śmiertelny dla pacjentów i stanowi duży problem dla lekarzy. Duża róż-

norodność symptomów, jak również lekceważenie pierwszych oznak choroby, często prowadzi do później diagnozy. Jeśli czerniak zostanie wykryty we wczesnym stadium, może zostać wyleczony przez wycięcie chirurgiczne, jednakże wraz z wykształceniem przerzutów, terapia staje się bardziej złożona. Dążenia do opracowania bezpiecznej terapii systemowej zaowocowały odkryciem tzw. inhibitorów punktów kontrolnych (ang. *check-point inhibitors*), szczepionek opartych na komórkach dendrytycznych czy adaptywnego transferu komórek T. Ponieważ czerniak służy jako modelowy nowotwór do badań, większość najnowszych metod immunoterapii znalazło zastosowanie w jego leczeniu. Aktualne studia w obszarze onkoimmunologii zgłębiają mechanizmy odporności nowotworu na immunoterapię poprzez unikanie wykrycia, produkcję hamującego odpowiedź immunologiczną mikrośrodowiska, czy akumulacje regulatorowych limfocytów i komórek mieloidalnych. Metody leczenia celujące w mikrośrodowisko nowotworu, w przyszłości, mogą stać się nieodłącznym elementem standardowej terapii. Uwzględniając najnowsze odkrycia, immunoterapia stanowiłaby najbardziej obiecujące rozwiązanie dla pacjentów onkologicznych.

*Słowa kluczowe:* melanoma, immunoterapia, mikrośrodowisko nowotworu, onkologia medyczna

## INTRODUCTION

Though years of studies for cancer biology and immunology, melanoma in it's late stage still remains lethal for patients and cause a considerable problem to physicians. Melanoma of skin is responsible for 0.6% of deaths caused by tumor disease. With over 300,000 new cases and 60,000 deaths estimated globally, melanoma is one of the most frequently diagnosed cancer in North America, Australia and New Zealand [7].

Once melanoma has been detected in its early stage, it can be cured by surgical resection, but with further progression to metastatic stage it is extremely difficult to treat it. Sustained studies of cell–signaling and mechanisms of immune system have contributed to development of new therapeutic approaches [28].

In order to apply the modern therapeutic strategies, targeting cell signaling pathways and immune system response against melanoma, it is important to understand their mechanisms.

The path to success of immunotherapy was full of obstacles. The first applied immune therapeutics were vaccines. Although their development was a milestone in infectious disease treatment they didn't show similar effect in cancer therapy.

Nowadays the clinical application of immune therapeutics provides satisfactory results, significantly extending the survival of patients. However, still in the most of cases the response to treatment isn't evident [54]. As a consequence of that, the most present researches explore the mechanisms of resistance to immune treatment.

The understanding of complexity of immunology system is fundamental to overcome the immune resistance providing the effective and safe therapy. This review brings into discussion the mechanisms of immune response, the most present possibilities of immunotherapy together with strategies of melanoma cancer cells resistance.

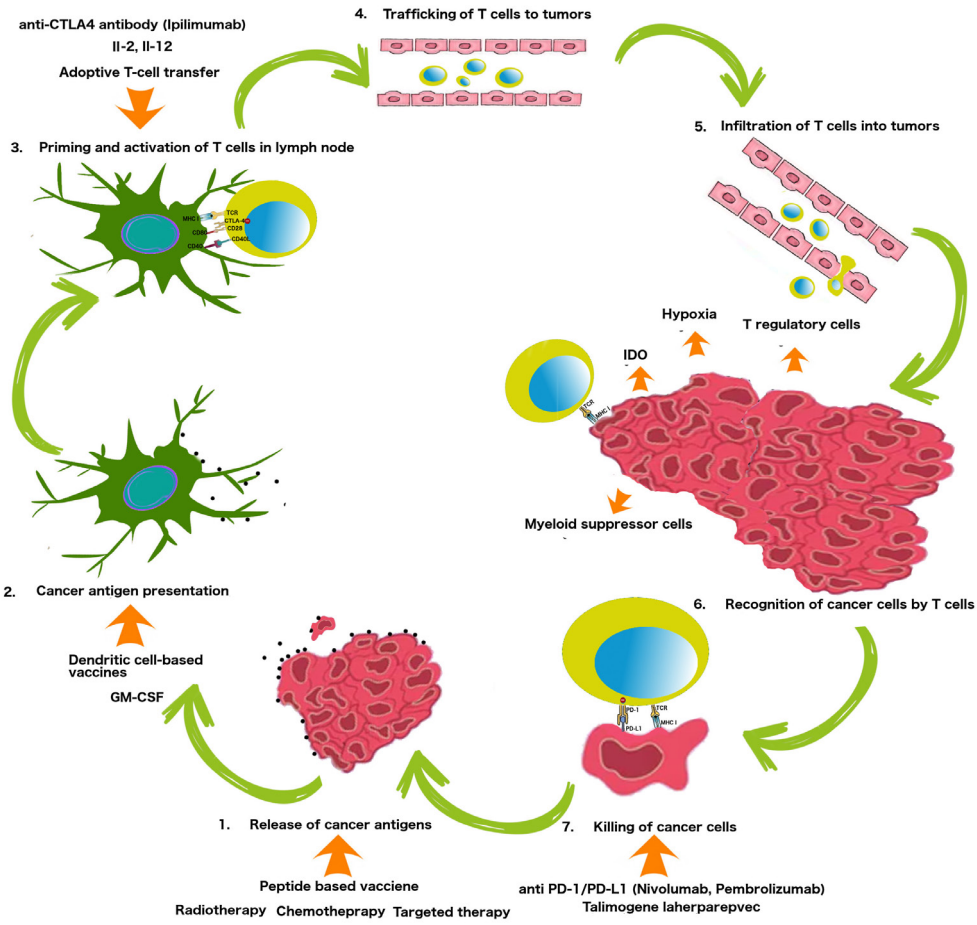


**FIGURE 1.** Cancer immunity cycle

The great insight into immune response mechanism was provided by Dan Chen and Ira Mellman [14]. They presented immunity cycle as a self-feeding system resulting in T-cell response. The immune therapy for melanoma can target almost every step of the cycle. The understanding of complexity of immunology system is essential to overcome the immune resistance and to provide effective and safe therapy to patients

**RYCINA 1.** Cykl immunologiczny nowotworu

Doskonały obraz mechanizmu odpowiedzi immunologicznej został zaprezentowany przez Dan Chen i Ira Mellman [14]. Przedstawili oni cykl immunologiczny jako samonapędzający się system skutkujący ondpowiedzią limfocytów T przeciw komórkom nowotworowym. Współczesne immunoterapeutyki mogą oddziaływać na każdy etap tego procesu. Zrozumienie złożoności systemu immunologicznego jest niezwykle istotne, żeby przezwyciężyć odporność nowotworu i zapewnić pacjentom bezpieczną i skuteczną terepię

## CANCER IMMUNITY CYCLE

The great insight into basis of onco-immunology was provided by Daniel Chen and Ira Mellman [14]. They presented immunity cycle as a self-feeding system resulting in T-cell response. The immune process was divided into seven steps. The present immune therapy approaches for melanoma can target almost every level of the cycle.

### RELEASE OF CANCER ANTIGENS

Immunogenic cell death triggers ejection of tumor specific antigens. The antigens can be formed either as the result of mutation or form non-mutate gens, preferably expressed by the specific type of tumor [54]. Therapies such us chemotherapy, radiation or targeted therapy trigger antigen ejection stimulating the immune response [94].

Tumor specific antigens are considered as a possible trigger of immune response against cancer. For example, the MAGE-A3 protein is known as a truly tumor-specific antigen for immunization against melanoma [8]. The other studies demonstrated that gp100 peptide vaccines was efficient to immunize patients with advanced stage melanoma [52].

### CANCER ANTIGEN PRESENTATION

The cancer antigens are detected by antigen presenting cells (APCs) among which the most important end effective are dendritic cells (DC). DCs have ability to recognize the antigens due to expression of pattern-recognition receptors (PRR) such as Toll-like receptors (TLR), NOD-like receptors (NLR) [43]. The DCs, in order to trigger the immune response, have to receive the specific activation signals such as type I IFN [43]. Salmon *et. al*. revealed that $CD103^+$ DC is the main intratumoral myeloid cell population in melanoma that transports cancer antigens to the lymph nodes [81]. During migration to lymph nodes, DCs downregulate their antigen-capturing capacity and upregulate the expression of MHC-II and costimulatory molecules such as Il-2, Il-12, Il-15 [6, 67, 99]. Maturing DCs acquire ability to trigger the antigen-specific T cells and thus initiate the immune response [67].

#### Dendritic cell vaccine

As dendritic cells play a key role in activating the immune response dendritic cell vaccines are considered as one of potential approach in immune treatment for melanoma.

Markowicz *et al.* evaluated the efficacy of peptide-loaded DC vaccine in high-risk stage III melanoma patients after lymph node dissection. They determined

that DC based vaccine was well tolerated and resulted in immune response to melanoma antigens [53].

In the study of Bol *et al.* vaccination with autologous DCs loaded with gp100 and tyrosinase also resulted in functional tumor-specific immune response. Moreover the overall survival (OS) achieved in the study group was significantly higher compared to control group [4].

Generally the vaccines that use DCs as immune response medium are composed of:

1) non-targeted peptides or nucleic acids which after administration are captured by DCs in vivo [65].

2) antigens directly coupled to antibodies recognized by DCs [65].

3) ex vivo generated DCs that are already loaded with tumor antigens [65].

The application of DC in immune therapy proved its efficiency in multiply small clinical trials [64, 80]. Thus the phase III clinical trials on dendritic cell vaccination as adjuvant therapy in advanced melanoma is undergoing [96].

One of the possible adjuvant that stimulate the growth of DC is granulocyte-macrophage colony-stimulating factor (GM-CSF). The research showed, that additional administration of GM-CSF as an adjuvant have an effect on $CD8^+$ T cell (CTL) response [30].

**High dose interferon alpha (HDI)**

Interferon alpha (IFN-α) is one of the first cytokine, which anti-tumor activity against melanoma was used in clinic. INFα is produced among others by $CD11c^+$ DCs in tumor-draining lymph nodes [24]. The direct mechanism of an impact of INF on immune response against melanoma is not well defined, but INF is known to have antiangiogenic, immunoregulatory, antiproliferative, and proapoptotic effects [89].

The study of Kirkwood *et al.* showed that cytotoxic functional activity against melanoma was elevated after injection of INFα [44]. Moreover Palmer *et al.* demonstrated that INFα can stimulate the generation of anti-melanoma CTL [63]. This finding was confirmed by Fuertes *et al.* [24]. who also revealed that presence of INFα promote the accumulation of the $CD8\alpha^+$ DCs in tumor microenvironment (TME).

Study of Kirkwood *et al.* reported that application of high dose INFα prolongs the relapse-free interval and OS of resected melanoma patients [45]. Based on this studies in 1994 the US Food and Drug Administration (FDA) approved the use of HDI [39].

To improve pharmacological properties of the INFα, such as clearance delay, pegylation was applied. Pegylation of interferon resulted in significant reduction of the frequency of dosing along with increased drug exposure [82]. In 2011, the US FDA approved PEG-IFN for stage III melanoma [39].

## PRIMING AND ACTIVATION OF T CELLS

Activated Dendritic cells, as mentioned, are capable of activating immune cells in lymph node. Among the population of the lymphoid compartment cells the CTL are known to have the major anti-cancer effect [3]. DCs take up cell-associated antigens and present them using MHC class I molecules to $CD8^+$T cells in a process referred to as cross-priming [31]. Moreover Dendritic cells express additional co-stimulatory molecules like B7 (CD80, CD86) and OX40L recognized respectively by CD28 and OX40 on T cells [54].

CD80 and CD86 are also ligands for cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), the specific antigen of CD8 T cells, known as the potent regulator of immune response. When CD28 provides costimulatory signals required for T-cell activation, CTLA-4 negatively modulates T-cells by raising the activation threshold for the priming [54].The therapeutic use of CTLA-4 antigens will be discussed later.

CTL can be generated either by stimulation of naive T cells or reprogramming of memory T cells [3]. Their activation is upregulated by Il-2, Il-12, released by DCs [67, 99]. As those interleukins have positive effect on immune response they were used in several clinical trials [66, 1].

### Interleukin-2

One of the relevant effects of Il-2 is the stimulation of CTL and NK-cell lysis [99]. However, paradoxically, it was also reported that Il-2 is responsible for the development of peripheral tolerance, promoting expansion of $CD4^+CD25^+$ regulatory T cells [61]. Nethertheless, the recombinant form of Il-2 showed potential and promoted significant immune activity against melanoma in clinical trials [74]. In 1998 the exogenous cytokine Il-2 was approved by the US FDA for treatment of advanced metastatic melanoma [74].

## TRAFFICKING OF T CELLS TO TUMORS

The amount of circulating tumor antigen-specific T cells does not necessarily correspond with melanoma regression in patients [75]. In order to perform anti-tumor activity the lymphocytes need to migrate into tumor microenvironment [27].

Research revealed the correlation between the presence of lymphocytes in TME and expression of defined chemokine genes. Among the subset of chemokines, CCL2, CCL3, CCL4, CCL5, CXCL9, and CXCL10 were confirmed to be preferentially express in melanoma metastatic tumors that contained T cells [34]. Moreover corresponding chemokine receptors were expressed on human $CD8^+$ effector T cells (Teff). In vitro research confirmed that each of this chemokines can activate the trafficking of $CD8^+$ Teff to tumor sites [34].

## INFILTRATION OF T CELLS INTO TUMORS

To kill the tumor cells, antigen-specific T cells not only have to detect the tumor localization, but also gain access to TME. To infiltrate the TME and overcome so-called tumor endothelial barrier, CTL require, apart form T-cell receptor engagement, the interaction of either CD103 with E-cadherin or Lymphocyte function-associated antigen 1 (LFA-1) with Intercellular Adhesion Molecule 1 (ICAM-1) [23].

Avoiding the immune response tumor endothelial cells suppress the immune cell adhesion down-regulating ICAM-1 and producing vascular endothelial growth factor (VEGF), which inhibits expression of major adhesion molecules [5, 51].

## RECOGNITION OF CANCER CELLS BY T CELLS

Human CD8+ Teff can recognize human leukocyte antigens (HLA) class I molecules on the surface of tumor cells. The HLA binds peptides, which are generated mostly from endogenous proteins (tumor-associated antigens) and present them on cell's surface. The recognition of these peptides by CTLs triggers a series of events that can result in tumor cell lysis [37].

Mutations of HLA class I genes, and defect in their regulation end expression, can provoke the HLA class I downregulation providing the obstacle for CTL to detect cancer cells [37].

## KILLING OF CANCER CELLS

The CTL are main anti-cancer immune effectors. High affinity for HLA on tumor cells and large diversity of surface molecules, enable them to recognize and traffic into the tumor cell. The cytotoxicity of CTL is provided by granzymes A and B and perforin. Moreover extended memory enable CTL to generate long lasting immune response [3].

Tumors can produce a variety of surface molecules in order to suppress immune response like programmed death receptor 1 (PD-1). Its ligands PD-L1 or PD-L2 engage receptors on the surfaces of activated T cells, causing T-cell anergy or exhaustion [32]. The effect of PD-1/PD-L1 checkpoint blockade on melanoma patients will be discussed later.

### Adoptive T-cell transfer

Well promising approach for melanoma treatment is adoptive T-cell transfer. The administration of $10^9$ to $10^{11}$ autologous tumor-infiltrating lymphocytes (TIL) represents one of the most personalized immunotherapy. Principally, this treatment consists in administration of special prepared lymphocytes infusion obtained form patients cancer. After multiplication, the lymphatic cells undergo

the selection. The research showed that cells expressing CD8, CD131, and PD-1 receptors are the most effective in melanoma treatment [72]. Moreover, in order to enhance the income of therapy and to avoid T-cell exhaustion the lymphocytes undergo genetic modifications [83]. Another strategy to improve efficiency of the T-cell transfer is lymphodepleting preconditioning. The aim of this procedure is depletion of $CD4^{+}CD25^{+}$ regulatory T cells (Treg) population [46]. Combination of T-cell transfer with Il-2 infusion in order to support T-cell activity has also been explored in several therapy protocols [2]. Adoptive cell therapy using autologous TIL has demonstrated a great potential for treatment of advanced tumors in many phase I/II clinical trials [73]. The phase III clinical trial of T-cell transfer for advanced melanoma treatment is currently undergoing [72].

## STRATEGIES OF PRESENT IMMUNE THERAPY

### CTLA-4 BLOCKADE (IPILIMUMAB)

Ipilimumab, one of the first clinically used antibody, in immune therapy against melanoma, target CTLA-4 antigens presented on T-cells. As described above CTLA-4 receptors rise the activation threshold of immune response. Hodi *et al*. proved that CTLA-4 blockade with Ipilimumab showed significant objective response rate in patients witch metastatic melanoma [38]. In 2011 FDA approved the clinical use of Ipilimumab for patients with Stage III or Stage IV melanoma [85].

### ANTIBODIES TARGETING PD-1 (NIVOLUMAB AND PEMBROLIZUMAB)

The breakthrough in immune therapy against melanoma was idea of inhibiting PD-1. The first clinical trial of PD-1 antagonist pembrolizumab included total of 135 patients with advanced melanoma. The notable objective response was observed with metastatic melanoma. Grade 3 or 4 drug-related adverse events were reported in 13% of patient [33]. In 2014 FDA approved pembrolizumab for patients with stage III and IV melanoma [15]. The second antibody used against PD-1 was nivolumab. In the 3$^{rd}$ phase of studies also reported remarkably objective responses in patients with metastatic melanoma. The grade 3-4 drug-related serious adverse events were reported in 5% of nivolumab-treated patients [95]. In 2014, the FDA granted approval to nivolumab for patients with unresectable or metastatic melanoma [36]. In, 2015 FDA granted approval to nivolumab and Ipilimumab combination treatment for unresectable Stage III melanoma and Stage IV melanoma. The decision was supported by the studies, which showed increased objective response to the combined therapy [9].

The recent investigations in immune inhibitory pathways extend our knowledge about response regulation mechanisms. The T-cell immunoreceptor with Ig and ITIM domains (TIGIT) appear to be the new target for checkpoint blockade therapy. TILs expressing TIGIT show different level of dysfunction [98]. TIGIT is an inhibitory ligand of costimulatory molecules CD155/PVR and CD112 that are highly expressed in melanoma environment by cancer cells and DCs [13]. The combination of TIGIT blockade with PD-1 blockade improved clinical benefits in patients with melanoma [13].

**TABLE 1.** Present immune therapeutics approved by FDA for melanoma treatment
**TABELA 1.** Obecne immunoterapeutyki zatwierdzone przez FDA do leczenia czerniaka

| NAME OF MEDICAMENT | COMMERCIAL NAME | DATE OF FDA APPROVAL | CLINICAL TRIALS |
|---|---|---|---|
| | | | |
| Talimogene laherparepvec | Imlygic® | October, 2015 | [20], [35] |
| Ipilimumab | Yervoy® | March, 2011 | [38] |
| Nivolumab | Opdivo® | December, 2014 | [95] |
| Pembrolizumab | Keytruda® | September, 2014 | [33] |
| IL-2 | Proleukin® | 1998 | [66], [76] |
| High dose INF-alpha | Intron® A | 1994 | [45] |
| Peg INF | Sylatron® | March, 2011 | [82] |
| Nivolumab and Ipilimum ab combination | Opdivo® , Keytruda® | September, 2015 | [9] |

## TALIMOGENE LAHERPAREPVEC

Another type of therapeutic vaccines against tumor cells is based on genetically modified oncolytic viruses. Currently used in clinic talimogene laherparepvec (TVEC) includes herpes simplex type 1 virus (HSV1) engineered to be specific for tumor cells and to use oncogenic pathways to replicate [26]. Additionally, in order to stimulate immune response granulocyte-macrophage colony-stimulating factor (GM-CSF) gene was incorporated into virus so its replication involves production of GM-CSF [26].

Moreover, application of genetic modifications, increased vaccine efficiency providing 1) reduction of infection of healthy cells [17], 2) improvement of lytic effect on tumor cells [17], 3) upregulation of antigen presentation by infected tumor cells [17], 4) stimulation of DCs by production of GM-CSF [17].

In phase III of clinical trial patients were treated either with TVEC or GM-CSF. TVEC had significantly higher durable response (25,2% versus 1.2%) in pa-

tients with stage III or IV melanoma [35]. In 2015 TVEC received FDA approval for local treatment of unresectable cutaneous, subcutaneous, and nodal lesions in patients with melanoma [29].

The new studies reported that combination of TVEC and checkpoint inhibitors provide better outcome compered to monotherapy. The phase II of studies of TVEC and Ipilimumab showed better overall response rate (ORR) of combined therapy compered to single Ipilimumab administration [20].

Phase III of TVEC and Pembrolizumab are ongoing, but so far, phase I showed promising results. ORR of combined therapy with Pembrolizumab and TVEC was significantly higher compering to effect of either therapy alone [17].

## TUMOR IMMUNOSUPPRESSIVE DEFENSE MECHANISMS

The history of countless clinical trials of immune agents against melanoma show us how difficult is to predict the outcome of immune therapy. The results depend strictly on the clinical state of patient, personalized features of cancer cells, but also on the whole environment of the tumor. One of the role of tumor microenvironment (TME), is to suppress the immune response against cancer and provide the best conditions for cancer development. TME can be characterized by physical features of tumors stroma, its metabolic landscape (hypoxia, acidosis) and cellular compartment (myeloid cells, Treg). Additional targeting of tumor microenvironment might help to overcome the natural resistant of tumor and provide the better outcome to melanoma patients. Alternations of microenvironment with tumor development indicate that the therapy must be strictly personalized. The purpose of this chapter is to gather and summarize the most recent discoveries on melanoma's TME.

### HYPOXIA AND GLUCOSE RESTRICTION

The metabolic landscape shows significant heterogeneity even within one type of tumor [70]. Heightened oxidative metabolism of tumor cells effect the development of intratumoral hypoxia [60]. The studies on impact of sufficient level of glucose and oxygen on T $CD8^+$ lymphocyte showed that hypoxia and glucose restriction might determine the limitation for immune response [12]. High level of oxygen is required for aerobic glycolysis performed by T cell [11]. It was observed that tumors using glycolysis, as a main metabolic pathway, are often resistant to immunotherapy for melanoma. Upregulation of glycolysis protected melanoma from T cells infiltration and impaired T cell cytotoxicity for tumor cells [10]. Moreover the clear dependence between the local hypoxia and localization of TIL inside tumor was observed. Immune cells were excluded from tumor regions with insufficient oxygen supply [60]. The type of up regulated metabolism in cancer cells can be used as a prognostic factor and as a potential target in anti-tumor therapy [10].

Insufficient supply in oxygen can also induce the production of inhibitory molecules by DCs and tumor cells. One of hypoxia-associated products is indoleamine 2,3-dioxygenase (IDO) [90]. IDO overproduction significantly lowers the concentration of the essential amino acids such as tryptophan and tryptophan metabolites in DCs, impairing their role as a APCs [86]. Moreover tryptophan depletion impairs T-cell proliferation and supports T-cell autophagy, consequently increasing Teff apoptosis [97]. Under normal condition, production of IDO helps to maintain the immunohomeostasis, but in TME IDO rather suppress immune response.

In recent publications in the area of oncoimmunology IDO was described as a promising target that might improve clinical outcome of immune therapy [92]. First inhibitors of IDO (Indoximod and Epacadostat) in combination with checkpoints inhibitors entered the clinical trials.

Although inhibiting IDO seemed to be promising strategy of enhancing the checkpoint inhibitors effect, recently, the study showed that Epacadostat with pembrolizumab had no effect on progression-free survival compared with pembrolizumab monotherapy. Due to this finding Epecadostat failed the phase III of ECHO 301 trial [58].

The next potential inhibitor of IDO is Idoximod. Phase II clinical trial of the IDO pathway inhibitor indoximod in combination with pembrolizumab has shown promising effect improving ORR of the therapy [50]. After promising results of phase II, Indoximod entered phase III of studies investigating an effect of administration of Indoximod with checkpoint inhibitors in patients with unresectable stage III /IV Malignant Melanoma [50].

## THE IMMUNOLOGICAL FEATURES OF Treg IN MELANOMA

$CD4^{+}CD25^{high}FoxP3^{+}$ Treg are known as a main immune suppressor in TME. Treg perform regulatory and immunosuppressive function using various strategies. Expressed on T regulatory cell surface PD-L1 and CTLA-4 impair the effective T cell function via cell-to-cell contacts mechanism [21, 77]. Moreover Treg can direct transfer cyclic adenosine monophosphate (cAMP) using a gap junction intercellular communication [47]. cAMP, the ligand of adenosine receptor A2A, is known to be potent negative regulator of Teff and DCs [19]. Some of strategies do not require cell-to-cell contact and can be mediated by Il-10 and TGF-h1 [87]. Eventually, Treg express the CD25 molecule with high affinity for Il-2. This receptor enables Treg to compete with Teff for the cytokine, limiting immune response [88].

Melanoma TME is known to by highly invaded by $CD4^{+}CD25^{high}FoxP3^{+}$ Treg. The cells were detected not only in primary lesions, but also in affected lymph nodes and metastatic lesions [57].

The immunosuppression mediated by Treg is one of the main mechanisms providing immunosuppression in TME. The several trials were investigating an issue weather the presence of Treg may be used as a prognostics factor for mel-

anoma patients. Miracco *et al.* found correlation between presence of Treg and local recurrence of cutaneous melanoma [56]. Knol *et al*. proved that Foxp3 expression is associated with a decrease in progression-free survival in melanoma patients [49]. Jacobs *et al.* reported that the ratio between CTL and Treg would be useful marker for predicting the immunotherapy outcome [40].

Due to the fact that Treg are key players in maintaining immunosuppressive environment inside tumor, the next trials investigated whether Treg depletion might provide additional advantages to the patients with melanoma.

Some strategies targeting Treg were based on CD25 receptor antibody daclizumab [69].

In mice, anti-CD25 antibody is an effective method of depleting $CD25^+$ $Foxp3^+$ Treg [93]. In clinical trials, metastatic melanoma patients were given daclicumab and DC vaccine. Although daclizumab depleted the $CD4^+FoxP3^+CD25$ high Treg from the peripheral circulation, it did not enhance the efficacy of the DC vaccine [41].

Quezada *et al*. tried to understand the reason of failure of anti-CD25 therapy. They suggested that the main cause is not the lack of elimination of $CD25^+$ Treg, but the failure of Teff to infiltrate the TME [68].

The other anti-Treg therapeutic approaches are focused on Foxp3. Differently to upon described CD25, Foxp3 is a transcription regulator and is not expressed on the cell surface [79]. Nair *et al.* investigated the impact of Foxp3 mRNA-transfected dendritic cells vaccination on melanoma mice model. Similar to anti-CD25 therapy, vaccination led to depletion of Foxp3-expressing Treg. Surprisingly, Treg residing inside the tumor were preferably affected by the vaccination. That fact might indicate the grater safety of therapy [59].

Miguel *et al*. evaluate the possible additional anti-tumor effect of FOXP3 and CTLA-4 gene silencing treatment before therapeutic vaccination with GM-CSF-engineered cells [55]. FOXP3 gene silencing appeared to have remarkably higher effect on Treg than CTLA-4 gene silencing and contributed to the significantly higher survival of mice's with melanoma.

Although above mentioned therapeutic approaches were potent in depletion of Treg, this effect doesn't correlate with clinical outcome of this therapies on melanoma patients [40].

Another strategy against Treg targets glucocorticoid-induced TNF receptor (GITR). GITR is a transmembrane protein normally poorly expressed by $CD8^+$ T cells, but present on $CD4^+CD25^+Foxp3^+$ Treg [48]. Cohen *et al*. investigated the anti-GITR monoclonal antibody DTA-1 on Treg and Teff on melanoma mouse model. They showed that DTA-1 antibody alter Treg accumulation in TME and results in loss of foxp3 expression, modifying Treg suppressive capacity [16]. Moreover Côté *et al.* demonstrated that DTA-1 stimulate $CD8^+$ cells and derives additional anti-tumor response [18]. As the anti-GITR antibody showed in vivo promising results, clinical trials on melanoma patients are currently undergoing.

## MYELOID CELLS – MYELOID-DERIVED SUPPRESSOR CELLS (MDSCs)

Another cells enriched in TME that have important role in tumor immunosuppressive environment are myeloid-derived suppressor cells (MDSCs). MDSCs represent the extremely heterogeneous population of granulocytes, macrophages, and DCs precursors [25]. The spectrum of MDSCs localized in tumor and also circulating MDSCs varies depending on the tumor type and response to the immunotherapy [84]. Large heterogeneity of cell populations indicates the vast diversity of immunosuppressive strategies of myeloid cells.

MDSC promote T cell apoptosis by production of nitric oxide and reactive oxygen species [42, 62]. Moreover, production of arginase-1 lowers the local concentration of L-arginine suppressing the T cell function [71]. The multiple mechanisms involved MDSC to exhibit immunosuppressive activity in melanoma tumors were reviewed by Umansky *et al.* [91].

Filipazzi *et al.* detected the $CD14^{+}HLA\text{-}DR^{-/lo}$ cells in all metastatic melanoma patients. The main suppressive activity of those cells was mediated by transforming growth factor beta (TGFβ), Moreover the further expansion of $CD14^{+}HLA\text{-}DR^{-/lo}$ cells was enhanced after administration of an anti-tumor vaccine with GM-CSF [22]. In this regard, the changes in myeloid compartment might provide the information about the response to anti-tumor therapy.

Sade-Feldman *et al.* suggested the use of $CD33^{+}CD11b^{+}HLA\text{-}DR^{-}$ MDSCs as a predictive and prognostic biomarker for patients with stage IV melanoma treated with Ipilimumab. Detection of MDSCs before therapy begin was associated with minimal response and no benefit in OS [78]. The evaluation myeloid alterations in tumor would be a useful tool to predict the response to immune therapy and to follow the disease progression.

## SUMMARY

The cancer immunity cycle presented the basis of immune response in oncoimmunology context. Due to undisputed complexity of this process, immune response modulation requires use of combined approaches. Nowadays the available treatment strategies can significantly improve the outcome of patients with advanced melanoma disease. Moreover, in the nearest future, the checkpoint inhibition combined with dendritic cell vaccines or adoptive T-cell transfer might provide even higher rate of response to the therapy.

Another, still widely investigated aspect is tumor microenvironment. Recent progress in technologies enables more precise tracking of changes in TME. Expanded knowledge in this area might provide clinicians key information about

patient's prognosis and indicate the most effective therapy. Inhibition of Treg cells and other immunosuppressive elements of TME may by unenviably use as an adjuvant to the standard therapy. Regarding all recent successes, immunotherapy seems to be the most promising treatment for cancer patients.

## ACKNOWLEDGMENTS

The work was created as part of the activity of the Student Research Group "Biology of Cancer Cell" at the Wroclaw Medical University (SKN No. K 148) and Statutory Funds of Department of Molecular and Cellular Biology.

## REFERENCES

[1] Alatrash G, Hutson TE, Molto L, Richmond A, Nemec C, Mekhail T. Clinical and Immunologic Effects of Subcutaneously Administered Interleukin-12 and Interferon Alfa-2b : Phase I Trial of Patients With Metastatic Renal Cell Carcinoma or Malignant Melanoma. *J Clin Oncol*. 2014; **22**(14): 2891-2900.

[2] Andersen R, Donia M, Ellebaek E, Borch Th, Kongsted P, Hendel HW. Long-Lasting Complete Responses in Patients with Metastatic Melanoma after Adoptive Cell Therapy with Tumor-In fi ltrating Lymphocytes and an Attenuated IL2 Regimen. *Clin Experymental Immunol*. 2016; **22**(15): 3734-3745.

[3] Appay V, Douek DC, Price DA. CD8 + T cell efficacy in vaccination and disease. *Nat Med*. 2008; **14**(6): 623-628.

[4] Bol KF, Aarntzen EHJG, Florentien EM, et al. Favorable overall survival in stage III melanoma patients after adjuvant dendritic cell vaccination. *Oncoimmunology*. 2016; **5**(1): 1-8.

[5] Bouzin C, Brouet A, Vriese JDE, et al. Effects of Vascular Endothelial Growth Factor on the Lymphocyte-Endothelium Interactions: Identification of Caveolin-1 and Nitric Oxide as Control Points of Endothelial Cell Anergy. *J Immunol*. 2007; **178**: 1505-1511.

[6] Brandt K, Bulanova E, Mirghomizadeh F, Paus R, Bulfone-Paus S. Dendritic cell-derived IL-15 controls the induction of CD8 T cell immune responses Ren e. *Eur J Immunol*. 2003;33:3493-3503.

[7] Bray F, Jacques F, Isabelle S, Rebecca LS, Lindsey AT, Jemal Ahmedin. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA CANCER J CLIN*. 2018; **68**: 394-424.

[8] Brichard Vg, Lejeune D. GSK ' s antigen-specific cancer immunotherapy programme : Pilot results leading to Phase III clinical development. *Vaccine*. 2007; **25**: 61-71.

[9] Carlino MS, Long GV. Ipilimumab Combined with Nivolumab: A Standard of Care for the Treatment of Advanced Melanoma ? *Clin Cancer Res*. 2016; **22**(16): 3992-3999.

[10] Cascone T, Mckenzie JA, Mbofung RM, Heymach JV, Hwu P, Peng W. Increased Tumor Glycolysis Characterizes Immune Resistance to Adoptive T Cell Therapy Clinical and Translational Report Increased Tumor Glycolysis Characterizes Immune Resistance to Adoptive T Cell Therapy. *Cell Metab*. 2018; **27**: 1-11.

[11] Cham CM, Driessens G, Keefe JPO, Gajewski TF. Glucose deprivation inhibits multiple key gene expression events and effector functions in CD8+T cells. *Eur J Immunol*. 2008; **38**: 2438-2450.

[12] Chang C, Qiu J, Sullivan O, et al. Metabolic Competition in the Tumor Microenvironment Is a Driver of Cancer Progression Article Metabolic Competition in the Tumor Microenvironment Is a Driver of Cancer Progression. *Cell*. 2015; **162**(6): 1229-1241.

[13] Chauvin J, Korman AJ, Zarour HM, et al. TIGIT and PD-1 impair tumor antigen – specific CD8 + T cells in melanoma patients. *J Clin Invasitgation*. 2015; **125**(5): 2046-2058.

[14] Chen DS, Mellman I. Oncology meets immunology: The cancer-immunity cycle. *Immunity*. 2013; **39**: 1-10.
[15] Chuk MK, Chang JT, Theoret MR, et al. FDA Approval Summary : Accelerated Approval of Pembrolizumab for Second-Line Treatment of Metastatic Melanoma. *Clin Cancer Res*. 2017; **23**(19): 5666-5671.
[16] Cohen AD, Schaer DA, Liu C, et al. Agonist Anti-GITR Monoclonal Antibody Induces Melanoma Tumor Immunity in Mice by Altering Regulatory T Cell Stability and Intra-Tumor Accumulation. *PLoS One*. 2010; **5**(5).
[17] Conry RM, Westbrook B, Mckee S, Graham T. Talimogene laherparepvec : First in class oncolytic virotherapy. *Hum Vaccin Immunother*. 2018; **14**(4): 839-846.
[18] Côté AL, Zhang P, Sullivan JAO, et al. Stimulation of the Glucocorticoid-Induced TNF Receptor Family-Related Receptor on CD8 T Cells Induces Protective and High-Avidity T Cell Responses to Tumor-Specific Antigens. *J Immunol*. 2011; **186**: 275-283.
[19] Cremwehbi VL, Taksen K. Molecular Mechanisms for cAMP-Mediated immunoregulation in T cells – Role of Anchored Protein Kinase A Signaling Units. *Front Immunol*. 2016; **7**(222): 1-19.
[20] Dummer R, Hoeller C, Pezzani I, Michielin O. Combining talimogene laherparepvec with immunotherapies in melanoma and other solid tumors. *Cancer Immunol Immunother*. 2017; **66**: 683-695.
[21] Feng C, Cao L-J, Song H-F, Xu p, Chen H, Xu J-C. Expression of PD-L1 on CD4+CD25+Foxp3+ Regulatory T Cells of Patients with Chronic HBV Infection and Its Correlation with Clinical Parameters. *Viral Immunol*. 2015; **28**(8): 418-424.
[22] Filipazzi P, Valenti R, Huber V, et al. Identification of a New Subset of Myeloid Suppressor Cells in Peripheral Blood of Melanoma Patients With Modulation by a Granulocyte-Macrophage Colony--Stimulation Factor – Based Antitumor Vaccine. *J Clin Oncol*. 2007; **25**(18): 2546-2553.
[23] Franciszkiewicz K, Le A, Boutet M, Vergnon I. CD103 or LFA-1 Engagement at the Immune Synapse between Cytotoxic T Cells and Tumor Cells Promotes Maturation and Regulates T-cell Effector Functions. *Microenviron Immunol*. 2013; **73**(2): 617-629.
[24] Fuertes MB, Kacha AK, Kline J, et al. Host type I IFN signals are required for antitumor CD8 + T cell responses through CD8a+ dendritic cells. *J Exp Med*. 2016; **208**(10): 2005-2016.
[25] Gabrilovich DI. Myeloid-derived suppressor cells. *Cancer Immunol Res*. 2017; **5**(1): 3-8.
[26] Garnock-Jones KP. Talimogene Laherparepvec : A Review in Unresectable Metastatic Melanoma. *BioDrugs*. 2016; **30**(5): 461-468.
[27] Gooden MJM, Bock GH DE, Leffers N, Daemen T, Nijman HW. The prognostic influence of tumour-infiltrating lymphocytes in cancer : a systematic review with meta-analysis. *Br J Cancer*. 2011; **105**: 93-103.
[28] Gray-Schopfer V, Wellbrock C, Marais R. Melanoma biology and new targeted therapy. *Nature*. 2007; **445**(7130): 851-857.
[29] Greig SL. Talimogene Laherparepvec: First Global Approval. *Drugs*. 2016; **76**: 147-154.
[30] Grotz TE, Kottschade L, Pavey ES, Markovic SN, Jakub JW. Adjuvant GM-CSF Improves Survival in High-risk Stage IIIC Melanoma. *Am J Clin Oncol*. 2014; **37**(5): 467-472.
[31] Haan BJMM DEN, Lehar SM, Bevan MJ. CD8 + but Not CD8 – Dendritic Cells Cross-prime Cytotoxic T Cells In Vivo. *J Experymental Med*. 2000; **192**(12): 1685-1695.
[32] Hamanishi J, Mandai M, Iwasaki M, et al. Programmed cell death 1 ligand 1 and tumor- infiltrating CD8+T lymphocytes are prognostic factors of human ovarian cancer. *PNAS*. 2007; **104**(9): 3360-3365.
[33] Hamid O, Robert C, et al. Safety and Tumor Responses with Lambrolizumab (Anti – PD-1) in Melanoma. *N Engl J Med*. 2013; **369**(2): 134-144.
[34] Harlin H, Meng Y, Peterson AC, et al. Chemokine Expression in Melanoma Metastases Associated with CD8+ T-Cell Recruitment. *Cancer Res*. 2009; **69**(7): 3077-3086.
[35] Harrington Kj, Andtbacka RHI, Collichio F, Downey G, Chen L. Efficacy and safety of talimogene laherparepvec versus granulocyte-macrophage colony- stimulating factor in patients with stage IIIB / C and IVM1a melanoma : subanalysis of the Phase III OPTiM trial. *Onco Targets Ther*. 2016; **9**: 7081-7093.
[36] Hazarika M, Chuk MK, Theoret MR, et al. U . S . FDA Approval Summary : Nivolumab for Treatment of Unresectable or Metastatic Melanoma Following Progression on Ipilimumab. *Clin Cancer Res*. 2017; **23**(14): 3484-3489.

[37] Hicklin Dj, Marincola Fm, Ferrone S. HLA class I antigen downregulation in human cancers : T-cell immunotherapy revives an old story. *Mol Med Today*. 1999; **5**: 178-186.
[38] Hodi SF, O'day SJ, Mcdermott DF, et al. Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. *N Engl J Med*. 2010; **363**(8):711-723.
[39] Ives NJ, Suciu S, Eggermont AMM, et al. Adjuvant interferon- a for the treatment of high-risk melanoma : An individual patient data meta-analysis. *Eur J Cancer*. 2017; **82**: 171-183.
[40] Jacobs Jfm, Nierkens S, Figdor CG, Vries IJM De, Adema GJ. Regulatory T cells in melanoma : the final hurdle towards effective immunotherapy? *Lancet*. 2012; **13**: 32-42.
[41] Jacobs JFM, Punt CJA, Lesterhuis WJ, Sutmuller RPM. Dendritic Cell Vaccination in Combination with Anti-CD25 Monoclonal Antibody Treatment : A Phase I / II Study in Metastatic Melanoma Patients. *Clin Cancer Res*. 2010; **16**(20): 5067-5079.
[42] Jia W, Jackson-Cook C, Graf M. Tumor-infiltrating, myeloid-derived suppressor cells inhibit T cell activity by nitric oxide production in an intracranial rat glioma + vaccination model. *J Neuroimmunol*. 2011; **223**(1-2): 20-30.
[43] Joffre O, Nolte MA. Inflammatory signals in dendritic cell activation and the induction of adaptive immunity. *Immunol Rev*. 2009; **227**: 234-247.
[44] Kirkwood JM, Richards T, Zarour HM, et al. Immunomodulatory Effects of High-Dose and Low- Dose Interferon α2b in Patients with High-Risk. *Cancer*. 2002; **95**(5): 1101-1112.
[45] Kirkwood JM, Strawderman MH, Ernstoff MS, et al. Interferon alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: the Eastern Cooperative Oncology Group Trial EST 1684. *J Clin Oncol*. 1996; **14**(1): 7-17.
[46] Klebanoff CA, Khong HT, Antony PA, Palmer DC, Restifo NP. Sinks, suppressors and antigen presenters: how lymphodepletion enhances T cell-mediated tumor immunotherapy. *Trends Immunol*. 2015; **26**(2): 111-117.
[47] Klein M, Bopp T, Bopp T. Cyclic AMP Represents a Crucial Component of Treg Cell-Mediated immune Regulation. *Front Immunol*. 2016; **7**(315): 1-5.
[48] Knee DA, Hewes B, Brogdon JL. Rationale for anti-GITR cancer immunotherapy. *Eur J Cancer*. 2016; **67**: 1-10.
[49] Knol AC, Nguyen JM, Brocard A, Khammari A, Dre B. Prognostic value of tumor-infiltrating Foxp3 + T-cell subpopulations in metastatic melanoma. *Exp Dermatol*. 2011; **20**: 430-434.
[50] Komiya T, Huang CH. Updates in the Clinical Development of Epacadostat and Other Inhibitors ( IDO1 ) for Human Cancers. *Front Immunol*. 2018; **8**(423): 1-7.
[51] Lanitis E, Irving M, Coukos G. Targeting the tumor vasculature to enhance T cell activity. *Curr Opin Immunol*. 2015; **33**: 55-63.
[52] Markovic S, Suman V, Ingle J, Kaur J. Peptide Vaccination of Patients With Metastatic Melanoma. *Am J Clin Oncol*. 2006; **29**(4): 352-360.
[53] Markowicz S, Nowecki ZI, Rutkowski P, Lipkowski AW, Walewski J. Adjuvant vaccination with melanoma antigen-pulsed dendritic cells in stage III melanoma patients. *Med Oncol*. 2012; **29**: 2966-2977.
[54] Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age (Finally!). *Nature*. 2011;480:480-489.
[55] Miguel A, Sendra L, Noé V, Dasí F, Hervas D, Herrero MJ. Silencing of Foxp3 enhances the antitumor efficacy of GM-CSF genetically modified tumor cell vaccine against B16 melanoma. *Onco Targets Ther*. 2017; **10**: 503-514.
[56] Miracco C, Mourmouras V, Biagioli M, et al. Utility of tumour-infiltrating CD25+FOXP3+ regulatory T cell evaluation in predicting local recurrence in vertical growth phase cutaneous melanoma. *Oncol Rep*. 2007; **18**: 1115-1122.
[57] Mourmouras V, Fimiani M, Rubegni P, Epistolato MC, Malagnino V, Cardone C. Evaluation of tumour-infiltrating CD 4 + CD 25 + FOXP 3 + regulatory T cells in human cutaneous benign and atypical naevi , melanomas and melanoma metastases. *Br J Dermatol*. 2007; **157**: 531-539.
[58] Mullard A. Ido takes a blow. *Nat Rev*. 2018;17(5):307.

[59] Nair S, Boczkowski D, Fassnacht M, Pisetsky D, Gilboa E. Vaccination against the Forkhead Family Transcription Factor Foxp3 Enhances Tumor Immunity. *Cancer Res*. 2007; **67**(1): 371-381.
[60] Najjar YG, Kirkwood JM, Delgoffe GM. Tumor cell oxidative metabolism as a barrier to PD-1 blockade immunotherapy in melanoma. *JCI Insight*. 2019; **4**(5): 1-18.
[61] Nelson BH, Nelson BH. IL-2, Regulatory T Cells, and Tolerance. *J Immunol*. 2004; **172**: 3983-3988.
[62] Ohl K, Tenbrock K. Reactive Oxygen Species as Regulators of MDSC-Mediated Immune Suppression. *Front Immunol*. 2018; **9**(2499):1 -7.
[63] Palmer KJ, Harries M, Gore ME. Interferon-alpha ( IFN- a ) stimulates anti-melanoma cytotoxic T lymphocyte ( CTL ) generation in mixed lymphocyte tumour cultures ( MLTC ). *Clin Experymental Immunol*. 2000; **119**: 412-418.
[64] Palucka AK, Ueno H, Connolly J, et al. Dendritic Cells Loaded With Killed Allogeneic Melanoma Cells can Induce Objective Clinical Responses and MART-1. *J Immunother*. 2006; **29**(5): 545-557.
[65] Palucka K, Banchereau J. Dendritic cell-based cancer therapeutic vaccines. *Immunity*. 2014; **39**(1): 38-48.
[66] Parkinson BDR, Abrams JS, Wiernik PH, et al. Interleukin-2 Therapy in Patients With Metastatic Malignant Melanoma: A Phase II Study. *J Clin Oncol*. 1990; **8**(10): 1650-1656.
[67] Pulend Ran B. The Varieties of Immunological Experience : Of Pathogens , Stress , and Dendritic Cells. *Annu Rev Immunol*. 2015; **33**: 563-606.
[68] Quezada SA, Peggs KS, Simpson TR, Shen Y, Littman DR, Allison JP. Limited tumor infi ltration by activated T eff ector cells restricts the therapeutic activity of regulatory T cell depletion against established melanoma. *J Exp Med*. 2008; **205**(9): 2125-2138.
[69] Rech AJ, Vonderheide RH. Clinical Use of Anti-CD25 Antibody Daclizumab to Enhance Immune Responses to Tumor Antigen Vaccination by Targeting Regulatory T cells. *Cancer Vaccines*. 2009;1174:99-106.
[70] Robertson-Tessi M, Gillies RJ, Gatenby RA. Impact of metabolic heterogeneity on tumor growth, invasion, and treatment outcomes. *Cancer Res 2015*. 2015; **75**(8): 1567-1579.
[71] Rodriguez PC, Ernstoff MS, Hernandez C, et al. Arginase I – Producing Myeloid-Derived Suppressor Cells in Renal Cell Carcinoma Are a Subpopulation of Activated Granulocytes. *Cancer Res*. 2009; **69**(4): 1553-1561.
[72] Rohaan MW, Berg JH, Kvistborg P, Haanen JBAG. Adoptive transfer of tumor-infiltrating lymphocytes in melanoma : a viable treatment option. *J Immunother Cancer*. 2018; **6**(102): 1-16.
[73] Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. *Science (80- )*. 2015;348(6230):62-68.
[74] Rosenberg SA, Rosenberg SA. IL-2: The First Effective Immunotherapy for Human Cancer. *J Immunol*. 2014; **192**: 5451-5458.
[75] Rosenberg SA, Sherry RM, Kathleen E, et al. Tumor Progression Can Occur despite the Induction of Very High Levels of Self/Tumor Antigen-Specific CD8+ T Cells in Patients with Melanoma. *J Immunol*. 2005;175:6169-6176.
[76] Rosenberg SA, Yang JC, Topalian SL, et al. Treatment of 283 Consecutive Patients With Metastatic Melanoma or Renal Cell Cancer Using. *JAMA*. 1994; **271**(12): 907-913.
[77] Rudd CE. CTLA-4 co-receptor impacts on the function of Treg and CD8 + T- cell subsets. *Eur J Immunol*. 2009; **39**(3): 687-690.
[78] Sade-Feldman M, Kanterman J, Klieger Y, et al. Clinical Significance of Circulating CD33+ CD11b+ HLA-DR- Myeloid Cells in Patients with Stage IV Melanoma Treated with Ipilimumab. *Clin Cancer Res*. 2016; **22**(23): 5661-5672.
[79] Sadlon TJ, Wilkinson BG, Pederson S, et al. Genome-Wide Identification of Human FOXP3 Target Genes in Natural Regulatory T Cells. *J Immunol*. 2010; **185**: 1071-1081.
[80] Salcedo M, Bercovivi N, Taylor R, Laporte M, Bruyns C. Vaccination of melanoma patients using dendritic cells loaded with an allogeneic tumor cell lysate. *Cancer Immunol Immunother*. 2006; **55**: 819-829.

[81] Salmon H, Idoyaga J, Rahman A, et al. Expansion and activation of CD103+ dendritic cell progenitors at the tumor site enhances tumor responses to therapeutic PD-L1 and BRAF inhibition. *Immunity*. 2017; **44**(4) :924-938.
[82] Schilling B, Vaubel J, Schadendorf D. Adjuvant pegylated interferon α-2b therapy for melanoma. *Oncol Lett*. 2010; **1**: 237-241.
[83] Singh N, Shi J, June CH, Ruella M. Genome-editing technologies in adoptive T cell immunotherapy for cancer. *Curr Hematol Malig Rep*. 2018; **12**(6): 522-529.
[84] Solito S, Marigo I, Pinton L, Damuzzo V, Mandruzzato S. Myeloid-derived suppressor cell heterogeneity in human cancers. *Ann N Y Acad Sci*. 2014; **1319**: 47-65.
[85] Sondak VK, Smalley KSM, Kudchadkar R, Grippon S, Kirkpatrick P. Ipilimumab. *Nat Rev Drug Discov*. 2011; **10**: 411-412.
[86] Song X, Zhang Y, Zhang L, Song W, Shi L. Hypoxia enhances indoleamine 2 , 3-dioxygenase production in dendritic cells. *Oncota*. 2018;9(14):11572-11580.
[87] Strauss L, Bergmann C, Szczepanski M, Gooding W, Johnson JT, Whiteside TL. Human Cancer Biology A Unique Subset of CD4 + CD25 high Foxp3 + T Cells Secreting Interleukin-10 and T ransforming Growth Factor- B 1Mediates Suppression in the T umor Microenvironment. *Clin Cancer Res*. 2007; **13**(15): 4345-4355.
[88] 88. Sukiennicki TL, Fowell DJ, Sukiennicki TL, Fowell DJ. Distinct Molecular Program Imposed on CD4 + T Cell Targets by CD4 + CD25 + Regulatory T Cells. *J Immunol*. 2019; **177**: 6952-6961.
[89] Tarhini AA, Gogas H, Kirkwood JM. IFN-α in the Treatment of Melanoma. *J Immunol*. 2012; **189**(8): 3789-3793.
[90] Theate I, Baren N Van, Pilotte L, et al. Extensive Profiling of the Expression of the Indoleamine 2 , 3-Dioxygenase 1 Protein in Normal and Tumoral Human Tissues. *Cancer Immunol Res*. 2015; **3**(2): 161-173.
[91] Umansky V, Gebhardt C. Myeloid-derived suppressor cells in malignant melanoma. *J Ger Soc Dermatology*. 2014:1021-1027.
[92] Vacchelli E, Aranda F, Eggermont A, et al. Trial watch : IDO inhibitors in cancer therapy. *Oncoimmunology*. 2014; **3**(10).
[93] Vargas FA, Furness AJS, Solomon I, et al. Fc-Optimized Anti-CD25 Depletes Tumor-Infiltrating Regulatory T Cells and Synergizes with PD-1 Blockade to Eradicate Established Tumors. *Immunity*. 2017; **46**: 577-586.
[94] Wargo JA, Alexandre R, Zooper ZA, Oh KS, Sullivan RJ. Immune Effects of Chemotherapy, Radiation, and Targeted Therapy and Opportunities for Combination With Immunotherapy. *Semin Oncol*. 2015; **42**(4): 601-616.
[95] Weber JS, Angelo SPD, Minor D, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment ( CheckMate 037 ): a randomised, controlled, open-label, phase 3 trial. *Lancet*. 2015; **16**: 375-384.
[96] Willigen WW VAN, Bloemendal M, Gerritsen WR, Schreibelt G. Dendritic Cell Cancer Therapy : Vaccinating the Right Patient at the Right Time. *Front Immunol*. 2018; **9**(2265): 1-13.
[97] Yentz S, Smith D. Indoleamine 2,3 Dioxygenase ( IDO ) Inhibition as a Strategy to Augment Cancer Immunotherapy. *BioDrugs*. 2018; **32**: 311-317.
[98] Yu X, Harden K, Gonzalez LC, et al. The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. *Nat Immunol*. 2009; **10**(1): 48-57.
[99] Zelante T, Fric J, Wong AYW, Ricciardi-Castagnoli P. Interleukin-2 production by dendritic cells and its immuno-regulatory functions. *Front Immunol*. 2012; **3**: 1-5.

*Redaktor prowadzący – Maciej Zabel*

*Otrzymano: 09.05.2019*
*Przyjęto: 24.06.2019*
*Aleksander Kiełbik*
*phone: +48 606970564*
*e-mail: akielbik6@gmail.com*