

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Immunotargeting of Melanoma

Jacek Mackiewicz<sup>1</sup> and Andrzej Mackiewicz<sup>1,2</sup>

<sup>1</sup>*Department of Cancer Immunology, Chair of Medical Biotechnology, Poznan University of Medical Sciences and Greater Poland Cancer Center, Poznan,*

<sup>2</sup>*BioContract Sp. z o.o. Poznan, Poland*

## 1. Introduction

The main task of the immune system is to defend the body against pathogens. The ability of the immune system to recognize and eliminate cancer cells is the basis for cancer immunotherapy. There is ample evidence of how important role plays the immune system to fight cancer: (i) spontaneous remission in patients with certain cancers; (ii) the presence of specific cytotoxic T lymphocytes in the environment of the tumor or regional lymph nodes, (iii) the presence of monocytes, lymphocytes and plasma cells infiltrating the tumor, (iv) increased incidence of some malignancies in immunosuppressed patients, (v) documented remissions of the disease after use of immunomodulators. Better understanding of the molecular and cellular mechanisms that control the immune system has enabled the development of many innovative and promising therapeutic strategies that modulate the immune response. It seems that in the next 5 to 10 years past surgery, radiotherapy and chemotherapy, immunotherapy will find a permanent position in the treatment of cancer modalities.

## 2. Anti-tumour immune response

Cancerogenesis is closely associated with non-lethal damage of genetic material. Such genetic damage (mutations) can stem from environmental factors, e.g. chemical, radioactive or viral, or can be hereditary. Neoplastic transformation occurs due to accumulation of mutations, predominantly in two gene classes: protooncogenes and suppressor genes. Mutated protooncogenes, termed oncogenes, promote autonomous cell proliferation. Proteins produced by the oncogenes transmit signals to cell nucleus and induce cell division. In contrast, mutated suppressor genes become inactivated and their protein products, deprived of their suppression properties, are not capable of controlling incorrect proliferation. Mutations in apoptosis-regulation genes via the synthesis of improper proteins develop mechanisms preventing programmed cell death.

Burnet's and Thomas' immune surveillance theory assumes that newly formed neoplastic cells are continually monitored by the human immune system which recognises and eliminates them. At some point, however, tumour cells escape immune surveillance, what may result in fully-fledged tumours. During the last twenty years, a number of mechanisms which enable tumour cells to evade effector immune mechanisms have been identified. They include:

- reduced expression/absence of expression of major histocompatibility complex antigens class I and II – (MHC I and II) (Resifo *et al.*, 1993; Garrido *et al.*, 1993; Ward *et al.*, 1990)
- loss of tumour antigens (Knurth *et al.*, 1989; Uyttenhove *et al.*, 1983; Ward *et al.*, 1989)
- improper intracellular antigen processing to prepare antigens for presentation (defects in proteosome function or adenosine-tri-phosphatate (ATP) dependent proteins transporting TAP peptides (Selinger *et al.*, 1998)
- reduction/loss of costimulatory signals of e.g. B7 molecules on the surface of tumour cells
- suppressed expression of adhesion molecules
- inhibited expression of the Fas receptor and/or Fas ligand resulting in the apoptosis of cytotoxic T lymphocytes (CTL) and/or natural killer (NK) cells (Strand *et al.*, 1996; Hahne *et al.*, 1996; Walker *et al.*, 1997)
- synthesis and secretion of immunosuppressive factors, e.g. interleukin (IL)10, TGF-beta, prostaglandine E<sub>2</sub> suppressing immune response (Boon *et al.*, 1995; Schmidt-Wolf *et al.*, 1995)
- expression of TRIAL (TNF-related apoptosis inducing ligand) on the tumour cell surface leading to T lymphocyte apoptosis (Baker *et al.*, 1998)
- prolonged stimulation of specific T cells resulting in clonal exhaustion or AICD (activation-induced cell death) (Overwijk *et al.*, 2001)

The term “cancer immunosurveillance” is no longer sufficient to precisely describe the difficult interactions that arise among a developing tumor and the immune system of the host. It was believed that cancer immunosurveillance was protecting the host by the adaptive immune system in the early phase of cell transformation. Dunn and coworkers described that not only the adaptive but also innate immune system plays a role in the process and serve not only to defend the host from tumor development but also to sculpt, or edit, the immunogenicity of tumors that may finally form. The authors use a wider term - “cancer immunoediting” to more suitably stress the double roles of immunity in not only preventing but also shaping neoplastic disease. According to this theory cancer immunoediting comprises of three phases: elimination, equilibrium and escape.

Elimination phase is based on the original concept of cancer immunosurveillance. If during this phase the developing tumor can be successfully eliminated the immunoediting process is completed without progression to the subsequent phases.

If the neoplasm circumvents the immune surveillance system, it may enter the next sub-clinical stage, termed the *equilibrium phase*, in which tumour tries to establish itself and withstand the growing pressure exerted by the immune system. Although lymphocytes and the secreted IFN-gamma attack the tumour cell, its genetic instability and multiple mutations protect it against destruction. The equilibrium phase is a long-term process and may last for years. The next stage is the *escape phase* which can stem from the exhaustion or suppression of the immune system, reduced expression of type I MHC on neoplastic cells or decreased sensitivity to interferon (IFN) gamma. The equilibrium phase does not occur in non-immunogenic tumours and the modified cell automatically enters the escape phase. This clinical stage is associated with fast growth and progression of the tumour (Dunn *et al.*, 2002 & 2006).

In tumours induced by viruses and chemical substances it has been found that tumour-related antigens are immunogenic and trigger specific cellular and humoral responses. Cytotoxic cells, including CD8<sup>+</sup> CTL, NK cells and CD4<sup>+</sup> T helper cells, are presumed to

play a decisive role in the process. Cellular immune response also involves neutrophil granulocytes (Cavallo *et al.*, 1992) and macrophages. Humoral response is executed by antibodies which are directed against tumour antigens produced by activated B lymphocytes – plasmocytes. The process leading to the lysis of tumour cells in this mechanism may comprise activation of the complement system or induction of ADCC (Antibody Dependent Cell-mediated Cytotoxicity).

The role of different subpopulations of helper T lymphocytes (Th cells) has not been fully explored yet (Nishimura *et al.*, 1999). Cytokines secreted by helper T cells activate humoral or cellular anti-tumour immune response. Depending on the profile of secreted cytokines, Th cells are divided into two sub-groups: Th1 producing IL-2, IFN-gamma and IL-12 inducing cellular immune response; and Th2 secreting IL-4, IL-5, IL-6 and IL-10 which stimulate the humoral response and suppresses the cellular response (Swain *et al.*, 1995). Moreover, the subpopulation of regulatory T cells CD4<sup>+</sup>/CD25<sup>+</sup>highFoxp3 may inhibit immune response by paracrinous secretion of immunosuppressive cytokines IL-10 and TGF-beta. Th17 lymphocytes belong to the most recently described CD4<sup>+</sup>Th . A characteristic feature of these cells is the secretion of interleukin-17 but also IL-22, IL-26, IL-6, TNF-alfa. So far, no unequivocal influence of Th17 lymphocytes for the development of cancer has been described. It was shown that IL-17 may promote tumor cell growth by inducing tumor vascularization or enhancing inflammation. Meanwhile, a lot of data suggests the antitumor activity of Th17 cells. It therefore appears that Th17 cells may have different effects on tumor growth depending on its immunogenicity, clinical stage (different role in the early and late stages), as well as the origin of cancer and the influence of inflammation and angiogenesis in tumor microenvironment (Hus *et al.*, 2010).

Effective anti-tumour response elicited by the immune system consists of two phases, induction and effector. The induction phase is marked by stimulation of specific anti-tumour response, while the effector phase involves a selective elimination of tumour cells. The following mechanisms are sequentially activated in the induction phase:

1. Presentation of tumour antigens in the context of HLA (Human Leukocyte Antigens) class I to CD8<sup>+</sup> lymphocytes, or HLA class II to CD4<sup>+</sup> lymphocytes;
2. Providing the costimulatory signal for T lymphocytes, e.g. binding of B7.1 molecules (CD80, CD86) with the CD28 receptor on the surface of T lymphocytes (Janeway *et al.*, 1994)
3. Providing the proliferation signal for immune cells in the area of tumour antigen presentation (typically cytokines or growth factors) (Pardoll *et al.*, 1995)

Induction of immune response is initiated by antigen presentation by dendritic cells (DCs) (Banchereau *et al.*, 1998). DCs phagocyte antigens from necrotic or apoptotic tumour cells and then migrate into regional lymph nodes, where they undergo maturation. In the lymph nodes, they present the antigen on their surface to T lymphocytes CD8<sup>+</sup> (in the context of HLA I) and to T lymphocytes CD4<sup>+</sup> (in the context of HLA II). This is where CD8<sup>+</sup> and CD4<sup>+</sup> CTLs are formed together with antibodies directed against specific tumour antigens.

The stimulation of humoral response requires a presentation of tumour antigen by a B lymphocyte to the specific CD4<sup>+</sup>T lymphocyte. Direct lymphocyte interaction, as well as production of cytokines by CD4<sup>+</sup>, triggers transformation of the B lymphocyte into a plasma cell and secretion of antibodies.

The effector phase of anti-tumour response may include the following cell destruction mechanisms by:

1. Specific activated CD8+ and CD4+ CTLs
2. Activated NK cells
3. Tumour-infiltrating granulocytes and macrophages
4. Specific antibodies determining activation of the complement system or ADCC
5. Inhibition of tumour neoangiogenesis by cytokines, such as IFN-gamma secreted by activated T lymphocytes (CD8+ and CD4+) into the tumour microenvironment.

Absence of one or several components listed above makes it possible for tumour cells to escape immune surveillance, which leads to tumour progression. Mechanisms involved in the induction and effector phases of anti-tumour response which have been identified so far makes it possible to apply immunotherapy to restore the capacity to recognise and eliminate tumour cells in immune defence mechanisms (Nanda *et al.*, 1995).

### 3. Tumour immunotherapy

Tumour immunotherapy is a treatment strategy based on intentional interference with the human immune system in order to enhance or modify the body's defence mechanisms against the developing tumour. Immune therapy can be divided into two major categories: passive and active. Within each category the therapy might be either specific or non-specific.

#### 3.1 Passive non-specific immunotherapy

Passive non-specific immunotherapy involves transfer of factors or activated effector cells to elicit a non-specific activation of the immune system provoking anti-tumour response. Immunotherapeutic approaches may use e.g. cytokines or LAK (Lymphokine Activated Killers) cells.

Cytokines are low molecular weight proteins which play a vital role in all phases of immune response, both humoral and cellular. In order to display biological activity, cytokine must target a specific receptor on immune cells (T and B lymphocytes, NK cells, monocytes/macrophages and granulocytes). Various cytokines may demonstrate an antagonistic, agonistic, additive or synergistic effects in biological processes. Known anti-tumour effects of cytokines include (i) direct cytotoxic effect (TNF-sensitivity of cancer cells to cytotoxic effects of various biological or chemical factors (IFN-gamma, TNF-alpha), (ii) inhibition of tumour cells proliferation (IFN-alpha, IFN-gamma) and (iii) activation of NK cells (Granulocyte-Macrophage Colony-Stimulating Factor – GM-CSF, IL-2, IL-6).

The first recombinant cytokine registered for clinical application was IFN-alpha which has been used in the treatment of hairy-cell leukaemia, chronic myeloid leukaemia, melanoma, Kaposi's sarcoma, metastatic renal cell cancer and malignant lymphoma.

Several randomised phase III trials evaluating IFN-alfa-2a and IFN-2b in low, medium and high dose have been conducted. Only in two of them statistically significant improvement of OS (*overall survival*) was observed. High dose IFN-alfa-2b (Intron®) has been approved by the U.S. FDA (*Food and Drug Administration*) based on the results of ECOG 1684 trial. Intron is indicated in patients after resection of high-risk melanoma (stage IIB and stage III). In the registration trial 287 patients after surgical removal of melanoma were randomised into two study arms: INF-alfa-2b vs observation. At a median follow-up of 6.9 years, a statistically significant improvement in survival was demonstrated for the patients treated with IFN-alfa-2b. However at 12,6 years of follow-up, OS was not significantly different between the two study groups, even though there was a significant benefit for relapse free survival (RFS). About 80% of patients developed grade 3 and 4 toxicity (Kirkwood *et al.*, 1996). In



another phase III study (ECOG 1694) efficacy of high-dose IFN- $\alpha$ -2b was compared with an experimental vaccine GM2-KLH21. After 2 years of median follow-up the median RFS and OS were significantly longer in patients treated with IFN- $\alpha$ -2b (Kirkwood *et al.*, 2000). Concerns raising the vaccine control group used in ECOG 1694 lead to initiation of another randomized phase III trial (EORTC 18961). The study which enrolled 1314 patients with stage II melanoma evaluated efficacy of GM2-KLH21 compared with observation. The trial was closed early by the data monitoring committee because of longer survival in the observation arm (Eggermont *et al.*, 2008a). Recently (March 2011) pegylated-IFN- $\alpha$ -2b (Sylatron®) has been approved for the treatment of patients with melanoma with microscopic or gross nodal involvement after definitive surgical resection including complete lymphadenectomy. The approval was based on the results of EORTC 18991 trial published in Lancet in the year 2008. The study enrolled 1256 patients with resected stage III melanoma to either observation or pegylated-IFN- $\alpha$ -2b. The estimated median RFS was 34.8 months (95% confidence interval (CI): 26.1, 47.4) and 25.5 months (95% CI: 19.6, 30.8) in the Sylatron and observation arms, respectively [hazard ratio (HR) 0.82 (95% CI: 0.71, 0.96); unstratified log-rank  $p = 0.011$ ]. Unfortunately, there was no difference in OS between the Sylatron and the observation arms [HR 0.98 (95% CI: 0.82, 1.16)]. The grade 3 and 4 adverse events were less frequent in patients treated with pegylated-IFN- $\alpha$ -2b than observed in subjects receiving IFN- $\alpha$ -2b in earlier trials (Eggermont *et al.*, 2008b).

Another cytokine registered in the USA for palliative treatment of renal cancer and melanoma is IL-2, however, it has recently been demonstrated that high doses of IL-2 stimulate Treg lymphocytes, what in fact suppresses immune response (Ahmadzadeh *et al.*, 2006).

IL-2 was approved by FDA in 1998 for the treatment of patients with metastatic melanoma. Overall objective response rates in patients treated with high dose IL-2 was 17% (Rosenberg *et al.*, 1994a). In a highly selected patient population with metastatic melanoma (270 patients) complete response (CR) was observed in 6% with median duration of response over 59 months. Partial response (PR) was seen in 10% of IL-2 treated patients. Disease did not progress in any patient responding for more than 30 months. Grade 3 and 4 toxicity was very frequent (Atkins *et al.*, 1993 & 1994). In another trial 684 patients with metastatic melanoma received high-dose IL-2 either alone or in combination with a variety of melanoma vaccines. The overall objective response rates were highest in patients treated with gp100:209-217(210M) peptide vaccine (22%) compared to IL-2 (13%) alone ( $P=0.01$ ) (Smith *et al.*, 2008). Number of clinical trials evaluating the efficacy of biochemotherapy (combination of chemotherapy and biological agents) have been conducted. In a small phase III trial the investigators compared sequential CVD (dacarbazine, cisplatin, vinblastine) chemotherapy with IL-2 and interferon  $\alpha$  with CVD alone. Patients treated with biochemotherapy responded more frequently (48%) to the treatment than receiving chemotherapy alone (25%). The median OS was 11,9 months vs. 9,2 months for biochemotherapy and CVD alone respectively (Eton *et al.*, 2002). In another similar phase III randomized trial (E3695) the observation seen in the previous study was not confirmed – no improvement in OS between both study arms (CVD + IL-2 + IFN- $\alpha$ -2b vs CVD) (Atkins *et al.*, 2008). A recent meta-analysis of 18 trials involving 2621 patients with metastatic melanoma showed that biochemotherapy improves overall response rate without benefit of survival (Ives *et al.*, 2007).

At the American Society of Clinical Oncology (ASCO) 2010 annual meeting Lawson *et al.* presented results of a phase III trial (E4697) evaluating efficacy of GM-SCF in the adjuvant

treatment in patients with resected high risk melanoma (stage III and IV). Patients were injected with 250 µg of GM-CSF or placebo s.c. daily for 14 consecutive days in a 4 week intervals. The treatment duration was 1 year. Median disease free survival (DFS) of patients treated with GM-CSF was significantly longer than of patients receiving placebo – 11,8 vs. 8,8 months ( $p=0,034$ ). Median OS was 72,1 vs. 59,8 months respectively in the study arm evaluating GM-CSF and placebo, but the difference was not statistically significant ( $P=0,551$ ). Toxicity was consistent with known effects of GM-CSF (Lawson *et al.*, 2010).

Another type of passive non-specific immunotherapy is the transfer of LAK cells. In this method, mononuclear cells are isolated from the blood of a patient, stimulated with IL-2 *ex vivo* and injected back with a simultaneous administration of high doses of IL-2 for continuous lymphocyte stimulation. LAK-based therapy was used by Rosenberg in a clinical trial of renal cancer and melanoma. It was found, however, that the therapeutic effect was rather achieved by IL-2, but not LAK cells, which is why further trials were discontinued (Rosenberg *et al.*, 1985 & 1993).

### 3.2 Passive specific immunotherapy

Passive specific immunotherapy is a treatment method based on the administration of factors or effector cells targeting specific tumour cells. Examples include application of antibodies directed against antigens present on tumour cells or cell therapies using tumour-infiltrating lymphocytes (TIL) which are isolated, cultured, activated and then transferred back into the patient. High expectations are currently held for the recently developed therapy based on modification of autologous lymphocytes isolated from PBLs (Peripheral Blood Lymphocytes) (Morgan *et al.*, 2006).

Passive immunotherapy employing antibodies was first described as far back as 100 years ago. However, it was not until the development of a viable technique of generating monoclonal antibodies (mAb) (Kohler *et al.*, 1975) that the method found more extensive applications in tumour therapy. The dynamic development of genetic engineering techniques has enabled generation of humanised and human mAb (technology using transgenic mice – Xenomouse®) (Green, 1999) deprived to the maximum extent possible of toxic effects associated with the induction of HAMA (Human Anti Murine Antibody) reaction.

Modified specific mAb used in immunotherapy act by binding directly to the tumour antigen, activate ADCC and CDC (Complement Dependent Cytotoxicity). Monoclonal antibodies can also block receptors on tumour cells, e.g. growth factor receptors. Antibodies conjugated with radioisotopes, cytostatics, enzymes, cytokines or toxins immediately destroy cells which they target.

The first mAb registered by the FDA in 1997 was Rituximab (MabThera®) used in the treatment of patients with low malignancy B-cell non-Hodgkin lymphomas. To date there are several mAb (trastuzumab, cetuximab, panitumumab, bevacizumab, ibritumomab, tiuxetan, tositumomab, gemtuzumab, ozogamicin, alemtuzumab) approved for the treatment of various malignancies. Ipilimumab, anti-CTLA4 (Cytotoxic T-lymphocyte-associated antigen 4) mAb has been recently registered for the treatment of metastatic melanoma (described in section: active non-specific immunotherapy).

Angiogenesis is of profound importance in melanoma development (Streit *et al.*, 2003). Malignant melanocytes excrete vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) (Kurzen *et al.*, 2003, Lacal *et al.*, 2000, Lev *et al.*, 2003 & 2004). The VEGF is the fundamental regulator of new vessels formation in the tumour and its expression is

related to poorer prognosis in melanoma patients (Ugurel *et al.*, 2001). Bevacizumab is a mAb directed against VEGF. Its effectiveness has been proven in the treatment of colon and kidney cancer (Saltz *et al.*, 2008, Melichar *et al.*, 2008). In the treatment of advanced melanoma its efficacy was evaluated in combination with low dose interferon- $\alpha$ -2b in a phase II trial which did not show extension of survival in studied patients (Varker *et al.*, 2007). In another phase II trial enrolling 62 metastatic melanoma patients, the effectiveness of bevacizumab in combination with temozolomide was tested. Objective clinical responses were observed in 26% of patients, while 30% of the patients developed SD (*stabilization disease*) lasting for 1.5–7.5 months (median of 3 months) (Von Mos R *et al.*, 2007). In a subsequent phase II trial with 214 randomized patients, the therapy with bevacizumab in combination with carboplatin and paclitaxel (first line treatment) improved effectiveness as the median OS was significantly longer (12.3 months) than in the control group (8.6 months) treated with chemotherapy alone. The toxicity of treatment was similar in both study arms (the number of grade 3–5 adverse events was 2% greater in the group treated with bevacizumab) (O'Day *et al.*, 2009). At the ASCO 2009 annual meeting, preliminary results were presented on the effectiveness of treatment with bevacizumab in combination with nab-paclitaxel in 41 patients with non-resectable III and IV stage melanoma (50% in M1c stage). Nab-paclitaxel is composed of paclitaxel and albumin. This combination alleviates the adverse events caused by administration of paclitaxel alone. The percent of 6 and 12-months survival was 91 and 83%, while the median OS was not reached (Boasberg *et al.*, 2009). The preliminary results of a phase II trial evaluating the efficacy of bevacizumab in combination with everolimus in first line treatment of advanced melanoma were presented at ASCO 2009 conference. Fifty six patients were qualified to the study, but the preliminary analysis included 31 subjects. PR was observed in 1 patient, SD in 19 patients and the median PFS was 3.5 months. The most frequent adverse event was mucositis. Grade 3 toxicity was observed in 13% of patients, other grade 3 adverse events were noted in less than 10% of subjects (Peyton *et al.*, 2009).

$\alpha_v$  integrin family such as  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  are related to the promotion of angiogenesis in the tumor and contribute to its growth. Human mAb CNTO 95 (intetumumab) directed against integrin has been found to show anticancer and antiangiogenic activity in animal models (Tripathi *et al.*, 2004). At the ASCO 2009 annual meeting results of the phase II trial with 129 metastatic melanoma patients enrolled, were reported. The patients were randomised into four study arms: (i) intetumumab 5 mg/kg; (ii) intetumumab 10 mg/kg; (iii) intetumumab 10 mg/kg + DTIC; (iv) *placebo* + DTIC. Median OS of patients respectively in the above-mentioned groups was 9.8; 14; 10.9 and 7.6 months. The treatment was well tolerated and the percent of patients with serious adverse events was the highest in the arm treated with dacarbazine (DTIC) plus *placebo* (Loquai *et al.*, 2009). Volociximab is a chimeric monoclonal antibody that combines with  $\alpha_5\beta_1$  integrin, inhibiting angiogenesis in tumors by damaging the bonds between endothelial cells and fibronectin in the intracellular cytoplasm. Effectiveness of volociximab was evaluated in a phase II trial which enrolled 19 patients diagnosed with advanced melanoma after progression during the first line treatment. Objective clinical responses were observed only in 5% of the patients. Responses to the treatment were associated with the expression of integrin  $\alpha_5\beta_1$  in patients tumor samples. The treatment was relatively well tolerated. Grade 1 and 2 toxicity (nausea, vomiting, diarrhoea) occurred in 68% of patients (Linette *et al.*, 2008).

Overexpression of glycoprotein NMB (GPNMB) was observed in many malignancies including melanoma. CRO11-vcMMAE is a human mAb targeting extracellular domain of



GPNMB, combined with the tubulin destabilising factor – monomethyl auristatin E (MMAE). The whole complex is stable in the blood, while MMAE is released within the tumour (Tse *et al.*, 2006). CRO11-vcMMAE was evaluated in phase I/II clinical trials. At ASCO 2009 the phase II study results were reported (MTD 1.88 mg/kg); this trial enrolled 36 patients with metastatic melanoma treated earlier with systemic therapy. 4 PR and 19 SD were observed, while the median PFS was 4 months. The most often noted grade 3 and 4 included neutropenia (22% patients) and rash (19%). Rash grade 2 and higher correlated with longer PFS (Hwu *et al.*, 2009).

Another therapeutic strategy of specific immunotherapy is the use of TIL cells which are extracted from patients tumors and incubated *ex vivo* in the presence of IL-2. Next they are transferred back into corresponding patients with a simultaneous administration of IL-2. In 1994, *et al.* observed objective clinical responses in 34% of patients with metastatic melanoma (Rosenberg *et al.*, 1994b). However, therapeutic benefit was assigned to i.v. injected IL-2 which stimulated lymphocytes.

To increase the efficacy of adoptive cell therapy, prior to TIL infusion, chemotherapy and/or total body irradiation (in order to obtain lymphodepletion – elimination of suppressor T-cells) followed by administration of IL-2 was applied. Although effectiveness of this strategy was limited in the treatment of leukemia (Curti *et al.*, 1998; Schultze *et al.*, 2001) some activity in the therapy of melanoma was observed (Oble *et al.*, 2009). Patients with metastatic melanoma prior to TIL and IL-2 infusion received a lymphodepleting dose of cyclophosphamide and fludarabine (Dudley *et al.*, 2005). In 51% (n=35) of treated patients objective response rate was observed. A very high overall response rate (70%) was seen in another trial where in melanoma patients prior to TIL transfusion a total body irradiation with a dose of 1200 cGy (in 3 fractionated doses) was obtained (Rosenberg *et al.*, 2008). Clinical trials, which tested peripheral blood lymphocytes stimulated with cytokines did not produce the desired result due to low numbers of antigen-specific lymphocytes. In order to increase the number of antigen-specific lymphocytes an *in vitro* stimulation with DC loaded with particular antigen can be obtained. In a phase I study patients with metastatic melanoma were treated with CD8+ T cell isolated from peripheral blood and incubated *ex vivo* with mature autologous DC pulsed with melanoma antigen Melan-A. Objective responses were seen in 3 out of all 11 patients. (Mackensen *et al.*, 2006). It has been proven that administration of CD4+ T-cells instead of CD8+ lymphocytes may turn out to be more effective in adoptive cell therapy (Perez-Diez *et al.*, 2002). Remission was observed in a patient with metastatic melanoma who received autologous CD4 + T cells specific for the melanoma antigen NY-ESO-1 (Hunder *et al.*, 2008).

Morgan *et al.* isolated PBLs which were then modified *ex vivo* with genes coding for TCR specific for a number of tumour-associated antigens (MART-1, gp100, NY-ESO-1 or p53). They demonstrated that modified PBLs recognise, in the context of HLA-A2, the above-mentioned tumour antigens present on melanoma, lung and breast cancer cells. The interaction of TCR and tumour peptides was associated with the production of a large quantity of IFN-gamma. In the clinical trial, Morgan *et al.* enrolled 31 patients with advanced melanoma resistant to IL-2 therapy. All subjects received autologous PBLs engineered with the anti-MART-1 TCR gene. Two patients showed a prolonged remission lasting more than 20 months. Patients who achieved a clinical response also exhibited high levels of modified circulating lymphocytes after 1 year from the injection of transduced PBLs. No toxicity was found to be associated with this type of therapy (Morgan *et al.*, 2006).

### 3.3 Active non-specific immunotherapy

Active non-specific immunotherapy is a therapeutic method based on stimulation of the immune system, cell-mediated immune response in particular, with antigens that are not present in tumour cells. Historically, the therapy has involved microorganisms, microbial fragments, enzymes and hormones. In recent years, due to the development of advanced genetic engineering technology and growing understanding of immune mechanisms involved in tumour growth, immune response-modulating mAbs were constructed.

Substances which contribute to the stimulation of immune processes include non-specific immunostimulators and immunomodulators. Immunostimulators include (i) intact microorganisms (living BCG – *Bacillus Calmette-Guérin*, killed *Corynebacterium parvum*), (ii) cell wall elements (BCG, *Nocardia*), (iii) microbial glycoproteins derived from *Klebsiella*, (iv) synthetic components, e.g. endotoxins (lipopolysaccharides). Immunomodulators include (i) thymus extracts (TPI, THF, TFX), (ii) synthetic thymus hormones (thymosin alpha-1, thymopoietin), (iii) tuftsin, (iv) enkephalins and endorphins (v) lymphocyte extracts (transfer factor, immunogenic RNA) (Hersh *et al.*, 1991).

Some of the substances listed above, when injected directly into the tumour, have triggered local inflammatory reaction associated with the infiltration of APCs, neutrophils, and T and B lymphocytes. Regression of injected tumours was often accomplished, however, the method was not capable of producing a specific systemic anti-tumour responses.

No active non-specific immunotherapy modality has entered routine clinical practice as yet. Passive non-specific immunotherapy includes also monoclonal antibodies modulating the immune system. CTLA-4 is an immune checkpoint molecule that is up-regulated on activated T-cells, which suppresses further activation of specific CD4+ and CD8+ T-cells by interaction with DC or directly as a result of a contact between suppressor and effector T lymphocytes. The anti-CTLA4 monoclonal antibody by blocking the interaction of CTLA-4 with CD80/86 switches off the mechanism of immune suppression and enables continuous, unrestrained stimulation of T-cells by DC (J. Mackiewicz & Kwinta, 2010). Two IgG monoclonal antibodies directed against CTLA-4 – ipilimumab and tremelimumab have been tested in number of clinical trials in patients with melanoma. In 2011 (March) ipilimumab (Yervoy®) has been approved by FDA for the treatment of metastatic melanoma patients that failed previous systemic therapy. The approval was based on the results of a randomized phase III trial which included 676 HLA-A\*0201-positive patients with unresectable stage III or IV melanoma. Patients enrolled were treated previously with IL-2 or chemotherapy and were randomly assigned for administration of ipilimumab plus glycoprotein (gp)100 vaccine (403 patients), ipilimumab alone (137), or gp100 alone (136). Ipilimumab, at a dose of 3 mg per kilogram of body weight, was administered with or without gp100 every 3 weeks for up to four treatments. Patients receiving ipilimumab plus a peptide vaccine had a median survival of 10 months, compared with 6.4 months of patients receiving the gp100 alone ( $P < 0.001$ ). Subjects treated with ipilimumab alone had a nearly identical median survival – 10.1 months – in the 3-group clinical trial ( $P < 0.003$ ). Immune related adverse events (grade 3 and 4) occurred in 10-15% of patients treated with ipilimumab and in 3% treated with gp100 alone (Hodi *et al.*, 2010).

Another monoclonal antibody targeting CTLA-4 is tremelimumab (administered in the dose of 15 mg/kg every 3 months), which effectiveness has been evaluated in a phase II trial in previously treated patients. From among 256 patients with metastatic melanoma enrolled into the study, objective clinical responses were observed in 8.3% patients, while the median OS was 10.2 months (Kirkwood *et al.*, 2008). The above results inclined the next phase III

trial in which 643 patients were treated with tremelimumab in monotherapy or in combination with DTIC/TMZ in the first line setting. Analysis of preliminary results did not show the advantage of tremelimumab over the standard therapy (OS 11.8 *vs* 10.7) and the trial was terminated (Ribas *et al.*, 2008). The effectiveness of treatment with tremelimumab in combination with high doses interferon-alfa-2b was evaluated in phase II trial in which from among 16 patients with inoperable stage III and IV melanoma, clinical response was observed in 19%. The most frequent grade 3 and 4 adverse events included: neutropenia (3 patients, 19%), elevated level of the liver enzymes (2, 13%), fatigue (6, 38%), anxiety (2, 13%) (Tarhini *et al.*, 2008).

Another human mAb modulating the immune system is MDX-1106 (Medarex) directed against PD-1 (a molecule close to that of CTLA-4), which undergoes expression on activated T lymphocytes. Results of the phase I trial have shown regression of the tumours in patients with advanced melanoma and low toxicity of the treatment (Brahmer *et al.*, 2008).

The monoclonal antibody BMS-663513 targeting co-stimulating molecule CD137 (4-1BB) acts according to a different mechanism. Binding of the ligand or anti-CD137 antibody with 4-1BB receptor on the surface of T lymphocytes provides a co-stimulating signal enhancing the cell's activation and triggering its proliferation. The phase I trial enrolling 54 patients with solid tumors has shown acceptable toxicity level and a certain clinical activity of BMS-663513 (Sznol *et al.*, 2008). We look forward to the results of large randomised phase II study which has just been completed (US National Institutes of Health [NIH], 2008a). CP-870.893 is a human agonistic mAb against co-stimulating molecule CD40 that is up-regulated on the surface of the APC. Phase I trial has shown PR in 4 (27%) out of 15 patients with advanced melanoma and 1 CR lasting 18 months after single administration of the drug (Vonderheide *et al.*, 2006). Currently, the trial evaluating the efficacy of CP-870,893 in combination with carboplatin and paclitaxel has been completed and the results probably will be disclosed soon (US National Institutes of Health [NIH], 2008b).

### 3.4 Active specific immunotherapy

Active specific immunotherapy is a method of treatment which stimulates immune response to antigens specific for a given tumour type.

Active specific immunotherapy includes therapeutic cancer vaccines which encompass cell and non-cell based products. Cell based vaccines comprise: cancer cell lysates, whole cancer cells with adjuvants, gene modified whole cancer cells, DCs pulsed with DNA, RNA, peptides, proteins or cell lysates, pulsed DCs modified with immune stimulators, fused cancer cells with DCs cells or B-lymphocytes. Non-cell based vaccines include DNA vaccines (naked, plasmid), peptide vaccines, protein vaccines, viral-vector vaccines, anti-idiotypic antibody vaccines, particle based vaccines (Table 1) (J. Mackiewicz & A. Mackiewicz, 2009).

#### 3.4.1 Therapeutic cancer vaccines

As early as in 1883, a New York surgeon William Colley was the first to make an attempt at administering a cancer vaccine. Colley injected bacterial toxins into sarcoma patients and observed disease remission.

First-generation cancer vaccines were created from irradiated autologous or allogeneic tumour cells. Cell lysates or natural cell-surface tumour antigens (such as gangliosides GD-2 and GM-2) were also used. In first-generation vaccines, injected cells or antigens were phagocytosed and degraded by mononuclear cells which are then presented to T and B

lymphocytes in the context of MHC molecules class I and II. Second generation vaccines include gene-modified tumour vaccines (GMTV) which use (autologous, allogeneic or mixed) tumour cells modified with genes encoding tumour antigens, immunostimulating factors, e.g. cytokines. Their functions include supply and presentation of tumour antigens together with providing co-stimulatory signal for the stimulation of specific anti-tumour mechanisms.

Vaccine type	Vaccine	Reference
Peptide	Glycoprotein 100 (gp100)/tyrosinase complex with Incomplete Freund Adjuvant (IFA) and granulocyte macrophage colony-stimulating factor (GM-SCF)	Weber et al., 2003
	Melanoma specific peptide (MART-1) + Incomplete Freund Adjuvant (IFA)	Wang et al., 2003
	Melanoma specific peptide MAGE-A3	Goldman & DeFrancesco 2009
Heat Shock Protein (HSP)	Autologous, tumor-derived heat-shock protein peptide complex glicoprotein96 + granulocyte macrophage colony-stimulating factor (GM-CSF)+ineterferon-alfa	Pilla et al., 2006
DNA	DNA coding epitopes of melanoma antigens: MELAN-A/MART-1	Weber et al., 2008
	DNA coding co-stimulating molecule – B7	Fynan et al., 1993
Viral vector	Vaccinia virus expressing three costimulatory molecules, B7.1, ICAM-1, and LFA-3 (rV-TRICOM)	Kaufman et al., 2006
Anti-idiotypic antibody	BEC2 anti-idiotypic monoclonal antibody vaccine that mimics GD3 ganglioside	Chapman et al., 2004

Tabele 1. Selected clinical non-cellular based vaccine studies in patients with melanoma

The strategy of immunisation with genetically unmodified autologous DCs is based on *ex vivo* preincubation of DCs with tumour antigens, followed by administration to patients. This type of immunotherapy activates an antigen-specific cell-mediated response.

3.4.1.1 Non-modified cell vaccines

Vaccines based on whole tumour cells and stimulating factors (adjuvants) were one of the first and fundamental specific tumour immunotherapy strategies. Berd *et al.* (1990) evaluated the effects of immunisation of forty late-stage melanoma patients with a vaccine consisting of irradiated autologous melanoma cells mixed with BCG. Objective clinical response was observed in 5 patients, whereas median survival time was 10 months. The next stage was the use of established cell lines (allogeneic vaccines) which present antigens specific for a given tumour type. Their immunogenicity was enhanced by response to alloantigens present on vaccine cells. Allogeneic vaccines have superseded autologous vaccines due to the difficulties in obtaining the sufficient number of cells for repeated



vaccinations. A vaccine consisting of three established allogeneic melanoma lines (Cancervax®) and BCG as an adjuvant was developed by Morton *et al.* (1992). The ensuing phase II trial involved 157 advanced melanoma patients. Objective clinical response was observed in 15-20% of trial subjects (Chan *et al.*, 1998). On the other hand, the outcome of randomised phase III clinical trials of patients treated with Cancervax failed to confirm prolonged survival of patients in comparison with the control group which received only BCG (Morton *et al.*, 2007).

Melacine is a melanoma tumor cell lysate vaccine consisting of two allogeneic melanoma cell lines (MSM-M-1 and MSM-M-2) combined with Detox® adjuvant (Vaishampayan *et al.*, 2002). After encouraging early phase studies the vaccine failed the phase III trial. Though, retrospective analysis showed that patients receiving Melacine and expressing at least two of five HLA antigens present on the vaccine cells developed longer RFS and OS ( $p = 0.0002$  and  $p = 0.0001$ , respectively). For that reason, the HLA pattern of the patient served here as a biomarker and allowed stratification of patients who would respond to the treatment. The Melacine may serve as an example of personalized therapeutic vaccine (J. Mackiewicz & A. Mackiewicz, 2010).

Wallach *et al.* in a multicenter randomized phase III trial assessed the efficacy of VMO – Vaccinia Melanoma Oncolysate. This preparation is a melanoma cell lysate (four cell lines) derived by infection of these lines by the vaccinia virus. The study enrolled 217 patients after resection of metastases to the lymph nodes. Test results showed no significant differences in RFS and OS (Wallack *et al.*, 1998).

### 3.4.1.2 Intracellular gene transfer

Systems of intracellular gene transfer can be broadly divided into non-viral (physical) and viral. The former category includes (i) electroporation (mechanical introduction of DNA in the electric field), (ii) “gene gun” (injection of DNA-coated gold beads into cells by means of pressurised helium). Non-viral chemical strategies are based on modifications of cell membrane permeability for macromolecules under the influence of cationic compounds or enabling penetration of liposome-encapsulated genes marked by high affinity to cell membranes. The most common gene transfer systems used in genetic therapy of human cancer nowadays are based on viral vectors: retroviral, adenoviral, adeno-associated viruses (AAV) and lentiviral.

Recombinant retroviruses are predominantly based on Moloney murine leukaemia virus (MoMLV). They are used to transduce cells both *in vitro* and *in vivo*. Retroviruses transfer genetic material to dividing cells, with viral DNA becoming permanently incorporated into the host’s genome, thus producing constant expression of the therapeutic gene in target cells and their descendants. Retroviruses are vectors of choice for constructing cellular cancer vaccines. When administered *in vivo* to humans, murine-enveloped retroviruses are quickly eliminated via complement activation. Consequently, human vectors or human-enveloped vectors were constructed.

Adenoviral vectors are used to modify tumour cells *in vivo*. A typical feature of adenoviral vectors is highly efficient transduction of target cells. On the other hand, as there is no interaction of genetic material with the host’s genome, gene expression is transient. In the case of serial administration of adenoviral vectors, they are eliminated from the human body due to the presence of specific adenoviral antibodies in the human serum. The main feature distinguishing adenoviral carriers from retroviral AAV is the ability to deliver genes into non-dividing cells. Adeno-associated vectors (AAV) have a low capacity and an

ability of episomal (extrachromosomal) replication of genetic material and simultaneous integration of inserted genetic material with the host cell’s DNA. Initially, AAV vectors contained “contaminants” in the form of immunogenic adenoviral particles necessary for AAV packaging, however new techniques have recently been developed to purify AAV vectors.

Lentiviral vectors are based on the human immunodeficiency virus type 1 (HIV-1). They have a capacity of permanent integration of genetic material with dividing and non-dividing cell genomes and are thus an efficient tools used to transduce early CD34+ stem cells (approximately 40%).

3.4.1.3 Genetically modified cell vaccines

Rapid development of genetic engineering and gene transfer systems, combined with increasing knowledge of tumour immunology, have triggered a dynamic development of

Immunostimulating factor	Dominant type of anti-tumour effect
IL-2	Direct anti-tumour effect Stimulation and proliferation of CD8+ lymphocytes and NK cells
IFN-gamma	Direct anti-tumour effect Stimulation of macrophages, induction of immune response, stimulation of expression of MHC and B7 cells Induction of chemokines which suppress neo-angiogenesis: IP-10, MIG
IL-12	Secreted by DCs, powerfully stimulates CD4+, CD8+ and NK cells. Secondary secretion of IFN-gamma and polarisation towards Th1-type response
IL-18	Secondary secretion of IFN-gamma and polarisation towards Th1-type response Suppressed secretion of IL-10 by stimulated T lymphocytes Stimulation of cytotoxic activity of NK cells by the Fas ligand
GM-CSF	Differentiation and maturation of DCs Stimulation of expression of MHC proteins, tumour antigens and co-stimulatory cells in antigen-presenting cells
IL-4	Tumour infiltration by macrophages and eosinophils Differentiation and maturation of DCs Stimulation of CD4+ cells
TNF	Direct anti-tumour effect (cytotoxic effect) Stimulation of specific and non-specific cell-mediated and humoral response
IL-7	Stimulation of CD4+ and CD8+ cells
IL-6+sIL6R	Stimulation of CD4+, CD8+ and NK cells, DCs maturation, presentation of cryptic antigens by DCs, inhibition of Treg formation, induction of GM-CSF secretion by lymphocytes
B7	Co-stimulatory signal for T cells
HLA-B7	As an allogeneic HLA molecule – stimulation of local production of interferons and cytokines in the tumour microenvironment

Table 2. Factors stimulating anti-tumour immune response used in genetic cancer therapy

gene-modified tumour cell vaccines (GMTV). GMTV vaccines are based on whole tumour cells that can be modified with genes encoding (i) immunostimulatory cytokines (such as IL-2, IL-4, IL-6, IL-7, IL-12, IFN-gamma, TNF) (Table 2), (ii) costimulatory molecules (CD80, CD86), (iii) adhesion molecules, (iv) histocompatibility (MHC) antigens. The aim of genetic modification of cancer cells is to augment their immunogenicity, e.g. via phenotype modifications (increased expression of MHC I and II) or activation of effector mechanisms of the immune system via the delivery of costimulatory signals (J. Mackiewicz & A. Mackiewicz, 2010). GMTV have been tested in many early phase clinical trials in the adjuvant and therapeutic settings (Table 3).

Type of modified cells	Vector	Gene	Trial phase	Number of patients	Clinical response (stable disease) [mixed disease]	Author and publication
Autologous	Liposomes	HLA-B7	I	5	1	Nabel GJ <i>et al.</i> , 1993
Autologous	Retrovirus	IL-7	I	10	0 (4) [2]	Moller <i>et al.</i> , 1998
Autologous	Gene gun	IL-12	I	6	0 (3)	Sun Y <i>et al.</i> , 1998
Autologous	Retrovirus	GM-CSF	I	29	0	Soiffer <i>et al.</i> , 1998
Autologous	Retrovirus	GM-CSF	I	35		Soiffer <i>et al.</i> , 2003
Autologous	Retrovirus	IL-2	II	12	(3)	Palmer <i>et al.</i> , 1999
Allogeneic	?	IL-2	I	12	[3]	Arienti <i>et al.</i> , 1996
Allogeneic	?	IL-4	I	12	[2]	Arienti <i>et al.</i> , 1999
Allogeneic	?	IL-2	I/II	33	2 (7)	Osanto <i>et al.</i> , 2000

Table 3. Results of gene therapy in metastatic melanoma – phase I/II trials

Mackiewicz et al. at the European Society of Medical Oncology (ESMO) meeting 2010 presented results of two phase II clinical trials conducted in almost 200 patients after resection of stage III and IV melanoma. In both studies patients were vaccinated with AGI-101 composed of two irradiated melanoma cell lines modified to express Hyper-IL-6 – a fusion protein composed of interleukin 6 (IL-6) and soluble IL-6 receptor . AGI-101 (5 x 10<sup>7</sup> cells per dose) was administered 8 times at 2-week intervals (induction phase) and then monthly (maintenance phase). At disease progression the induction phase (+/- surgery) was restarted, followed by a second maintenance phase. At progression 43 (Trial 3) and 39 (Trial 5) patients were re-induced +/- surgery followed by a second maintenance phase; of those 11 and 16 patients respectively are alive following re-induction. The 5-year survival in Trial 3 was 66,7%, 43,8% and 26,1% respectively in stage IIIB, IIIC and IV. In Trial 5 the 5-year survival was as follows 56,3%, 39,8% and 41,2% correspondingly in stage IIIA/B, IIIC and IV. The OS observed in trial 3 was 4,4 years and 3,1 year in trial 5 (A. Mackiewicz *et al.*, 2010). The vaccine was well tolerated as no vaccine related toxicity of CTC>2 was detected. Intensive research of melanoma vaccines is currently carried out in a number of countries worldwide. However, no vaccine, has been approved by regulatory authorities so far.

3.4.1.4 Vaccines based on dendritic cells

Extensive research of DCs has shown that they are the most efficient APCs (Banchereau *et al.*, 1998; Hart *et al.*, 1997). DCs play a vital role in inducing immune response. They are the only representatives of APCs that are capable of inducing primary response of virgin T lymphocytes. The use of DCs for antigen presentation offers an opportunity to trigger

immune response even to weakly immunogenic tumour antigens and break immune tolerance.

Human DCs are generated by isolation of immature DCs from blood (Fong *et al.*, 2000) and differentiation *ex vivo* in the presence of IL-4 and GM-CSF, myeloid progenitor cells (CD34 +) or monocytes (CD14 +) (Sallusto *et al.*, 1994). Obtained immature DCs can be pulsed with tumor cell lysates (Nair SK *et al.* 1997) or synthetic peptides (Gitlitz *et al.*, 2003), modified with genes encoding tumor antigens or tumor cells RNA (Ashley *et al.*, 1997). For immunization are also used hybrids of DCs with tumor cells (Avigan *et al.*, 2004).

In a small trial, a group of 11 patients with advanced-stage melanoma were immunised with autologous DCs previously incubated with the MAGE-3 peptide presented by HLA-A1. Regression of cancerous lesions in the skin, lungs and the liver was achieved in 6 cases (Thurner *et al.*, 1999). In one of the few randomized phase III studies, Schadendorf *et al* injected metastatic melanoma patients with autologous DC pulsed with peptides presented in the context of HLA class I and II. However, preliminary analysis has not demonstrated superiority of vaccine over dacarbazine (control arm) and the study was terminated (Schadendorf *et al.*, 2006). Nevertheless, only 53 patients in the vaccine group and 55 in the control arm were participating in the trial and the vaccine was administered depending on the amount of DC cells, usually only from two to several times. Though, subsequent analysis showed that immunized patients with HLA-A2 +/HLA-B44 haplotyp lived longer than those treated with dacarbazine (Engel-Noerregaard *et al.*, 2009).

Peptide-pulsed DCs have certain limitations as well, including (i) short period of antigen presentation, dependent on the half-life of the MHC-peptide complex, (ii) the fact that using a given peptide is only limited to patients with an appropriate MHC haplotype (Amoscato *et al.*, 1998). It is believed that the strategy of modified DCs can be both more efficient and more universal. DCs can also be loaded with genes encoding tumour antigens, immunostimulatory factors or cytokines. Furthermore, DCs derived from different patients can be modified with the same genetic sequence (no need to match appropriate MHC haplotypes), while expression of a given introduced sequence is long-term.

Metharom *et al.* immunised mice with dendritic cells transduced with the mTRP-2 gene (tumour antigen of B16 murine melanoma cells). Tumour regression was observed in 4 out of 7 mice (Metharom *et al.*, 2001).

In a phase I/II trial, melanoma patients were immunised with autologous DCs transduced with tumour-derived mRNA, which enables lymphocytes to present – beside shared melanoma antigens – a wide range of tumour antigens that are unique for a given patient, as well as previously unknown antigens. The trial showed that the vaccine is completely safe. Response of T lymphocytes against tumour antigens encoded by tumour-derived mRNA *in vivo* was observed in the majority of cases (Kyte *et al.*, 2006).

Melanoma DC vaccines tested to date, despite encouraging results noted in phase I and II trials, have not proven effective in phase III randomised trials.

#### 4. Cancer immunotherapy clinical trial design

Results of many promising early-phase clinical studies evaluating the effectiveness of immunotherapy have not been confirmed in phase III trials. It is becoming clear that these failures could be related to the design of clinical trials (Finke *et al.*, 2007). At present, clinical trials design of new therapeutic strategies including immunotherapy are based on criteria developed for cytotoxic drugs. However immunotherapeutics, especially of active



immunotherapy have very different clinical characteristics, mechanism of action and toxicity profile than chemotherapeutic agents. Accordingly, new clinical immunotherapy trial design paradigm was developed (Hoos, *et al.*, 2007). Here we outline some aspects which should be considered while designing clinical trials for the assessment of the effectiveness of immunotherapy.

Patients qualified to earlier clinical trials evaluating the efficacy of immunotherapeutics were in late stage of the disease often treated previously with chemo- or radiotherapy. However, the highest clinical benefit was observed in patients with minimal residual disease and such patient population should be considered as candidates for immunotherapy trials (mainly cancer vaccine trials).

Clinical trial end points evaluating chemotherapeutic agents drugs may not be adequate for immunotherapy trials. The Response Evaluation Criteria in Solid Tumors (RECIST) in the assessment of objective clinical response to chemotherapy may not reflect the benefit from immunotherapy. The time points between tumor assessments in trials evaluating immunotherapeutics should be longer (in contrast to chemotherapy trials) giving time for the immune system to mount response to the treatment. In a recent phase II metastatic melanoma study, patients treated with AGI-101H (genetically modified melanoma vaccine secreting Hyper-IL-6) developed clinical response usually after 3-4 months of treatment. However, in many patients tumor shrinkage was observed after several months or even years following SD (Nawrocki & A. Mackiewicz, 2007, Nawrocki *et al.*, 2000). Nevertheless, in many studies assessed tumors tend to enlarge first due to infiltration of inflammatory cells and then shrink. Furthermore immunotherapy may fail to induce tumor decrease, but yet still be effective in slowing the rate of progression giving the patient benefit in OS. The cancer Vaccine Clinical Trial Working Group (CVCTWG) propose that patients after initial clinically not significant progression should not be excluded from the treatment and following tumor regression their response rate could be scored based on the largest tumor volume measured after the start of treatment, not necessarily from baseline tumor volume (Hoos *et al.*, 2007). Based on the observations from phase II clinical trial evaluating earlier described ipilimumab in metastatic melanoma patients' new guidelines for evaluation of immune-related response criteria (irRC) have been developed. In some patients treated with ipilimumab responses after initial increase in total tumor burden in the presence of new lesions were observed. Those patterns were associated with favorable survival. According to irRC: (i) new, nonmeasurable lesions do not define progression; (ii) new measurable lesions are not defined as progression but are incorporated into tumor burden; (iii) progression of the disease has to be confirmed by a repeat consecutive assessment no less than 4 weeks from the first documented date (Wolchok, 2009).

OS is a "gold standard" used for efficacy evaluation of new drugs and is a primary end point of choice in phase III clinical studies. OS is a very good end point, but might be affected by subsequent therapies, is time consuming, and requires large samples of patients. In efficacy randomized phase II trials using adoptive component might be required.

DFS and PFS used respectively in adjuvant and metastatic disease setting are acceptable surrogate primary end points which can shorten the time of randomized efficacy trials. However, definitions of DFS and PFS might not be consistent for immunotherapy trials. While as mentioned before patient's immune system needs time to develop clinical activity and even patients after progression of disease still may benefit from treatment. In terms of classical DFS/PFS description patients with relapse or progression of the disease could be excluded from the trial too early. Modifications of these definitions have been proposed:

confirmation of progression after at least second tumor assessments; not taking into account early progression within a defined time-interval (eg, three month from the beginning of the treatment). In patients who developed early progression with subsequent response to study treatment, the time of DFS and PFS should be calculation from the first day of drug administration (Hoos *et al.*, 2007). Moreover, in some malignancies like melanoma where there is no optional effective second line treatment, immunotherapy should be continued.

In patients developing progression, changes in immunotherapy schedule (induction phase) or metastasectomy may need to be performed. In our own melanoma study patients treated with AGI-101H vaccine received induction phase (8 doses in 2 week interval), with subsequent maintenance phase (1 dose monthly). After developing progression induction phase schedule followed by maintenance was performed. We observed that patients undergoing re-induction benefit from the treatment (Nawrocki & A. Mackiewicz, 2007, A. Mackiewicz *et al.*, 2010). Similar observation was seen in the ipilimumab phase III study conducted in metastatic melanoma patients. Ipilimumab was administered four times in 3 week intervals (induction phase). Patients with SD for 3 months duration after week 12 or a confirmed PR or CR were offered reinduction after developing progression. Among 31 patients given reinduction therapy with ipilimumab, a CR, PR or SD was achieved by 21 patients.

A properly designed immunotherapy clinical trial is very important, while these agents in contrast to chemical agents or small molecules like tyrosine kinases inhibitors might cure patients with immunogenic malignancies (eg. melanoma, renal cell carcinoma) even when the disease is disseminated. In a highly selected patient population with metastatic melanoma (270 patients) treated with IL-2, CR was observed in 6% with median duration of response over 59 months. PR was seen in 10% of IL-2 treated patients. Disease did not progress in any patient responding for more than 30 months (Atkins *et al.*, 1993 & 1994). Another example is a study evaluating ipilimumab in metastatic melanoma patients, where best overall response (CR+PR) rate of only 10.9% was observed. However 60.0% of patients developed an objective response for at least 2 years (26.5 to 44.2 months[ongoing]) (Hodi *et al.*, 2010).

## 5. Conclusions and further directions of the development

Recent approval of DC based prostate cancer vaccine – Stipuleucel-T or immunostimulatory antibody – Yervoy prove the potential of active immunotherapeutic approaches to treat cancer. Recent better understanding of immune tolerance mechanisms and their braking, development of vaccine design and the paradigm of immunotherapy clinical trials design will lead to the boosting of progress in the field of active cancer immunotherapy. Since examples already include melanoma, certainly immune targeting especially specific active immunotherapy approaches such as melanoma therapeutic vaccine will continue to be tested and finally successful. Most likely immunotherapy will need to be combined with other modalities such as for ex. small molecules, but certainly without support of the immune system elimination of cancer may not be possible.

## 6. References

Ahmadzadeh, M. & Rosenberg, S.A. (2006). Blood. *IL-2 administration increases CD4+ CD25(hi) Foxp3+ regulatory T cells in cancer patients*. Vol. 107, No. 6, (March 2006), pp. 2409-2414.

- Amoscato, A.A., Prenovitz, D.A. & Lotze, M.T. (1998). *J Immunol Rapid extracellular degradation of synthetic class I peptides by human dendritic cells*. Vol. 161, No. 8, (October 1998), pp. 149-157.
- Arienti, F., Sulé-Suso, J. & Belli, F. et al. (1996). *Hum Gene Ther. Limited antitumor T cell response in melanoma patients vaccinated with interleukin-2 gene-transduced allogeneic melanoma cells*. Vol. 7, No. 16, (October 1996), pp. 1955-1963.
- Arienti, F., Belli, F. & Napolitano, F. et al. (1999). *Hum Gene Ther. Vaccination of melanoma patients with interleukin 4 gene-transduced allogeneic melanoma cells*. Vol. 10, No. 18, (1999 December), pp. 2907-2916.
- Ashley, D.M., Faiola, B., Nair, S., Hale, L.P., Bigner, D.D. & Gilboa, E. (1997). *J Exp Med. Bone marrow-generated dendritic cells pulsed with tumor extracts or tumor RNA induce antitumor immunity against central nervous system tumors*. Vol. 186, No. 7, (October 1997) pp. 1177-1182.
- Atkins, M.B., Robertson, M.J. & Gordon, M. (1997). *Clin Cancer Res. Phase I evaluation of intravenous recombinant human interleukin 12 in patients with advanced malignancies*. (March 1997), Vol. 3, No. 3, pp. 409-417.
- Atkins, M.B., Lotze, M.T. & Dutcher, J.P. et al. (1999). *J Clin Oncol. High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated with 1985 and 1993*. (July 1999), Vol. 17, No 7, pp. 2105-2116.
- Atkins, M.B., Kunkel, L., Sznol, M. & Rosenberg, S.A. (2000.) *Cancer J Sci Am. High-dose recombinant interleukin 2 therapy in patients with metastatic melanoma: long-term survival update*. (February 2000), 6 Suppl. 1, pp. 11-14.
- Atkins, M.B., Hsu, J. & Lee, S. et al. (2008). *J Clin Oncol. Phase III trial comparing concurrent biochemotherapy with cisplatin, vinblastin, dacarbazine, interleukin-2, and interferon alfa-2b with cisplatin, vinblastin and dacarbazine alone (E3695): A trial coordinated by eastern cooperative oncology group*. (December 2008), Vol. 26, No. 35, pp. 5748-5754.
- Avigan, D., Vasir, B. & Gong, J., et al. (2004). *Clin Cancer Res. Fusion cell vaccination of patients with metastatic breast and renal cancer induces immunological and clinical responses*. (July 2004), Vol. 10, No. 14, pp. 4699-4708.
- Baker, A.B., Philips, J.H. & Figer, C.G. et al. (1998). *J Immunol. Killer cell inhibitory receptors for MHC class I molecules regulate lysis of melanoma cells mediated by NK cells, gamma-delta T cells and antigen specific CTL*. (Jun 1998), Vol. 160, No. 11, pp. 5239-5245.
- Banchereau, J. & Steinman, R.M. (1998). *Nature. Dendritic cells and the control of immunity*. (March 1998), Vol. 392, No. 6673, pp. 245-252.
- Berd, D., Maguire, H.C. Jr & McCue, P., et al. (1990). *J Clin Oncol. Treatment of metastatic melanoma with an autologous tumor-cell vaccine. Clinical and immunologic results in 64 patients*. (November 1990), Vol. 8, No. 11, pp. 1858-67.
- Boasberg, P., Cruickshank, S., Hamid, O., O'Day, S., Weber, R. & Spitler, L. (2009). *J Clin Oncol. Nab-paclitaxel (Abraxane) and bevacizumab as first line therapy in patients with unresectable stage III nad IV melanoma; Suppl. 27. Abstr 9061*.
- Boon, T., Gajewski, T.F. & Coulie, P.G. (1995). *Immunol Today. From defined human tumor antigens of effective immunization?* (July 1995), Vol. 16, No. 7, pp. 334-345.
- Brahmer, J.R., Topalian, S. & Wollner, I. et al. (2008) *J. Clin. Oncol. Safety and activity of MDX-1106 (ONO-4538), an anti-PD-1 monoclonal antibody, in patients with selected refractory or relapsed malignancies*. Suppl. 26, Abstract. 3006.

- Bristol Myers Squibb, (January 2008). A Randomized, Multi-Dose, Open-Label, Phase II Study of BMS-663513 as a Second-Line Monotherapy in Subjects With Previously Treated Unresectable Stage III or IV Melanoma. *US National Institutes of Health (NIH)*. 24.01.2011, Available from <http://clinicaltrials.gov/ct2/show/NCT00612664?term=4-1BB&rank=1>
- Cavallo, F., Giovarelli, M., Gulino, A., Vacca, A., Stoppacciaro, A., Modesti, A. & Forni, G. (1992). *J Immunol*. Role of the neutrophils and CD4+ T lymphocytes in the primary and memory response to nonimmunogenic murine mammary adenocarcinoma made immunogenic by IL-2 gene. (December 1992), Vol. 149, No. 11, pp.3627-3635.
- Chan, A.D. & Morton, D.L. (1998). *Semin Oncol*. Active immunotherapy with allogeneic tumor cell vaccines. Present status. (December 1998), Vol. 25, No. 6, pp. 611-622.
- Chapman, P.B., Wu, D., Ragupathi, G., Lu, S., Williams, L., Hwu, W.J., Johnson, D. & Livingston, P.O. (2004) *Clin. Cancer. Res*. Sequential immunization of melanoma patients with GD3 ganglioside vaccine and antiidiotypic monoclonal antibody that mimics GD3 ganglioside. (July 2004), Vol. 10, No. 14, pp. 4717-4723.
- Curti, B.D., Ochoa, A.C. & Powers, G.C. et al. (1998) *J. Clin. Oncol*. Phase I trial of anti-CD3-stimulated CD4+ T cells, infusional interleukin-2, and cyclophosphamide in patients with advanced cancer. (August 1998), Vol. 16, No. 8, pp. 2752-2760.
- Dudley, M.E., Wunderlich, J.R. & Yang, J.C. et al. (2005). *J Clin Oncol*. Adoptive cell transfer therapy following non myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. (April 2005), Vol. 23, No. 10, pp.2346-2357.
- Dunn, G.P., Bruce, A.T., Ikeda, H. & Old, L.J. Schreiber RD. (2002). *Nat Immunol* Cancer immunoediting: from immunosurveillance to tumor escape. (November 2002), Vol. 3, No. 11, pp. 991-998.
- Dunn, G.P., Koebel, C.M. & Schreiber, R.D. (2006). *Nat Rev Immunol* Interferons, immunity and cancer immunoediting. (November 2006), Vol. 6, No. 11, pp. 836-848.
- Eggermont, A.M., Suci, S. & Ruka, W. et al. EOTRC 18961: (2008a). *J Clin Oncol* Post-operative adjuvant ganglioside GM2-KLH21 vaccination treatment vs observation in stage II (T3-T4N0M0) melanoma: 2<sup>nd</sup> interm analysis led to an early disclosure of the results.; Suppl. 15, Abstract. 9004.
- Eggermont, A.M., Suci, S. & Santinami, M et al. (2008b). *Lancet*. Adjuvant therapy with pegylated interferon alfa-2b versus observation alone in resected stage III melanoma: final results of EORTC 18991, a randomised phase III trial. (July 2008), Vol. 372, No. 9633, pp. 117-126.
- Engel-Noerregaard, L., Hansen, T.H, Andersen, M.H., Straten PT & Svane IM. (2009) *Cancer Immunol. Immunother*. Review of clinical studies on dendritic cell-based vaccination of patients with malignant melanoma: assessment of correlation between clinical response and vaccine parameters. (January 2009), Vol. 58, No. 1, pp. 1-14.
- Eton, O., Legha, S.S. & Bedikian, A.Y. et al. (2002). *J Clin Oncol*. Sequential biochemiotherapy versus chemotherapy for metastatic melanoma: results from a phase III randomized trial. (April 2002), Vol. 20, No. 8, pp. 2045-2052.
- Finke, L.H., Wentworth, K., Blumenstein, B., Rudolph, N.S., Levitsky, H. & Hoos, A. et al. (2007). *Vaccine*. Lessons from randomized phase III studies with active cancer immunotherapies – Outcomes from the 2006 Meeting of the Cancer Vaccine Consortium (CVC). (September 2007), Suppl. 25, pp. 97-109.



- Fong, L. & Engleman, E.G. (2000). *AnnuRev Immunol. Dendritic cells in cancer immunotherapy.* (April 2000), Vol. 18, pp. 245-273.
- Fynan, E.F., Webster, R.G., Fuller, D.H., Haynes, J.R., Santoro, J.C. & Robinson, H.L. *Proc. Natl. Acad. Sci. USA* (1993). *DNA vaccines: protective immunizations by parenteral, mucosal, and gene-gun inoculations.* (April 1993), Vol. 90, No. 24, pp. 11478-11482.
- Garrido, F., Cabrera, T. & Concha, A. et al. (1993). *Immunol Today. Natural history of HLA expression during tumor development.* (October 1993), Vol. 14, No. 10, pp. 491-499.
- Gitlitz, B.J., Beldegrun, A.S. & Zisman, A., et al. (2003). *J Immunother. A pilot trial of tumor lysate-loaded dendritic cells for the treatment of metastatic renal cell carcinoma.* (October 2003), Vol. 26, No. 5, pp. 412-9.
- Goldman, B. & DeFrancesco, L. (2009). *Nature Biotech. The cancer vaccine roller coaster.* (November 2009), Vol. 27, No. 2, pp. 129-140.
- Green, L.L. (1999). *J Immunol Methods. Antibody engineering via genetic engineering of the mouse: XenoMouse strains are a vehicle for the facile generation of therapeutic human monoclonal antibodies.* (December 1999), Vol. 231, No 1-2, pp. 11-23.
- Hart, D.N. (1997). *Blood. Dendritic cells: unique leukocyte populations which control the primary immune response.* (November 1997), Vol. 90, No. 9, pp. 3245-87.
- Hahne, M., Rimoldi, D. & Schroter, M. et al. (1996). *Science. Melanoma cell expression of Fas ligand: implications for tumor immune escape.* (November 1996), Vol. 274, No. 5291, pp. 1363-1366.
- Hodi, F.S., O'Day, S.J. & McDermott, D.F. et al. (2010). *N Engl J Med. Improved survival with ipilimumab in patients with metastatic melanoma.* (August 2010), Vol. 363, No. 8, pp. 711-723.
- Hoos, A., Parmiani, G. & Hege, K. (2007) *J. Immunother. A clinical development paradigm for cancer vaccines and related biologics.* (January 2007), Vol. 30, No.1, pp. 1-15.
- Hunder, N.W., Wallen, H. & Cao, J. et al. (2008). *N Engl J Med. Treatment of metastatic melanoma with autologous CD4+ T cells against NY-ESO-1.* (Jun 2008), Vol. 358, No. 25, pp. 2698-2703.
- Hus, I., Maciąg, E. & Rolinski, J. (2010). *Postepy Hig Med Dosw. The role of Th17 cells in anti-cancer immunity.* (July 2010), Vol. 64, pp. 244-250 e-ISSN 1732-2693.
- Hwu, P., Sznol, M. & Pavlick, A. (2009). *J Clin Oncol. A phase I/II study of CR011-vcMMAE, an antibody-drug conjugate (DC) targeting glycoprotein NMB (GPNMB) in patients (pts) with advanced melanoma.* Suppl. 27. Abstr 9032.
- Ives, N.J., Stowe, R.L. & Lorigan, R. et al. (2007). *J Clin Oncol. Chemotherapy compared with biochemotherapy form the treatment of metastatic melanoma: a meta-analysis of 18 trials involving 2,621 patients.;* (December 2007), Vol. 25, No. 34, pp. 5426-5434.
- Janeway, C.A. Jr. & Bottomly, K.: (1994). *Cell. Signals and signs for lymphocyte responses.* (January 1994), vol. 76, No. 2, pp. 275-285.
- Kaufman, H.L., Cohen, S., Cheung, K., DeRaffele, G., Mitcham, J., Moroziewicz, D., Schlom, J. & Hesdorffer, C. (2006). *Hum. Gene Ther. Local delivery of vaccinia virus expressing multiple costimulatory molecules for the treatment of established tumors.* (February 2006), Vol. 17, No. 2, pp. 239-244.
- Kirkwood, J.M., Strawderman, M.H. & Ernstoff, M.S. et al. (1996). *J Clin Oncol. Interferon alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: the Eastern Cooperative Oncology Group Trial EST 1684.* (January 1996), Vol. 14, No. 1, pp. 7-17.

- Kirkwood, J.M., Ibrahim, J.G. & Sondak VK et al. (2000). *J Clin Oncol. High- and low-dose interferon alfa-2b in high-risk melanoma: first analysis of intergroup trial E1690/S911/C9190.* ; (Jun 2000), Vol. 18, No. 12, pp. 2444-2458.
- Kirkwood, J.M., Lorigan, P. & Hersey, P. et al. . (2008). *J Clin Oncol. A phase II trial of tremelimumab (CP-675,206) in patients with advanced refractory or relapsed melanoma.* (July 2008), Suppl. 26: Abstr 9023.
- Knurth, I., Horsten, U. & Pflanz, S. et al. (1999). *J Immunol. Activation of the signal transducer glycoprotein 130 by both IL-6 and IL-11 requires two distinct binding epitopes.* (January 1999), Vol. 162, No. 3, pp. 1480-1487.
- Kohler, G. & Milstein, C. (1975). *Nature. Continuous cultures of fused cells secreting antibody of predefined specificity.* (August 1975), Vol. 256, No. 5517, pp. 256-475.
- Kurzen, H., Schmitt, S., Naher H, Mohler T. *Anticancer Drugs.* (2003). *Inhibition of angiogenesis by non-toxic doses of temozolomide.* (August 2003), Vol. 14, No. 7, pp. 515-522.
- Kyte, J.A., Mu, L. & Aamdal, S. et al. (2006). *Cancer Gene Ther. Phase I/II trial of melanoma therapy with dendritic cells transfected with autologous tumor m-RNA.*(October 2006), Vol. 13, No. 10, pp. 905-18.
- Lacal, P.M., Failla, C.M. & Pagani, E. et al. (2000) *J. Invest. Dermatol. Human melanoma cells secrete and respond to placenta growth factor and vascular endothelial growth factor.* (December 2000, Vol. 115, No. 6, pp. 1000-1007.
- Lawson, D.H., Lee, .S.J. & Tarhini, A.A. et al. (2010). *J Clin Oncol. E4697: Phase III cooperative group study of yeast-derived granulocyte macrophage colony-stimulating factor (GM-CSF) versus placebo as adjuvant treatment of patients with completely resected stage III-IV melanoma.* Suppl. 15, Abstract 8504.
- Lev, D.C., Ruiz, M. & Mills, L. et al. (2003). *Mol. Cancer Ther. Dacarbazine causes transcriptional up-regulation of interleukin 8 and vascular endothelial growth factor in melanoma cells: a possible escape mechanism from chemotherapy.* (August 2003), Vol. 2, No. 8, pp. 753-763.
- Lev, D.C., Onn, A. & Melinkova, V.O. et al. (2004) *J. Clin. Oncol. Exposure of melanoma cells to dacarbazine results in enhanced tumor growth and metastasis in vivo.* (Jun 2004), Vol. 22, No. 11, pp. 2092-2100.
- Linette, G., Cranmer, L. & Hodi, S. et al. (2008). *J Clin Oncol. A multicenter phase II study of volociximab in patients with relapsed metastatic melanoma.* (July 2008), Suppl. 26. Abstract 3505.
- Loquai, C., Pavlick & A. Lawson, D. et al. (2009). *J Clin Oncol. Randomised phase 2 study of the safety and efficacy of a human anti-  $\alpha_v$  integrin monoclonal antibody Intetumumab (CNTO 95) alone and in combination with dacarbazine in patients with stage IV melanoma: 12 month results.* (July 2009), Suppl. 27. Abstract 9029.
- Mackensen, A., Meidenbauer, N. & Vogl S et al. (2006). *J Clin Oncol. Phase I study of adoptive T-cell therapy using antigen specific CD8+ T cells for the treatment of patients with metastatic melanoma.* (November 2006), Vol. 24, No. 31, pp. 5060-5069.
- Mackiewicz, A., Wysocki, P. & Mackiewicz, J. et al. (2010). *Ann of Oncology. Long term survival of high risk melanoma patients immunized with an allogeneic whole cell genetically modified vaccine (AGI-101) after complete resection.* (October 2010), Vol. 21, Suppl. 8.
- Mackiewicz, J. & Mackiewicz, A. (2009) *Eur J Pharmacol. Design of clinical trials for therapeutic cancer vaccines development.* (December 2009), Vol. 625, No. 1-3, pp. 84-89.

- Mackiewicz, J. & Mackiewicz, A. (2010). *Contemporary Oncology. Immunotherapy of cancer and perspectives of its development.* (February 2010), Vol. 14, No. 2:59-71.
- Mackiewicz, J. & Kwinta, L. (2010). *Contemporary Oncology. New targeted therapies in the treatment of patients with metastatic melanoma.* (January 2010), Vol. 14, No. 1, pp. 15-22.
- Mackiewicz, J. & Mackiewicz, A. (2010). *Transplant Proc. Gene-modified cellular vaccines: technologic aspects and clinical problems;* (October 2010), Vol. 42, No. 8, pp. 3287-3292.
- Melichar, B., Koralewski, B. & Ravaud, A. et al. (2008). *Ann Oncol. First-line bevacizumab combined with reduced dose interferon-alpha2a is active in patients with metastatic renal cell carcinoma.* (August 2008), Vol. 19, No. 8, pp. 1470-1476.
- Metharom, P., Ellem, K.A., Schmidt, C. & wie, M.Q. (2001). *Hum Gene Ther. Lentiviral vector-mediated tyrosine-related protein 2 gene transfer to dendritic cells for the therapy of melanoma.* (December 2001), Vol. 18, No. 12, pp. 2203-2213.
- Möller, P., Sun, Y. & Dorbic, T. et al. (1998). *Br J Cancer. Vaccination with IL-7 gene-modified autologous melanoma cells can enhance the anti-melanoma lytic activity in peripheral blood of patients with a good clinical performance status: a clinical phase I study.* (November 1998), Vol. 77, No. 11, pp. 1907-1916.
- Morgan, R.A., Dudley, M.E. & Wunderlich, JR. et al. (2006). *Science. Cancer Regression in Patients After Transfer of Genetically Engineered Lymphocytes.* (October 2006), Vol. 314, No. 5796, pp. 126-129.
- Morton, D.L., Foshag, L.J. & Hoon, D.S. et al. (1992). *Ann Surg. Prolongation of survival in metastatic melanoma after active specific immunotherapy with a new polyvalent melanoma vaccine.* (October 1992), Vol. 216, No. 4, pp. 463-482.
- Morton, D.L., Mozzillo, N. & Thompson, J.F. et al. (2007). *J Clin Oncol. An international, randomized, phase III trial of bacillus Calmette-Guerin (BCG) plus allogeneic melanoma vaccine (MCV) or placebo after complete resection of melanoma metastatic or regional or distant sites.* Suppl. 25:Abstr 8508.
- Nabel, G.J., Nabel, E.G. & Yang, Z.Y. et al. (1993). *Proc Natl Acad Sci U S A. Direct gene transfer with DNA-liposome complexes in melanoma: expression, biologic activity, and lack of toxicity in humans.* (January 1993), Vol. 90, No. 23, pp. 11307-11311.
- Nair, S.K., Snyder, D., Rouse, B.T. & Gilboa, E. (1997). *Int J Cancer. Regression of tumors in mice vaccinated with professional antigen-presenting cells pulsed with tumor extracts.* (March 1997), Vol. 70, No. 6, pp. 706-715.
- Nanda, N.K. & Sercarz, E.E. (1995). *Cell Induction of anti-self-immunity to cure cancer.* (July 1995), Vol. 82, No. 1, pp. 13-17.
- Nawrocki, S. & Mackiewicz A. (2007). *Expert. Opin. Investig. Drugs. Clinical trials of active cancer immunotherapy.* (August 2007), Vol. 16, No. 8, pp. 1137-1141.
- Nawrocki, S., Murawa, P. & Malicki, J. et al. (2000). *Immunol. Lett. Genetically modified tumour vaccines (GMTV) in melanoma clinical trials.* (September 2000), Vol. 74, No. 1, pp. 81-86.
- Nishimura, T., Iwakabe, K. & Sekimoto, M. et al. (1999). *J Exp Med. Distinct role of antigen-specific T helper type 1 (Th1) and Th2 cells in tumor eradication in vivo.* (September 1999), Vol. 190, No. 5, pp. 617-627.
- Oble, D.A., Loewe, R., Yu, P. & Mihm, Jr. M.C. (2009). *Cancer Immunol. Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in human melanoma.* (April 2009), Vol. 9, p. 3.

- O'Day, S.J., Kim, G.P. & Sosman, J.A. et al. BEAM: (2009). *Ann Oncol. A randomized phase II study evaluating the activity of bevacizumab In combination with carboplatin plus paklitaxel In patients with previously untreated Advanced Melanoma*. Suppl. 8. Abstract 23LBA.
- Osanto, S., Schiphorst, P.P., Weijl, N.I. et al. (2000). *Hum Gene Ther. Vaccination of melanoma patients with an allogeneic, genetically modified interleukin 2-producing melanoma cell line*. (May 2000), Vol. 11, No. 5, pp. 739-50.
- Overwijk, W.W. & Restifo, N. (2001). *Trends in Immunology. Creating therapeutic cancer vaccines: notes from the battlefield*. (January 2001), Vol. 22, No. 1, pp. 5-7.
- Palmer, K., Moore, J. & Everard, M. et al. (1999). *Hum Gene Ther. Gene therapy with autologous, interleukin 2-secreting tumor cells in patients with malignant melanoma*. (August 1999), Vol. 10, No. 8, pp. 1261-1268.
- Pardoll DM. Paracrine cytokine adjuvants in cancer immunotherapy. (1995). *Annu Rev Immunol*. Vol. 13, pp. 399-415.
- Perez-Diez, A., Joncker, N.T., Choi, K., Chan, W.F., Anderson, C.C., Lantz, O. & Matzinger, P. (2007). *Blood. CD4 cells can be more efficient at tumor rejection than CD8 cells*. (Jun 2007), Vol. 109, No. 12, pp. 5346-5354.
- Peyton, J.D., Spigel, D.R., Burris, H.A., Lane, C., Rubin, M., Browning, M., Trent, D. & Hainsworth, J.D. (2009). *J Clin Oncol. Phase II trial of bevacizumab and everolimus in the treatment of patients with metastatic melanoma: Preliminary results*. Suppl. 27. Abstract 9027.
- Pfizer, (January 2008). *A Phase 1 Study Of CP- 870,893 In Combination With Paclitaxel And Carboplatin In Patients With Metastatic Solid Tumors*. *US National Institutes of Health (NIH)*. 17.11.2009, Available from <http://clinicaltrials.gov/ct2/show/NCT00607048?term=CP-870%2C893&rank=1NIH>
- Pilla, L., Patuzzo, R. & Rivoltini, L. et al. (2006). *Cancer Immunol. Immunother. A Phase II trial of vaccination with autologous, tumor-derived heat-shock protein peptide complex Gp96, in combination with GM-SCF and interferon-alfa in metastatic melanoma patients*. (August 2006), Vol. 55, No. 8, pp. 958-968.
- Restifo, N.P., Kawakami, Y. & Marincola, F. et al. (1993). *J Immunother. Molecular mechanisms used by tumors to escape immune recognition: immunogenethrapy and the cell biology of major histocompatibility complex class I*. (October 1993), Vol. 14, No. 3, pp. 182-190.
- Ribas, A., Hauschild, A. & Kefford, R. et al. (2008). *J Clin Oncol. Phase III, open-label, randomized, comparative study of tremelimumab (CP-675,206) and chemotherapy (temozolomide [TMZ] or dacarbazine [DTIC]) in patients with advanced melanoma*. Suppl. 26, Abstr LBA9011.
- Rosenberg, S.A., Lotze, M.T. & Muul, L.M. et al. (1985). *N Engl J Med. Observation on the systemic administration of autologous lymphokine-activated-killer cells and recombinant interleukin-2 t patients with metastatic cancer*. (December 1985), Vol. 313, No. 23, pp. 1485-1892.
- Rosenberg, S.A., Lotze, M.T. & Yang, J.C. et al. (1993). *J Natl Cancer Inst. Porspective randomized trial of high-dose interleukin 2 alone or in conjugation with lymphokine-activated-killer cells for the treatment of patients with advanced cancer*. (April 1993), Vol. 85, No. 8, pp. 622-632.
- Rosenberg, S.A., Yang, J.C. & Topalian, S.L. et al. (1994a). *JAMA. Treatment of 283 consecutive patients with metastatic melanoma or renal cell cancer using high dose bolus interleukin 2*. (March 1994), Vol. 271, No. 12, pp. 907-913.

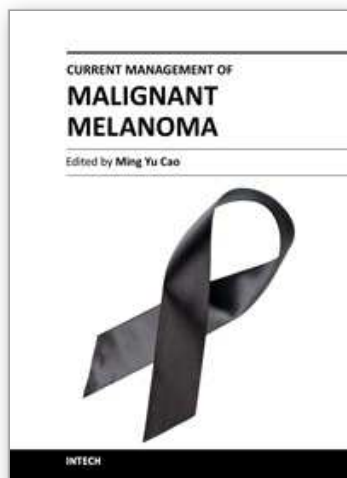


- Rosenberg, S.A. Yannelli, J.R., Yang, J.C. et al. (1994b). *J Natl Cancer Inst. Treatment of patients with metastatic melanoma with autologous tumor-infiltrating lymphocytes and interleukin-2.* (August 1994), Vol. 86,1 No. 15, pp. 1159-1566.
- Rosenberg, S.A., Restifo, N.P. & Yang, J.C. et al. (2008). *Nat Rev Cancer. Adoptive cell transfer: a clinical path to effective cancer immunotherapy.* (April 2008), Vol. 8, No. 4, pp. 299-308.
- Sallusto, F. & Lanzavecchia, A. (1994). *J Exp Med. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha.* (April 1994), Vol. 179, No. 4, pp. 1109-1118.
- Saltz, L.B., Clarke, S. & Díaz-Rubio, E. (2008). *J Clin Oncol. et al. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study.* (April 2008), Vol. 26, No. 12, pp. 2013-2019.
- Schadendorf, D., Ugurel, S., Schuler-Thurner, B. et al. (2006). *Ann Oncol. Dacarbazine (DTIC) versus vaccination with autologous peptide-pulsed dendritic cells (DC) in first-line treatment of patients with metastatic melanoma: a randomized phase III trial of the DC study group of the DeCOG.* (April 2006), Vol. 17, No. 4, pp. 563-570.
- Schmidt-Wolf, G. & Schmidt-Wolf, I.G.H. (1995). *Immunol Today. Cytokines and gene therapy.* (April 1995), Vol. 16, No. 4, pp. 173-175.
- Schultze, J.L., Anderson, K.C., Gilleece, M.H., Gribben, J.G. & Nadler, L.M. (2001). *Br. J. Haematol. A pilot study of combined immunotherapy with autologous adoptive tumour-specific T-cell transfer, vaccination with CD40-activated malignant B cells and interleukin 2.* (May 2001), Vol. 113, No. 2, pp. 455-460.
- Selinger, B., Hards, C., Lohmann, S., Momburg, F., Tampe, R. & Huber, C. (1998). *Eur J Immunol. Down regulation of the MHC class I antigen-processing machinery after oncogenic transformation of murine fibroblasts.* (January 1998), Vol. 28, No. 1, pp. 122-132.
- Smith, F.O., Downey, S.G. & Klapper, J.A. et al. (2008). *Clin Cancer Res. Treatment of metastatic melanoma using interleukin-2 alone or in conjunction with vaccines.* (September 2008), Vol. 14, No. 17, pp. 5610-5618.
- Soiffer, R., Lynch, T. & Mihm, M. et al. (1998). *Proc Natl Acad Sci U S A. Vaccination with irradiated autologous melanoma cells engineered to secrete human granulocyte-macrophage colony-stimulating factor generates potent antitumor immunity in patients with metastatic melanoma.* (October 1998), Vol. 95, No. 22, pp. 13141-13146.
- Soiffer, R., Hodi, F.S. & Haluska, F. et al. (2003). *J Clin Oncol. Vaccination with irradiated, autologous melanoma cells engineered to secrete granulocyte-macrophage colony-stimulating factor by adenoviral-mediated gene transfer augments antitumor immunity in patients with metastatic melanoma.* (May 2003), Vol. 21, No. 17, pp. 3343-3350.
- Strand, S., Hofmann, W.J. & Hug, H. et al. (1996). *Nature Med. Lymphocyte apoptosis induced by CD 95 (APO-1/CD95) ligand – expressing tumor cells – a mechanism of immune evasion.* (December 1996), Vol. 2, No. 12, pp. 1361-1366.
- Streit, M. & Detmar, M. (2003). *Oncogene. Angiogenesis, lymphangiogenesis, and melanoma metastasis.* (May 2003), Vol. 22, No. 20, pp. 3172-3179.
- Sun, Y., Jurgovsky, K., Möller, P., Alijagic, S., Dorbic, T., Georgieva, J., Wittig, B. & Schadendorf, D. (1998). *Gene Ther. Vaccination with IL-12 gene-modified autologous melanoma cells: preclinical results and a first clinical phase I study.* (April 1998), Vol. 5, No. 4, pp. 481-490.

- Swain, S.L. (1995). *J Leukoc Biol. CD4 T cell development and cytokine polarization: an overview.* (May 1995), Vol. 57, No. 5, pp. 797-819.
- Sznol, M., Hodi, F.S. & Margolin, K. (2008). *J Clin Oncol. Phase I study of BMS-663513, a fully human anti-CD137 agonist monoclonal antibody, in patients (pts) with advanced cancer (CA).* Suppl. 26. Abstract 3007.
- Tarhini, A.A., Moschos, S.S., Schlesselman, J.J. et al. (2008). *J Clin Oncol. Phase II trial combination biotherapy of high-dose interferon alfa-2b and tremelimumab for recurrent inoperable stage III or IV melanoma.* Suppl. 26, Abstract 9009.
- Thurner, B., Haendle, I. & Roder, C. et al. (1999). *J Exp Med. Vaccination with Mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma.* (December 1999), Vol. 190, No. 11, pp. 1669-1678.
- Trikha, M., Zhou, Z., Nemeth, J.A. et al. (2004). *Int. J. Cancer. CNTO 95, a fully human monoclonal antibody that inhibits  $\alpha v$  integrins, has antitumor and antiangiogenic activity in vivo.* (Jun 2004), Vol. 110, No. 3, pp. 326-335.
- Tse, K.F., Jeffers, M., Pollack, V.A. et al. (2006). *Clin. Cancer Res. CR011, a fully human monoclonal antibody-auristatin E conjugate, for the treatment of melanoma.* (February 2006), Vol. 12, No. 4, pp. 1373-1382.
- Ugurel, S., Rappl, G., Tilgen, W. & Reinhold, U. (2001). *J Clin Oncol. Increased serum concentration of angiogenic factors in malignant melanoma patients correlates with tumor progression and survival.* (January 2001), Vol. 19, No.2, pp. 577-583.
- Uyittenhove, C., Maryanski, J. & Boon, T. (1983). *J Exp Med. Escape of mouse mastocytoma P815 after nearly complete rejection is due to antigen-loss variants rather than immunosuppression.* (March 1983), Vol. 157, No. 3, pp. 1040-1052.
- Vaishampayan, U., Abrams, J. & Darrah, D. et al. (2002). *Clin Cancer Res. Active immunotherapy of metastatic melanoma with allogeneic melanoma lysates and interferon alpha.* (December 2002), Vol. 8, No. 12, pp. 3696-36701.
- Varker, K.A., Biber, J.E. & Kefauver, C. et al. (2007). *Ann. Surg. Oncol. A randomized Phase 2 trial of bevacizumab with or without daily low-dose interferon  $\alpha$ -2b in metastatic malignant melanoma.* (August 2007), Vol. 14, No. 8, pp. 2367-2376.
- Vonderheide, R.H., Flaherty, K. & Khalil, M. et al. (2006). *J Clin Oncol. Clinical activity and immune modulation in cancer patients treated with CP-870,893, a novel CD40 agonist monoclonal antibody.* (March 2006), Vol. 25, No. 7, pp. 876-883.
- Von Mos, R., Seifert, B. & Ochsenein, A. et al. (2009). *Ann Oncol. Temozolomide combined with bevacizumab in metastatic melanoma. A multicenter phase II trial (SAKK 50/07).* Suppl 20. Abstract 24LBA.
- Wallack, M.K., Sivanandham, M. & Balch & C.M. et al. (1998). *J Am Coll Surg. Surgical adjuvant active specific immunotherapy for patients with stage III melanoma: the final analysis of data from phase III, randomized, double-blind, multicenter vaccinia melanoma oncolysate trial.* (July 1998), Vol. 187, No. 1, pp. 69-77.
- Walker, P., Saas, P. & Dietrich, P.Y. (1997). *J Immunol. Role of Fas ligand in immune escape.* (May 1997), Vol. 158, No.10: pp. 4521-4524.
- Wang, F., Bade, E., Kuniyoshi, C., Spears, L., Jeffery, G., Marty, V., Groshen, S. & Weber, J. (1999). *Clin. Cancer Res. Phase I trial of a MART-1 peptide vaccine with incomplete Freund's adjuvant for resected high-risk melanoma.* (October 1999), Vol. 5, No. 10, pp. 2756-2765.

- Ward, P.L., Koeppen, H., Hurteau, T. & Schreiber, H. (1989). *J Exp Med. Tumor antigens defined by cloned immunological probes are highly polymorphic and are not detected in autologous normal cells.* (July 1989), Vol. 170, No. 1, pp. 217-32.
- Ward, P.L., Koeppen, H., Hurteau, T., Rowley, D.A. & Schreiber, H. (1990). *Cancer Res. Major histocompatibility complex class I and unique antigen expression by murine tumors that escaped from CD8+ T-cell-dependent surveillance.* (July 1990), Vol. 50, No. 13, pp. 3851-8.
- Weber, J., Sondak, V.K. & Scotland et al. (2003). *Cancer. Granulocyte-macrophage-colony-stimulating factor added to a multi-peptide vaccine for resected Stage II melanoma.* (January 2003), Vol. 97, No. 1, pp. 186-200.
- Weber, J., Boswell, W. & Smith, J. et al. (2008). *J. Immunother. Phase 1 trial of intranodal injection of a Melan-A/MART-1 DNA plasmid vaccine in patients with stage IV melanoma.* (February 2008), Vol. 31, No. 2, pp. 215-223.
- Wolchok, J.D., Hoos, A. & O'Day, S. (2009). *Clin Cancer Res. Guidelines for evaluation of immune therapy activity in solid tumors: immune-related response criteria.* (December 2009), Vol. 15, No. 23, pp. 7412-7420.

IntechOpen



## **Current Management of Malignant Melanoma**

Edited by Dr. Ming Yu Cao

ISBN 978-953-307-264-7

Hard cover, 274 pages

**Publisher** InTech

**Published online** 22, September, 2011

**Published in print edition** September, 2011

Management of melanoma is challenging, especially for the late stage of the disease. Development of new therapies and optimizing current treatments are being pursued in attempt to further improve the survival rate. The book provides up-to-date knowledge and experience in early diagnosis, prevention and treatment of melanoma as well as current ongoing clinical studies on melanoma. The book also provides the most recent perspectives of research on the molecular basis of melanoma, such as melanoma associated genes and a possible link between stress and melanoma.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Jacek Mackiewicz and Andrzej Mackiewicz (2011). Immunotargeting of Melanoma, Current Management of Malignant Melanoma, Dr. Ming Yu Cao (Ed.), ISBN: 978-953-307-264-7, InTech, Available from: <http://www.intechopen.com/books/current-management-of-malignant-melanoma/immunotargeting-of-melanoma>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821



© 2011 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License](https://creativecommons.org/licenses/by-nc-sa/3.0/), which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.

IntechOpen

IntechOpen