Immunotherapeutical approaches for multiple myeloma

Immunoterapia w szpiczaku plazmocytowym

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ABSTRACT

Multiple myeloma (MM) is characterized by deep immunodeficiency caused by many factors including uncontrolled production of monoclonal immunoglobulin and inhibitory effect of microenvironment. Despite the use of different therapeutic strategies as drug treatment, chemotherapy and stem cell transplantation still remains an incurable disease. Novel treatment modalities introduced for MM significantly increased overall survival, but it still reaches not more than 4 years. Therefore there is a necessity to generate novel therapeutical strategies that successfully improve quality of MM patients life and cause complete recovery. Tumor-associated antigens (TAA) are potential targets for cancer immunotherapy due to their limited expression on normal tissues or restriction to tumor cells. Epitopes derived from TAA induce specific, cytotoxic T lymphocytes which are able to recognize and eradicate myeloma cells with good efficacy. These features allow to construct various types of peptide-based vaccines, which could prolong MM patients life and lead to complete remission. In this work we have characterized (C/T), human telomerase reverse transcriptase (hTERT), X-box binding protein 1 (XBP-1), PAS domain-containing protein 1 (PASD-1), receptor for hyaluronic acid–mediated motility (RHAMM), mucin 1 (MUC-1), Wilms Tumor-1 (WT1), that might represent a target for peptide-based immunotherapy. Results from first clinical trials on immunotherapy in MM were also characterized.

Key words: multiple myeloma (MM), tumor-associated antigens (TAA), immunotherapy

Słowa kluczowe: szpiczak plazmocytowy, immunoterapia, antygeny

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Autorzy nie zgłaszają konfliktu interesu

Multiple myeloma (MM) is hematological malignancy characterized by neoplastic proliferation of the monoclonal plasma cells [1]. MM affects mostly people after 40 years of age and is estimated that annually constitutes about 1% of all new cancer cases in western countries [2]. The main symptoms related to abnormal hematopoietic stem cells function include osteolytic lesions (caused by activation of osteoclasts as well as inhibition of osteoblasts), anemia and immunosuppression. Furthermore, uncontrolled production and secretion of monoclonal immunoglobulin by myeloma cell (plasmocyte) contributes to immunodeficiency [3]. Specific genetic aberrations and abnormal gene expression of few protooncogenes also accompany MM [4]. Although novel treatment modalities introduced for MM significantly increased overall survival (OS), OS still reaches not more than 4 years [2]. MM still remains incurable disease and patients relapse in certain time-point. Therefore there is a necessity to generate novel therapeutical strategies. Results from allogenic stem cell transplantation (ASCT) did not prove survival benefit to MM patients mainly due to high treatment-related mortality. Interestingly, those patients who survived ASCT procedure benefited from graft-versus–leukemia effect, what points that activation of immune system might effectively treat myeloma and anti-myeloma effect successfully protects MM patients from relapse. Other methods to induce specific anti-myeloma immunity were also introduced in the clinical practice. Here we review the current status of immunotherapeutical approaches for MM.

Immune status of MM patients

Immune system in MM is deficient due to a lot of factors associated with this disease. Low levels of normal B cells are not enough to assure sufficient immune control on MM. Kay et al. observed decrease of normal B lymphocytes, as well as their functional deficit characterized by impaired response for the myeloma antigens [5–7]. Reduced production of polyclonal im-

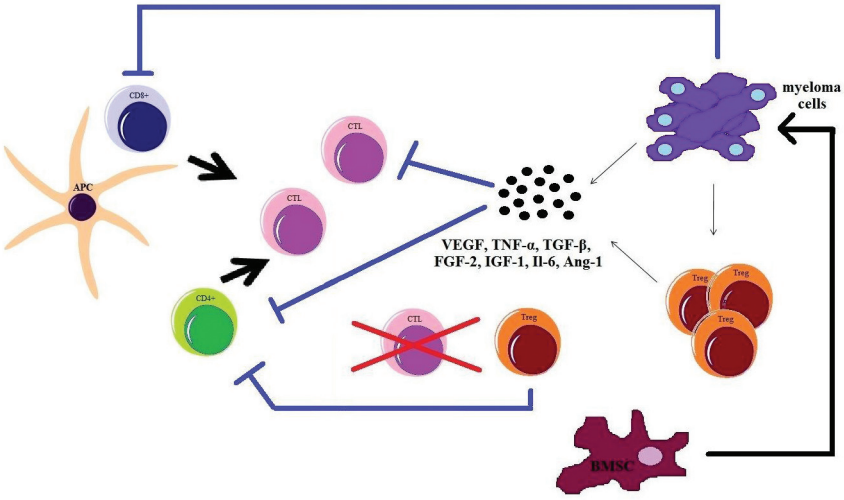

Fig. 1. Immune modulation in tumor microenvironment
Antigen presenting cells (APC) display melanoma antigens that stimulate cytotoxic T lymphocytes (CTLs) to immunological response against myeloma cells. Immunosuppressive cytokines such as vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF-β), tumor necrosis factor (TNF-α), interleukin 6 (IL-6), insuline-like growth factor 1 (IGF-1), fibroblast growth factor (FBF), angiopoietin 1 (Ang-1) which are present at microenvironment inhibit immune response and promote growth and migration of myeloma cells and angiogenesis. Bone marrow stem cells (BMSC) adhesive and secretion function maintain tumor growth. Treg – Regulatory T lymphocytes.

munoglobulin results in increased incidence of bacterial infections, which often contributes to death of MM patients [8]. It should be emphasized that level of CD19+ cells is lower in advanced stages than early stages. In contrast high levels of B lymphocytes were found in patients who responded for therapy [5].

The changes in T-cell subset are even more complex. Mellstedt et al. described changes in T-cell subsets including inversion of CD4:CD8 ratio, which is most pronounced in the progressive disease [9]. Lower Th1:Th2 of CD4+ ratios are due to decreased absolute number of Th2 cells [10]. Moss et al. observed oligoclonal expansion of CD8+ cells which occurs in MM patients with paraproteinemia [11]. Prabhalla et al. found decrease and functional impairment of FOXP3+ T regulatory cells (Tregs) characterized by inability to suppress anti-CD3- mediated proliferation [12]. In contrary we found increased frequencies of Tregs in MM that modulated survival. Patients with higher percentage of Tregs lived significantly shorter when compared to those with lower percentages of Tregs [13]. T cell receptor defects and down-regulation of co-stimulatory molecules that participate in signal transduction are also responsible for functional impairment of T cells [14]. MM dendritic cells (DCs) were characterized by low expression of HLA-DR as well as co-stimulatory molecules CD40 and CD80. They were also not able to present patient-specific tumor idiotype to autologous T cells [15, 16]. Additionally as a result of the disturbances in the amount of DCs and functional impairment, incapability to induction of T cells in MM patients occurs [15]. Finally, cells that are components of tumor microenvironment secrete certain cytokines that influence immune system. Immunosuppressive cytokines such as vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF-β), tumor necrosis factor (TNF-α), interleukin 6 (IL-6), insuline-like growth factor 1 (IGF-1), fibroblast growth factor (FBF), angiopoietin 1 (Ang-1) inhibit immune response, promote growth and migration of myeloma cells and angiogenesis (Figure 1). Thereby immune control is limited and ineffective in MM patients and this information should be taken into consideration while designing immunotherapy trials for MM patients [16].

Tumor-associated antigens

The fundamental step for immunotherapy constitutes the identification of the proper target antigen that is exclusively expressed on myeloma cells.

Initially, cancer/testis (C/T) antigens were discovered in patients with melanoma as favorable targets for immunotherapy presenting limited expression on normal tissue. Members of C/T family antigens were found in a lot of tumors including hematological malignances and MM. C/T antigens are presented only on germ and placenta cells. [17]. Andrade et al. found that expression of more than six C/T antigens on myeloma cells correlated with shorter OS in MM patients [18]. C/T antigens could stimulate T-cell responses effectively, therefore they could represent good targets in cancer immunotherapy [19, 20].

The proof of possibility to induce antigen-specific CTLs that are able to recognize epitope peptides derived from target antigen constitutes cornerstone for peptide-based immunotherapy. In patients in advanced stage III MM Jungbluth et al. showed that expression of melanoma-associated antigen C1 (MAGE-C1) increase with progression disease and melanoma antigen C1 (C/T-7, MAGE-C1) demonstrated 87% expression on mRNA and 82% at protein level [17]. Jungbluth et al. also showed that expression of MAGE-C1 with progression disease and also higher levels of this antigen correlated with elevated rate of plasma cell proliferation. Anderson et al. primarily identified 13 novel MAGE-C1-derived peptides. They immunized HLA-A2 transgenic mice with cell-based vaccine and detected CTL responses for 3 immunogenic peptides CT-7$_{959-968}$, CT-7$_{1083-1091}$ and CT-7$_{998-1006}$. CT-7$_{959-968}$ and CT-7$_{1083-1091}$ were recognized by specific CTLs, what represent an evidence that these molecules presented on myeloma cells are immunogenic in MM [21].

Another C/T antigen-MAGE-A3/6 showed 100% expression on mRNA and 70% protein expression on immunohistochemistry samples of third stage MM [17]. Gene expression levels of MAGE-C1 and MAGE-A3/6 demonstrated strong correlation with clinical status. Higher levels of MAGE-C1 were observed in stage III myeloma patients and MAGE -A3 was the most frequently detected C/T antigen of MAGE family in monoclonal gammopathy of undetermined significance (MGUS). Jungbluth et al. confirmed positive correlation between CT-7 expression and disease progression, as well as higher percentages of Ki-67-positive cells which indicate plasma cell proliferation. These results might suggest that CT-7 and MAGE-A3 play an important role in pathogenesis of MM and possibly participate in the deregulation of the cell cycle [17].

Moreno et al. found that MAGE-A3 gene expression is more frequent at relapse patients. High gene expression was associated with proliferative signature of the disease, which indicated poor clinical outcome [22]. They also proved that high expression of MAGE-A3 is significantly related to shorter OS and event-free survival (EFS).

Another C/T antigen LAGE-1 occurs in two alternative splicing variants a and b. Andrade et al. observed 49% expression in MM patients [18]. Their mRNA sequence is highly homologic with NY-ESO-1. Lethe et al. found LAGE-1 specific CTLs. Notably, only the LAGE-1a protein is very similar to NY-ESO-1 protein [23]. In study of Carvalho et al., authors found that LAGE-1a is expressed frequently in MM patients. According to them, LAGE could elicit immune response against NY-ESO-1 and high homology both of these C/T antigens might be useful for vaccine immunotherapy [20].

NY-ESO-1 is aberrantly expressed in certain percentage of primary myeloma cells, and its expression correlate with poor prognosis. Expression of NY-ESO-1 is very low in MGUS and smoldering myeloma. MM patients with genetic abnormalities characterized by conventional cytogenetic analyses demonstrate increase frequency of NY-ESO-1. van Rhee et al. discovered that this C/T antigen shows higher expression in relapsed patients than in newly diagnosed patients. They also found spontaneous NY-ESO-$_{1157-165}$-specific T cells in peripheral blood of NY-ESO-1 positive MM patients using HLA-A*0201/ NY-ESO-$_{1157-165}$ tetramers. NY-ESO-$_{1157-165}$-specific CTLs were able to kill primary MM cells. Moreover, they observed self-generated humoral response in MM patients also those with unfavorable cytogenetic abnormalities [24]. All of this features demonstrated that NY-ESO-1 might be an excellent target for immunotherapy.

SPAN-Xb is spermatid-specific protein which restricted expression was found in testis and on MM cells. Wang et al. were able to detect antibody response against this C/T antigen [25]. Frank et al. identified two immunogenic peptides and created the third heterolytic peptide which was even be more immunogenic [26]. They also observed CD8+ lymphocytes specific for all three SPAN-Xb-derived peptides and their cytotoxic function using ELISPOT assay [26, 27].

Sp-17 is spermatozoa protein involved in the reaction of acrosome during fertilization, which expression in healthy donors is limited to testis. This C/T antigen occurs on both mRNA and protein levels in 30% of patients with MM. Wang et al. found that SP-17 gene expression depended on promoter methylation [28]. Chriva-Internati et al. generated specific CTLs from MM patients using recombinant Sp-17 protein [29]. They found that these specific CTLs were able to lyse MM cells through perforin-mediated pathway. Due to ability to induce specific CTLs and limited expression in healthy tissue Sp-17 might be an excellent target for immunotherapy of MM patients.

Human telomerase reverse transcriptase (hTERT) represents widely expressed TAA, which activity is found in >85% tumors and protects telomeric ends of chromosomes from degradation [30]. Due to limited presence in other human tissues hTERT might constitute an excellent target for cancer immunotherapy [31]. Kryukov et al. found that hTERT specific CTLs, which were stimulated by hTERT-pulsed dendritic cells demonstrated complete cytotoxic activity against ARH77 hTERT-positive myeloma cell line [32]. ARH77 hTERT- specific immune response was manifested by secretion of INF-γ and effective lysis of MM cells.

X-box binding protein 1 (XBP-1) could represent another target for MM [33]. This antigen is a transcription factor that binds promoter of human major

histocompatibility complex class II [34]. Expression occurs constitutively in all MM cells and MM cell lines. Function of *XBP-1* is required for the terminal differentiation of B lymphocytes to plasma cells and enables immunoglobulin secretion [35]. Wen et al. found that IL-6 induces expression of XBP-1 gene in MM cells and XBP-1 participates in proliferation of myeloma cells [36]. Bagratuni et al. found that low expression of XBP-1 gene correlated with better outcome [37]. Furthermore, low level of this transcription factor allowed to predict beneficial effect of thalidomide treatment. Bae et al. observed and characterized two stable heterolytic XBP-1-derived HLA-A2 restricted peptides [38]. XBP-1 antigen-specific CTLs could secrete IFN-γ and revealed specific proliferation in response to myeloma cells in HLA-A2-restrickted and antigen-specific way. They also showed ability of XBP-1-specific CTLs to lyse myeloma cells in all tested MM cell lines (McCAR, MM1S and U266). These results confirmed that XBP-1 might represent specific target for vaccine-based immunotherapy in MM.

PAS domain-containing protein 1 (PASD-1) was investigated in diffuse large B-cell lymphoma cells [38]. Sahota et al. confirmed expression on mRNA and protein level on MM cells both in cell lines RPMI8226 and THIEL as well as patients samples [39]. PASD-1 protein level was present in almost of 95% all cells, but transcripts were demonstrated in only 27-40% of cells.

Receptor for hyaluronic acid–mediated motility (RHAMM) physiologically occurs in humans only in the thymus, placenta and testis. Initially Mohapatra et al. reported that this molecule participates in cell migration and transition cells through G2 phase to M phase in cell cycle [40, 41]. RHAMM plays significant role in formation of mitotic spindle and angiogenesis [42]. In MM the higher RHAMM expression is associated with poor prognosis [43]. Greiner et al. identified two immunogenic RHAMM epitopes R3 and R5 in acute myeloid leukemia (AML). They also generated RHAMM-R3-specific CTLs which were able to kill AML blast [44].

Mucin 1 (MUC-1) is transmembrane type I glycoprotein occurs in MM cell as well as in other hematological malignances [45]. Overexpression of this TAA contributes to increased tumor growth and survival through activate NF-κB and β-catenin pathway [46]. Choi et al. observed presence and functional activity of MUC-1- specific CTLs in MM patients [47].

Wilms Tumor-1 (WT1) is universal TAA that occurs in adults in a few normal tissue for example stromal cells, Sertolie cells, granulose cells and kidney's podocytes. This restricted expression pattern causes WT-1 to represent excellent target to cancer immunotherapy [48]. Azuma et al. generated CTLs WT-1-specific which were competent to lyse efficiently MM cells in HLA class I-restricted manner [49]. All of described TAA are detailed characterized in Table I.

Clinical trials of peptide-based vaccines

Kuball et al. described results of the clinical trial on peptide immunotherapy in 5 MM patients with advanced disease. Patients received two different types of vaccines. They were injected six times by vaccines

Table I. Tumor-associated antigens expressed in multiple myeloma

Antigen	Expression	Epitopes	CTL	References
MAGE-C1 (CT-7)	C/T	–	+	[19]
MAGE-A3	C/T	MAGE-A3/ HLA-A *6801	+	[19]
LAGE-1	C/T	–	+	[23]
NY-ESO-1	C/T	NY-ESO-$1_{157-165}$	+	[24]
SPAN-Xb	C/T	–	+	[26]
Sp17	C/T	–	+	[29]
hTERT	male germ cells, activated, lymphocytes,	–	+	[32]
XBP-1	skeletal muscles, secretory cells in pancreas and salivary glands	+	+	[37]
PASD-1	testis	–	–	[39]
RHAMM	thymus, placenta, testis	R3	+	[52]
MUC-1	cancers	MUC-1_{79-87}; MUC-$1_{138-178}$	–	[45]
WT-1	stromal cells, sertolie cells, podocytes,	–	+	[49]

C/T – cancer testis, CTL – specific cytotoxic T lymphocytes which are able to recognize tumor cells

containing CpG7909, Montanide ISA51 and either MUC-1_{79-87} and PADRE or the oligomer MUC-$1_{138-178}$ including epitopes for both $CD4^+$ and $CD8^+$ lymphocytes. Authors did not find any vaccine-reactive T lymphocytes after vaccination, however before both functional and non-functional reactive T cells were observed. It might suggest that vaccine could induce anergy or exhaustion of T cells. Subsequently in this study authors could not observe any clinical benefits [50].

Rapoport et al. studied whether adoptive cell transfer of vaccine-primed autologous T lymphocytes might induce immune response against TAA represented by hTERT and surviving. Patients are randomized based on HLA-A2 expression. 28 HLA-A2 positive patients received pneumococcal conjugate vaccine (PCV) and multipeptide tumor antigen vaccine containing peptides derived from hTERT and survivin. 26 HLA-A2 negative patients of control arm were only vaccinated against PCV. Patients received ASCT and subsequently they were vaccinated three times after infusion of T cells. Immune response to the tumor-derived antigen was detectable in 36% patients in HLA-A2 positive patients. Although the immunization of hTERT and survivin before and after ASCT improved cellular and humoral antitumor immunity, the clinical benefit of vaccination could not be noted [51].

Tsuboi et al. performed vaccination of 57 years old chemotherapy-resistant MM patient. Vaccine included Montanide ISA51 as an adjuvant and HLA-A*2402-restricted 9-mer WT1 peptide. Patient was injected intradermally during 12 weeks once a week. After vaccination 60% decrease of cancer cells in the bone marrow (BM) as well as lowering M protein level in urine from 3.6 to 0.6 g/day was observed. They also showed increased amount of WT-1-tetramer-specific T cells, as well as increase from 27% to 38.6% the frequency CD107a/b+ cells fraction responding to WT-1 peptide. Interestingly, the amount CXCR4 positive cells increased in BM and decreased in peripheral blood indicating that WT-1-specific CTLs might effectively migrate to tumor site after vaccination [48].

Schmitt et al. vaccinated AML, myelodysplastic syndrome (MDS) and four MM patients with RHAMM-derived peptide R3 with emulsificated incomplete Freund adjuvant followed by GM-CSF [52]. Patients received vaccine 4 times at biweekly intervals. They observed decrease of free light chains in serum in 50% MM patients. These results confirmed that RHAMM and their highly immunogenic peptide R3 might be novel target in cancer immunotherapy, due to their ability to induce clinical and immunological response. Two years later the same group conducted study on patients with hematological malignances including MM with higher dose of R3 peptide. They observed that higher dose did not improve response to therapy [53]. The clinical trials results are summarized in Table II.

Table II. Clinical trials

TAA	Immunological Response	Patients	References
MUC-1	–	5	[50]
hTERT and survivin	+ 10/28 –	28 HLA-A2 positive 26 HLA-A2 negative	[51]
WT-1	+	1	[48]
RHAMM	+ 3/4; + 2/3	4 (low* dose) 3 (high** dose)	[52]

* Low dose of 300μg RHAMM-derived peptide
** High dose of 1000μg RHAMM-derived peptide

This review summarizes identified targets for immunotherapy and results from first clinical trials in MM. MM is characterized by deep immunodeficiency caused by many factors including microenvironment. Limited efficacy of first peptide-based immunotherapeutical approaches point that more complex therapeutical modalities including incorporating simultaneous administration of drugs able to shape microenvironment, should be added to the treatment.

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