Iran J Cancer Prev. 2015 October; 8(5): e3755. doi: 10.17795/ijcp-3755

Published online 2015 October 27. **Review Article**

Immunotherapy in Multiple Myeloma Using Cancer-Testis Antigens

Soudeh Ghafouri-Fard,[1,*] Mahnaz Seifi-Alan,[1] Roshanak Shamsi,[1] and Ali Esfandiary[1]

[1]Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran

*Corresponding author: Soudeh Ghafouri-Frad, Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, P. O. Box: 1985717443, Tehran, IR Iran. Tel/Fax: +98-2123872572, E-mail: s.ghafourifard@sbmu.ac.ir

Received 2015 August 4; **Revised** 2015 August 29; **Accepted** 2015 September 22

Abstract

Context: Multiple myeloma (MM) is a B-cell malignancy characterized by monoclonal expansion of abnormal plasma cells in the bone marrow. It accounts for 10% of hematological malignancies. Although patients respond to a wide range of anticancer modalities, relapse occurs in a significant number of the cases. Immunotherapeutic approaches have been evolved to tackle this problem. Cancer-testis antigens CTAs as a group of tumor-associated antigens are appropriate targets for cancer immunotherapy as they have restricted expression pattern in normal tissues except for testis which is an immune-privileged site. Expression of these antigens has been assessed in different malignancies including MM.

Evidence Acquisition: We performed a computerized search of the MEDLINE/PubMed databases with key words: multiple myeloma, cancer-testis antigen, and cancer stem cell and immunotherapy.

Results: Several CTAs including NY-ESO-1, MAGE and GAGE family have been shown to be expressed in MM patients. Cellular and humoral immune responses against these antigens have been detected in MM patients.

Conclusions: The frequent and high expression level of CTAs in MM patients shows that these antigens can be applied as cancer biomarkers as well as targets for immunotherapy in these patients.

Keywords: Multiple Myeloma, Immunotherapy, Cancer-Testis Antigen, Cancer Stem Cell

1. Context

Multiple myeloma (MM) is the second most frequent hematological malignancy accounting for 10% of all hematological malignancies and 1% of all cancers (1). The main characteristic of this B-cell malignancy is the monoclonal expansion of abnormal plasma cells in the bone marrow which results in heterogeneous manifestations of the disorder including bone pain and fractures, symptoms of inadequate hematopoiesis, hypercalcemia, hyperviscosity, renal failure, infections and peripheral neuropathy (1, 2). Several chromosomal, genetic and epigenetic events have been known to contribute to plasma cell transformation as well as disease progression (3). The most significant epigenetic changes detected during the transformation of monoclonal gammopathy of undetermined significance (MGUS) to myeloma are global hypomethylation and gene-specific hypermethylation (3).

A wide range of anticancer modalities such as conventional cytotoxic chemotherapy, corticosteroids, radiation therapy, in addition to an increasing number of drugs with novel mechanisms of action such as lenalidomide and bortezomib are being used in MM patients. However, relapse and disease resistance occur in a considerable subset of patients (4, 5). This problem necessitates a search for novel modalities with the ability to target the population of cells which are responsible for relapse. Immunotherapy as a new strategy to tackle the problem of relapse has gained attention from researchers. In this field, cancer-testis antigens (CTAs) have been identified as appropriate targets for immunotherapy of cancer.

2. Evidence Acquisition

In order to collect data about expression of CTAs in MM, we performed a computerized search of the MEDLINE/PubMed databases with the key words: multiple myeloma, cancer-testis antigen, cancer stem cells and immunotherapy.

2.1. Cancer Stem Cells (CSCs) in MM

MM is one of the first tumors assumed to arise from proliferation of a rare population of CSCs (6). Evidences supporting the presence of CSCs in MM came from studies which showed that myeloma plasma cells are functionally competent and able to make monoclonal immunoglobulin. In addition, it has been shown that most of such cells are quiescent which implies that tumor growth is limited to a specialized cell population (4). CSCs have been shown to have some biological features of normal adult stem cells

such as self-renewal and expression of specific cell markers (7). MM stem cells have the ability of self-renewal in addition to producing differentiated effectors (specifically plasma cells) (4). Another characteristic of normal stem cells is resistance to toxic injury, which is believed to be shared by myeloma stem cells. The evidences for such idea have come from the results of studies showing the persistent risk of relapse among MM patients after treatment with standard therapies. Moreover, *in vitro* studies have shown that MM CSCs are relatively resistant even to novel agents compared with the myeloma plasma cells (4). The exact phenotypic characteristics of MM CSCs have not been clarified yet. However, it has been demonstrated that these cells do not express CD138. Such circulating clonotypic B-cell populations are responsible for the relative drug resistance in MM which is mediated by processes that guard normal stem cells against toxic injury. These processes include drug efflux and intracellular drug detoxification (8). Some CTAs have been shown to be preferentially expressed in CSCs derived from lung, colon and breast adenocarcinomas as well as glioma cell lines (9). However, the expression pattern of CTAs in MM CSCs remains to be elucidated.

2.2. Immunotherapy in MM

The role of immune system in the prevention of cancers and specifically hematological malignancies has been demonstrated by increased incidence of such malignancies in immunesuppressed patients. However, various tumor escape mechanisms contribute to the failure of immune system in such an attempt. Among them are abnormal number and function of dendritic cells (DCs), loss of expression of major histocompatibility complex (MHC), expansion of regulatory T cells (Treg), decreased T-cell cytotoxic activity and responsiveness to IL-2, as well as impairment of B-cell immunity which have been demonstrated in MM (10-12). In addition, a role for myeloid-derived suppressor cells in the pathogenesis of myeloma has been highlighted in recent years (13). Importantly, adaptive immune system has been shown to have a role in the control of MM given that donor-derived T cells participate in the effectiveness of allogeneic stem cell transplantation and when infused into MM patients can induce remission in relapsed patients (14). Consequently, diverse immunotherapeutic approaches such as monoclonal antibodies, idiotype or peptide vaccines, DC vaccines, adoptive T-cells immunotherapy as well as strategies to interrupt negative regulation of immune system have been developed and used in MM patients (15). Such approaches provide new tools for removal of residual disease after treatment (16). Although the ability of immunotherapeutic approaches to eliminate MM CSCs has not been evaluated yet, evidences suggest small fractions of MM cells which escape the action of chemotherapy and are invisible by conventional techniques can be destroyed by cytotoxic T lymphocytes (CTLs) (17). CTAs are appropriate targets for active-specific immunotherapy which has the important characteristic of inducing highly effective antitumor T lymphocytes and memory functions. As shown in MM patients, infusion of donor lymphocytes in relapsed and refractory patients could induce long-term remission (18). Consequently, researches are focused on feasibility and efficacy of CTA-based immunotherapeutic approaches in MM patients.

3. Results

3.1. Cancer-Testis Antigen (CTA) Expression in MM

CTAs are a group of tumor associated antigens (TAAs) with restricted expression in normal somatic tissues, with expression in gametogenic tissues as well as a wide variety of tumors (19-24). Such expression pattern has provided them with properties to be used as cancer biomarkers (25) as well as targets for cancer immunotherapy because of the immune-privileged status of the testis (26, 27). However, some of genes previously attributed to this family have been shown to be expressed in normal somatic tissues at a level comparable to gametogenic tissues which necessitate reevaluation of the expression of so called genes in normal tissues (28). According to their expression pattern, CTAs can be classified to three categories of testis-restricted, testis/brain-restricted and testis-selective (29). Assessment of CTAs expression in different tissues is important in estimation of probability of immunotherapy side-effects (16). Although the function of all CTAs has not been defined yet, some of them are shown to have relevant functions in the process of tumorigenesis. This is especially an important criterion for a TAA being used in immunotherapy to prevent alternative clonal progression. Other critical characteristics of appropriate targets for immunotherapy are restricted expression in normal tissues to reduce toxicity when targeted and expression in a large population of patients to increase applicability (15). Both of these properties have been demonstrated for CTAs. Expression of CTAs in spermatocytes and their function in meiosis imply that their aberrant expressions in cancer cells may cause abnormal chromosome segregation and aneuploidy, rationalizing their significance in the process of tumorigenesis (17). CTA expression in tumor cells has been assumed to be regulated by epigenetic mechanisms, namely demethylation (21). The absence or low expression of CTAs in differentiated somatic tissues implies that their expression in tumor tissue might be confined to cells that maintain stem cell properties i.e. CSCs (17). The expression of various CTAs has been assessed in MM samples and cell lines. These antigens have been proposed as applicable prognostic markers in newly diagnosed MM patients as well as relapse patients (16). In addition, longitudinal studies have indicated a strong correlation between CTA expression and the clinical course of MM in such a way that only a small percentage of patients in complete remission have been shown to express CTAs. On the contrary, half of the patients in partial remission have been shown to express CTAs (14). Table 1 summarizes a list of CTA genes whose expression has been identified in MM patients.

Table 1. Cancer-Testis Antigens With Expression in Multiple Myeloma (MM) Patients

Gene	Expression Pattern Among Normal Tissues	Chromosomal Location	Physiological Function	Expression Frequency in MM Patients	Reference
ADAM2	Testis-selective	8p11.2	Sperm-egg membrane binding	Analyzed only in MM cell lines	(30, 31)
AKAP4	Testis-selective	Xp11.2	Signal transduction via targeting cyclic adenosine monophosphate-dependent protein kinase-A	42%	(32, 33)
BAGE	Testis-selective	21p11.1	-	27%	(34, 35)
BRDT	Testis-selective	1p22.1	May have a role in spermatogenesis	2%	(36)
CCDC36	Testis/brain-restricted	3p21.31	-	56%	(16)
CDCA1(NUF2)	Testis-selective	1q23	Kinetochore-microtubule interactions, chromosome alignment and segregation	58%	(16, 37)
CEP290	Testis-selective	12q21.32	Microtubule organization and ciliogenesis	68%	(16, 38)
CEP55	Testis-selective	10q23.33	Has an important role in cytokinesis	77%	(16, 39)
CPXCR1	Testis-restricted	Xq21.3	-	51%	(16)
CRISP2	Testis-selective	6p21 - qter	Regulate ion channel activity	6%	(36)
CT45	Not available	Xq26.3	Modulation of cell morphology, cell adherence and cell motility	6 - 40%	(36)
CTAGE1	Testis/brain-restricted	18p11.2	-	56 - 92%	(16, 40, 41)
CTAGE5	Testis-selective	14q13.3	-	95%	(16)
CTNNA2	Testis/brain-restricted	2p12 - p11.1	Regulator for the stability of synaptic contacts	26.5-61%	(16, 42)
DDX43	Testis-selective	6q12 - q13	-	24%	(36)
DSCR8	Testis-selective	21q22.2	-	10%	(36)
FAM133A	Testis/brain-restricted	Xq21.32	-	79 - 86%	(16)
FATE1	Testis-selective	Xq28	-	39%	(16)
IGSF11	Testis-selective	3q13.32	Cell adhesion	60%	(16)
JARID1B	Testis-selective	1q32.1	A strong transcriptional repressor	82%	(16)
KM-HN-1	Not available	4q35.1	-	15 - 56%	(36)
LDHC	Testis-selective	11p15.3 - p15.5	A metabolic catalyst	11%	(30, 36)
LIP1	Testis-selective	21q11.2	-	Analyzed only in MM cell lines	(30)
MAGE-A1	Testis-restricted	Xq28	MAGE-A proteins interact with p53 proteins and may block the association of p53 with its cognate sites in chromatin.	16 - 22%	(16, 35)
MAGE-B2	Testis-restricted	Xp21.3	Activate RING E3 ubiquitin ligases	28 - 47%	(16)
MAGE-C1	Testis-restricted	Xq26	Activate RING E3 ubiquitin ligases	61 - 73%	(16)
MAGE-C2	Testis/brain-restricted	Xq27	Activate RING E3 ubiquitin ligases	9.5 - 29%	(16)
MORC	Testis-selective	3q13	Spermatogenesis	57%	(36)
NOL4	Testis-selective	18q12	-	54%	(16, 43)
NY-ESO-1	Testis-restricted	Xq28	-	7 - 36%	(31, 35)
PAGE1	Not available	Xp11.23	-	3%	(36)
PAGE2	Testis-restricted	Xp11.21	-	6%	(16)
PASD1	Testis-restricted	Xq28	Transcription factor	87%	(44, 45)
PBK	Testis-selective	8p21.2	Protein kinase, control of cell proliferation	94%	(16)
PRAME	Testis-selective	22q11.22	-	23%	(34)
PTPN20A	Testis-selective	10q11.22	Tyrosine phosphatase	25%	(43)
ROPN1	Testis-restricted	3q21.1	Testis specific anchoring protein	44%	(46)
SPACA3	Testis-selective	17q11.2	Binding of spermatozoa to the oocytes during fertilization	33 - 55%	(36)
SPAG9	Testis-selective	17q21.33	A scaffolding protein	100%	(16)
SPANXC	Testis-restricted	Xq27.1	-	0.1 - 5%	(16, 36)
SPO11	Testis-selective	20q13.2 - q13.3	Homologous recombination during meiosis	0.2 - 27%	(16, 36)
SSX1	Testis-restricted	Xp11.23	Transcriptional regulator	30%	(16)
SYCP1	Testis-selective	1p12 - p13	Assembly of the synaptonemal complexes	10%	(47)
TEX14	testis-restricted	17q22	Intercellular bridges in germ cells	7%	(16, 43)
TDRD1		10q25.3	-	0.5%	(36)
TSPY1	Testis-restricted	Yp11.2	Control of cell cycle progression, cell proliferation and tumorigenesis	10%	(16)
XAGE1	Testis-selective	Xp11.22	-	1 - 20%	(16, 36)
XAGE3	Testis-selective	Xp11	-	4 - 12.5%	(36)

3.1.1. CTAG2 (LAGE1)

In an expression study of CTAs in MM patients, it has been demonstrated to be the second most frequently expressed CTA after *MAGE-C1/CT7* (34). Its expression has been shown to result in recognition of tumor cells by LAGE specific autologous CTLs (48). Its expression in relapsed MM cases has been shown to be associated with shorter progression free survival (16). It is among the genes whose expressions in MM patients have been shown to be correlated with resistance to proteasome inhibitor bortezomib (40). Its amino acid sequence is highly similar to *NY-ESO-1* (34). It has been shown to be expressed in half of stage III myelomas and plasma cell leukemia (35) as well as about half of MM patients in another independent study (34). Its expression has been frequently detected in a selection of MM patients, despite the notably lower expression of its close homologue *NY-ESO-1*. It has been suggested that because of the high sequence homology, *LAGE-1* may also induce an immune response against *NY-ESO-1*. Moreover, the concomitant expression of *LAGE-1* and *NY-ESO-1* has been detected in 23% of the advanced stage MM patients (36).

3.1.2. GAGE Family

In total, expression GAGE family genes have been detected in one-third of MM patients. Their expression has been identified as an independent prognostic factor in MM patients (34). *GAGE-4* and *GAGE-8* are testis/brain-restricted antigens with higher expression frequency in relapse MM patients than in newly diagnosed ones (16). *GAGE-1* and *GAGE-12* have been shown to be over-expressed in a group of patients in which genes involved in cell cycle and proliferation were over-expressed, which verifies the association between CTA genes and prognosis (42).

3.1.3. MAGE Family

MAGE family members locate on the X chromosome and share a 200 amino acid common domain, named MHD (MAGE Homology Domain) (49). The expression of MAGE family members in tumor cells seems to participate in the malignant phenotype and poor response to treatment modalities (50). They may participate in tumor transformation or in some features of tumor progression for instance in tumor metastasis (51). A previous study has shown that about one-third of MM samples express at least 1 of the MAGE-A genes, and about two-third express at least 1 of the MAGE-type genes (35). Most of these genes have testis-restricted expression pattern. However, some of them have a less restricted expression pattern in normal tissues. As in most studies the expression pattern of these genes has been evaluated altogether. We will discuss them in a separate part.

3.1.3.1. MAGE-A Genes

MAGE-A1 and *MAGE-A2* expressions have been shown in new cases as well as relapse cases of MM with comparable frequencies (16). *MAGE-A3* gene has been shown to be involved in the survival of myeloma cells, reducing drug induced apoptosis (52). *MAGE-A3* has been previously suggested as a gatekeeper CT gene in solid tumors (53). However, a more recent study in MM patients has indicated that *MAGE-C1/CT7* strongly predict simultaneous expression of other CTAs, even of *MAGE-A3* (14). *MAGE-A3* has been shown to be expressed in more than half of MM patients with bone marrow plasma cell infiltration > 10% (14). *MAGE-A6* and *MAGE-A9* expressions in newly diagnosed MM patients have been associated with shorter progression free survival and shorter overall survival respectively. *MAGE-A9* has been expressed in relapse patients as well (16). *MAGE-A2*, *MAGE-A4*, *MAGE-A5*, *MAGE-A6*, *MAGE-A8* and *MAGE-12* have been shown by microarray analysis to be expressed in MM patient samples and cell lines but not in MGUS. However, *MAGE-A4* and *MAGE-A8* are expressed in a subset of normal peripheral blood memory B cells (36).

3.1.3.2. MAGE-B Genes

MAGE-B2 has been among the most frequent testis-restricted CTAs in both newly diagnosed and relapse MM patients with higher expression frequency in new cases. However, it has been proved to be expressed in a subset of normal plasma cells. *MAGE-B1* and *MAGE-B4* have also been demonstrated to be expressed in both patient sets (16).

3.1.3.3. MAGE-C Genes

MAGE-C1 gene is located in the region Xq26-27. Immunofluorescence staining has shown its protein in the cytoplasm as well as in the cell nucleus. In addition, *MAGE-C1/CT7* has physical interaction with *NY-ESO-1* protein, implying that the coordinated expression of these two genes is a frequent happening in many types of tumors, such as MM (54). It has been among the most common testis-restricted CTAs in both newly diagnosed and relapsed MM patients with expression in a small percentage of normal plasma cells (16). The expression of its protein has been demonstrated in most MM, medullary plasmacytoma, and extramedullary plasmacytoma samples (55, 56). Furthermore, its expression on the cell surface has been shown in the CAG myeloma cell line and one case of plasmacytoma (55). As *MAGE-C1/CT7* expression seems to arise early in the course of disease, it may have a function in the early stages of MM and may participate in plasma cell proliferation (34). The involvement of *MAGE-C1/CT7* in survival of malignant MM cells has been demonstrated in two independent studies aimed at *MAGE-C1/CT7* silencing. Both studies have shown that *MAGE-C1/CT7* expression in MM decreases drug induced apoptosis (52, 57). *MAGE-C1/CT7* expression has been frequently detected in osteolytic lesions of MM patients with higher expression frequency in patients with advanced stage of disease and with a chromosomal deletion of 17p13 (p53). In addition, an association has been found between the percentage of *MAGE-*

C1/CT7 expressing myeloma cells and higher proliferative rate (58). *MAGE-C1/CT7* expression has been shown to be high post therapy and in the relapse cases (16). Notably, it has been shown that if a patient expressed a *MAGE-C1/CT7* at least once, the likelihood for its expression in relapse is close to 100%, a finding that is important for selection of *MAGE-C1/CT7* as a target for immunotherapy. As it has been proved to be present even in remission phase, it is an appropriate target for immunotherapy in minimal residual disease (14). The correlation of *MAGE-C1/CT7* expression with disease stage, patient prognosis and survival has been assessed in various studies. For instance, higher expression of *MAGE-C1/CT7* has been observed in samples of MM stage III, compared to individuals with MGUS or lower stages suggesting its relation to disease progression in myeloma (34, 56). Another study has shown that *MAGE-C1/CT7* is more expressed in patients with newly diagnosed MM compared with patients with MGUS and its expression is associated with shorter survival (36). In addition, sub cellular localization of *MAGE-C1/CT7* has been shown to be correlated with prognosis in such a way that its pure cytoplasmic expression was associated with a better prognosis than combined nuclear-cytoplasmic or nuclear expression only (44). Furthermore, *MAGE-C1/CT7* expression in malignant plasma cells from bone marrow has a prognostic value in early recurrence and worse overall survival after allogeneic hematopoietic stem cells transplant (alloSCT) and correlates with disease burden after treatment (14). In another study, its expression has been identified as the only independent prognostic factor in non-transplanted patients (34). Its expression has been demonstrated to be more frequent in newly diagnosed MM cases than in relapse cases (16). *MAGE-C1/CT7*-specific T lymphocytes have been identified in MM patients implying the suitability of this antigen for immunotherapeutic approaches (46, 59, 60). *MAGE-C1* has been proposed as a gatekeeper gene for expression of other CTAs (14).

MAGE-C2 is a testis/brain-restricted antigen with higher expression frequency in newly diagnosed than relapse MM patients (16). Its expression has been frequently detected in osteolytic lesions of MM patients (58). It has been demonstrated to be expressed in about two-third of MM patients with bone marrow plasma cell infiltration > 10% (14). The high expression frequency of *MAGE-C2* in MM implies that this antigen might represent a potential target for cancer vaccines especially when considering the results of previous studies which showed its capability to elicit spontaneous humoral as well as CD8 + T cell responses in patients with *MAGE-C2* expressing solid tumor (30).

3.1.4. NY-ESO-1

It encodes the most immunogenic CTA. It has been proved to be expressed in about one-third of stage III myelomas and plasma cell leukemias (35) as well as the same percentage of total MM samples (34). In addition, its expression has been seen in an extramedullary plasmocytoma patient who also showed a strong immune response against *NY-ESO-1* (30). The increased expression of *NY-ESO-1* has been demonstrated in MM patients with cytogenetic abnormality (CA) compared to patients with normal cytogenetics. In addition, high *NY-ESO-1* expression has been seen in relapsing MM particularly those with CA. Spontaneous *NY-ESO-1*-specific antibodies have been detected in one-third of *NY-ESO-1* expression patients especially in CA patients. Furthermore, spontaneous *NY-ESO-1*-specific T cells have been found in the peripheral blood of *NY-ESO-1* expressing patients and these cells were able to kill primary MM cells after expansion (61).

3.1.5. ROPN1

It is a testis-restricted antigen which is shown to be expressed in MM cell lines as well as about half of the MM primary samples. The immunogenicity of ROPN1 has been verified by the occurrence of specific antibodies in patients' sera. Its expression at the cell surface of MM plasma cells makes it a promising target for MM immunotherapy. In addition, HLA class I-restricted cytotoxic lymphocytes have been generated with ability to kill autologous MM cells (62).

3.1.6. SSLP1

It is a novel CTA whose expression has been demonstrated in most MM cell lines at RNA and protein levels while it was not expressed in normal bone marrow. In addition, it has been proved to be expressed in a high percentage of MM patients at least once during the course of their disease as well as in considerable percentage of newly diagnosed patients. Of note, its expression was associated with adverse cytogenetics, negative prognostic factors as well as a reduced overall survival after allogeneic stem cell transplantation. About 10% of patients showed spontaneous anti-SLLP humoral immunity. In addition, *in vitro* stimulation with SLLP1 could result in induction of antigen-specific T cells (63).

3.1.7. SPANXB

Its expression as well as induction of high titer immunoglobulin G (IgG) antibodies against it has been demonstrated in a subset of MM patients (64). It has been shown by microarray analysis to be expressed in a small percentage of MM patient samples and cell lines as well as MGUS and normal bone marrow plasma cells (36). SPANXB-derived peptides have been shown to induce CD8 + T cell response in MM patients as well as healthy donors (65).

3.1.8. SSX Genes

Several members of SSX (synovial sarcoma, X chromosome) genes including SSX1, SSX2, SSX4, SSX5, and SSX8 have been shown to be frequently expressed in MM cell lines. In addition, SSX6 are SSX7 shown to be less fre-

quently expressed in such cell lines (30). These genes emerged as promising candidates for MM immunotherapy. Co-expression of *SSX1*, 2, 4, and 5 has been shown to be correlated with decreased survival (66). Although most of *SSX* genes are testis-restricted, some of them such as *SSX4* have a less restricted pattern among normal tissues.

3.1.8.1. SSX1

It has been among the most frequent testis-restricted CTAs in both newly diagnosed and relapse MM patients. In relapse MM patients, the expression of SSX1 has been associated with shorter overall survival as well as shorter progression free survival (16). It has been shown by microarray analysis to be expressed in MM patient samples and cell lines but not in MGUS (36). Its mRNA expression has been shown to be significantly higher in advanced stage MM patients than in early stages (67).

3.1.8.2. SSX2

Its expression has been shown in both newly diagnosed MM cases and relapse ones with comparable frequencies (16). It has been shown to be expressed in 12% of all MM samples with a bone marrow plasma cell infiltration of more than 10% (14). It has been shown by microarray analysis to be expressed in a small percentage of MM patient samples and cell lines but not in MGUS (36). Another study has revealed a strong association between the expression of SSX2 and reduced survival (66).

3.1.8.3. SSX3

Its expression has been shown in both newly diagnosed MM cases and relapse ones with comparable frequencies (16). It has been shown by microarray analysis to be expressed in a small percentage of MM patient samples and cell lines but not in MGUS (36).

3.1.8.4. SSX4

It has been shown by microarray analysis to be expressed in MM patient samples and cell lines but not in MGUS (36). However, another study has shown its expression in a minority of healthy bone marrow samples (30). Its mRNA expression has been shown to be significantly higher in advanced stage patients than in early stages (67).

3.2. CTA Immunogenicity in MM Patients

The existence of blood-testis barrier has made testis an immune-privileged site so sperms in the testis do not elicit immune responses. Such barrier is made by tight junctions between Sertoli cells along the basolateral aspect and between capillary endothelial cells. Since spermatogenesis initiates at puberty, novel cell surface antigens are expressed after finishing the time for immune system to recognize self from non-self. In addition, antigen-presenting cells are barely seen within the seminiferous tubules and human leukocyte antigen (HLA) class I is not expressed on the surface of germ cells (21). For these reasons, testis specific antigens are not presented to the immune system and if such antigens are expressed in other tissues rather than testis, they can elicit immune responses. However, some CTAs are shown to be expressed in some normal tissues in a level which is not comparable with their high expression level in the testis and tumor cells. Such mRNA expression in normal tissues other than testis does not usually lead to protein expression and immunogenicity. Instead, high expression of CTAs on cancer cells as what has been demonstrated in MM cells can elicit immune responses. Humoral as well as cellular immune responses against CTAs have been demonstrated in MM patients. It has been shown that immune responses against CTAs are induced by alloSCT. Notably, in about half of patients who developed humoral responses, antibodies were specific for *NY-ESO-1*, a CTA which is not very frequently expressed in MM. This implies an important role for *NY-ESO-1* in the immunology of MM. In addition, *NY-ESO-1*-specific CD4 + and CD8 + T-cell responses have been detected in a patient after alloSCT (30). Co-incubation of lymphocytes with *MAGE-C1*/*CT7*-transduced autologous myeloid DCs has resulted in an increase in INF-γ secretion by lymphocytes which implies that *MAGE-C1*/*CT7* expression in MM cells could induce a T-cell immune response (54). In addition, *MAGE-C1*/*CT7* has been shown to elicit spontaneous humoral responses in a subset of MM patients with all of them being in advance stages (68). Another study has shown CD8 + T cell activity against a number of CTAs including NY-ESO-1, LAGE-1 and some MAGE-A antigens in many MM patients (69). Collectively, these data show the ability of CTAs to elicit both humoral and cellular immune response in MM patients which implies the suitability of these antigens for immunotherapy of these patients.

3.3. Clinical Trials Using CTAs in MM

Frequent heterogeneous expression of CTAs within tumors and immune escape mechanisms of tumor cells imply that the use of different CTAs in a vaccine would be preferable (36). This strategy has been applied in some clinical trials. Such an approach would broaden the range of patients who benefit from a vaccine formulation. For instance, *MAGE-C1*/*CT7*, *MAGE-A3*/6 and *LAGE-1* have been suggested as good candidates for immunotherapy, given that it has been shown that they collectively cover 85% of MM cases (34). A previous report has shown that immunization of a healthy donor with *MAGE-A3* protein elicits strong antigen specific antibody, CD8 + and CD4 + T cell responses that can be transferred and expanded post-transplant in the recipient. Such an approach resulted in detectable *MAGE-A3* specific immune responses until one year and clinical remission up to 2.5 years after transplant (70) (2007). Numerous clinical trials vaccinating MM patients with MAGE family and *NY-ESO-1* are currently enrolling patients (71, 72). Table 2 shows a number of CTA-based clinical trials conducted in MM patients.

Table 2. Selected CTA-Based Clinical Trials in Multiple Myeloma Patients

Immunological Response/ Trial Status	Vaccine/Adjuvant	Number of Patients	Phase	Study, y	Reference
Not Mentioned/ Completed	*MAGE-A3* and *NY-ESO-1* peptides in combo With DTPACE chemo and auto transplantation/ GM-CSF	4 entered the study, 2 completed	II/III	2003-2004	(73)
Humoral and cellular (CD8+) immune responses were induced./ completed	MEL 200 tandem Tx (Tx1: auto, Tx2: syngeneic) and MAGE-3 recombinant protein/ ASO2B adjuvant	1	-	2005	(74)
Vaccine-specific T-cell responses were induced after transplant	*MAGE-A3* immunizations with Hiltonol® (Poly-ICLC) plus transfer of vaccine-primed autologous T cells followed by lenalidomide maintenance/ GM-CSF	27	II	2010-2014	(75, 76)
This study is ongoing, but not recruiting participants	recombinant *MAGE-A3* protein/AS15	16	I	2011-2014	(77)
Recruiting participants	Autologous T cells expressing a high affinity TCR specific for *MAGE-A3*/6 or *NY-ESO-1* administered post ASCT	26	I/II	2011-2031	(78)
Recruiting participants	CT7, *MAGE-A3*, and WT1 mRNA-electroporated Autologous Langerhans-type Dendritic Cells	20	I	2013-2015	(79)
Recruiting participants	Engineered Autologous T Cells Expressing an Affinity-enhanced TCR Specific for *NY-ESO-1* and LAGE-1	10	I/II	2013-2031	(80)
Not yet open for participant recruitment	TAA(*NY-ESO-1*, MAGEA4, PRAME, Survivin and SSX)-specific CTLs	36	I	2014-2020	(81)

3.4. Potential Side Effects Associated With Immunotherapy Modalities

Although immunotherapeutic approaches have been developed to lessen the side effects associated with conventional anti cancer modalities, they have been shown to cause adverse effects. For instance, although notable clinical responses were seen in a subset of patients treated with T cell receptor (TCR)-modified T cells redirected against two antigens including *NY-ESO-1*, on and off-target toxicity was associated with most of these clinical responses, and lethal complications have been detected in some patients (82). Of note, as some CTAs such as *NY-ESO-1* have been shown to be expressed in normal stem cells, some potential side effects can be attributed to this expression pattern (9). In addition, materials used in the preparation of ex-vivo expanded T cells such as complex media, serum and cytokines, and genetic modification, can increase the risk for infusion reactions (83). However, DC based vaccination has been shown to be associated with low toxicity (84). Finally, administration of monoclonal antibodies can result in immune reactions such as acute anaphylaxis, serum sickness, the generation of antibodies as well as numerous adverse effects that are related to their specific targets (85). Consequently, attempts are focused on the minimization of immunotherapy associated side effects.

4. Conclusions

The frequent and high expression level of CTAs in MM patients shows that these antigens can be applied as cancer biomarkers as well as targets for immunotherapy in these patients. As down-regulation of CTA expression over time may restrict their potential application for immunotherapeutic approaches in cancers, it is necessary to evaluate their expression during the course of disease (14). Consequently, longitudinal analyses of CTA expression are of value. Among CTAs, the highest expressions in MM have been reported for MAGE-A, *MAGE-C1* and *NY-ESO-1* (47, 86). Therefore, future studies should focus on application of these antigens in clinical settings especially when considering the role of *MAGE-C1* and *MAGE-A3* in promoting the survival of myeloma cells.

The identification of CSCs in MM and the resistance of such cells to conventional and even novel therapies necessitate the search for new treatment modalities for elimination of such cells. Future studies should focus on the analysis of CTA expression in MM CSCs to find whether these antigens are special markers for these cells. CTA based immunotherapy has the possible advantage of targeting such cells within the populations of MM cells, thus preventing disease recurrence.

Acknowledgments

Soudeh Ghafouri-Fard designed the study and wrote the manuscript. Mahnaz Seifi-Alan, Roshanak Shamsi and Ali Esfandiary contributed in electronic search and tables design. All authors read and approved the final manuscript.

Footnotes

Authors' Contribution: Soudeh Ghafouri-Fard designed the study and wrote the manuscript. Mahnaz Seifi-Alan, Roshanak Shamsi and Ali Esfandiary contributed in electronic search and tables design. All authors read and approved the final manuscript.

Conflict of Interest: No conflicts of interests exist.

Financial Disclosure: No financial support was utilized in this research.

References

1. Dimopoulos MA, Terpos E. Multiple myeloma. *Ann Oncol.* 2010;**21 Suppl 7**:vii143–50. doi: 10.1093/annonc/mdq370. [PubMed: 20943607]
2. Dimopoulos MA, Kastritis E, Anagnostopoulos A. Hematological malignancies: myeloma. *Ann Oncol.* 2006;**17 Suppl 10**:x137–43. doi: 10.1093/annonc/mdl251. [PubMed: 17018714]
3. Boyle EM, Davies FE, Leleu X, Morgan GJ. Understanding the multiple biological aspects leading to myeloma. *Haematologica.* 2014;**99**(4):605–12. doi: 10.3324/haematol.2013.097907. [PubMed: 24688108]
4. Huff CA, Matsui W. Multiple myeloma cancer stem cells. *J Clin Oncol.* 2008;**26**(17):2895–900. doi: 10.1200/JCO.2007.15.8428. [PubMed: 18539970]
5. Kumar SK, Rajkumar SV, Dispenzieri A, Lacy MQ, Hayman SR, Buadi FK, et al. Improved survival in multiple myeloma and the impact of novel therapies. *Blood.* 2008;**111**(5):2516–20. doi: 10.1182/blood-2007-10-116129. [PubMed: 17975015]
6. Brennan SK, Matsui W. Cancer stem cells: controversies in multiple myeloma. *J Mol Med (Berl).* 2009;**87**(11):1079–85. doi: 10.1007/s00109-009-0531-7. [PubMed: 19760278]
7. Tabarestani S, Ghafouri-Fard S. Cancer stem cells and response to therapy. *Asian Pac J Cancer Prev.* 2012;**13**(12):5951–8. [PubMed: 23464384]
8. Matsui W, Wang Q, Barber JP, Brennan S, Smith BD, Borrello I, et al. Clonogenic multiple myeloma progenitors, stem cell properties, and drug resistance. *Cancer Res.* 2008;**68**(1):190–7. doi: 10.1158/0008-5472.CAN-07-3096. [PubMed: 18172311]
9. Ghafouri-Fard S. Expression of cancer-testis antigens in stem cells: is it a potential drawback or an advantage in cancer immunotherapy. *Asian Pac J Cancer Prev.* 2015;**16**(7):3079–81. [PubMed: 25854409]
10. Costello RT, Rey J, Fauriat C, Gastaut JA, Olive D. New approaches in the immunotherapy of haematological malignancies. *Eur J Haematol.* 2003;**70**(5):333–45. [PubMed: 12694173]
11. Klippel ZK, Chou J, Towlerton AM, Voong LN, Robbins P, Bensinger WI, et al. Immune escape from NY-ESO-1-specific T-cell therapy via loss of heterozygosity in the MHC. *Gene Ther.* 2014;**21**(3):337–42. doi: 10.1038/gt.2013.87. [PubMed: 24451117]
12. Rutella S, Locatelli F. Targeting multiple-myeloma-induced immune dysfunction to improve immunotherapy outcomes. *Clin Dev Immunol.* **2012**:196063. doi: 10.1155/2012/196063. [PubMed: 22567028]
13. Gorgun GT, Whitehill G, Anderson JL, Hideshima T, Maguire C, Laubach J, et al. Tumor-promoting immune-suppressive myeloid-derived suppressor cells in the multiple myeloma microenvironment in humans. *Blood.* 2013;**121**(15):2975–87. doi: 10.1182/blood-2012-08-448548. [PubMed: 23321256]
14. Atanackovic D, Luetkens T, Hildebrandt Y, Arfsten J, Bartels K, Horn C, et al. Longitudinal analysis and prognostic effect of cancer-testis antigen expression in multiple myeloma. *Clin Cancer Res.* 2009;**15**(4):1343–52. doi: 10.1158/1078-0432.CCR-08-0989. [PubMed: 19190130]
15. Locke FL, Nishihori T, Alsina M, Kharfan-Dabaja MA. Immunotherapy strategies for multiple myeloma: the present and the future. *Immunotherapy.* 2013;**5**(9):1005–20. doi: 10.2217/imt.13.97. [PubMed: 23998734]
16. van Duin M, Broyl A, de Knegt Y, Goldschmidt H, Richardson PG, Hop WC, et al. Cancer testis antigens in newly diagnosed and relapse multiple myeloma: prognostic markers and potential targets for immunotherapy. *Haematologica.* 2011;**96**(11):1662–9. doi: 10.3324/haematol.2010.037978. [PubMed: 21791470]
17. de Carvalho F, Vettore AL, Colleoni GW. Cancer/Testis Antigen MAGE-C1/CT7: new target for multiple myeloma therapy. *Clin Dev Immunol.* 2012;**2012**:257695. doi: 10.1155/2012/257695. [PubMed: 22481966]
18. Bae J, Munshi NC, Anderson KC. Immunotherapy strategies in multiple myeloma. *Hematol Oncol Clin North Am.* 2014;**28**(5):927–43. doi: 10.1016/j.hoc.2014.07.002. [PubMed: 25212890]
19. Dianatpour M, Mehdipour P, Nayernia K, Mobasheri MB, Ghafouri-Fard S, Savad S, et al. Expression of Testis Specific Genes TSGA10, TEX101 and ODF3 in Breast Cancer. *Iran Red Crescent Med J.* 2012;**14**(11):722–6. doi: 10.5812/ircmj.3611. [PubMed: 23396665]
20. Ghafouri-Fard S, Abbasi A, Moslehi H, Faramarzi N, Taba Taba Vakili S, Mobasheri MB, et al. Elevated expression levels of testis-specific genes TEX101 and SPATA19 in basal cell carcinoma and their correlation with clinical and pathological features. *Br J Dermatol.* 2010;**162**(4):772–9. doi: 10.1111/j.1365-2133.2009.09568.x. [PubMed: 19886887]
21. Ghafouri-Fard S, Modarressi MH. Cancer-testis antigens: potential targets for cancer immunotherapy. *Arch Iran Med.* 2009;**12**(4):395–404. [PubMed: 19566358]
22. Ghafouri-Fard S, Modarressi MH. Expression of cancer-testis genes in brain tumors: implications for cancer immunotherapy. *Immunotherapy.* 2012;**4**(1):59–75. doi: 10.2217/imt.11.145. [PubMed: 22150001]
23. Seifi-Alan M, Shamsi R, Ghafouri-Fard S, Mirfakhraie R, Zare-Abdollahi D, Movafagh A, et al. Expression analysis of two cancer-testis genes, FBXO39 and TDRD4, in breast cancer tissues and cell lines. *Asian Pac J Cancer Prev.* 2014;**14**(11):6625–9. [PubMed: 24377578]
24. Ghafouri-Fard S, Modarressi MH, Yazarloo F. Expression of testis-specific genes, TEX101 and ODF4, in chronic myeloid leukemia and evaluation of TEX101 immunogenicity. *Ann Saudi Med.* 2012;**32**(3):256–61. doi: 10.5144/0256-4947.2012.256. [PubMed: 22588436]
25. Ghafouri-Fard S, Nekoohesh L, Motevaseli E. Bladder cancer biomarkers: review and update. *Asian Pac J Cancer Prev.* 2014;**15**(6):2395–403. [PubMed: 24761840]
26. Ghafouri-Fard S, Ghafouri-Fard S. siRNA and cancer immunotherapy. *Immunotherapy.* 2012;**4**(9):907–17. doi: 10.2217/imt.12.87. [PubMed: 23046235]
27. Ghafouri-Fard S, Shamsi R, Seifi-Alan M, Javaheri M, Tabarestani S. Cancer-testis genes as candidates for immunotherapy in breast cancer. *Immunotherapy.* 2014;**6**(2):165–79. doi: 10.2217/imt.13.165. [PubMed: 24491090]
28. Ghafouri-Fard S, Rezazadeh F, Zare-Abdollahi D, Omrani MD, Movafagh A. Are so-called cancer-testis genes expressed only in testis? *Asian Pac J Cancer Prev.* 2014;**15**(18):7703–5. [PubMed: 25292049]
29. Hofmann O, Caballero OL, Stevenson BJ, Chen YT, Cohen T, Chua R, et al. Genome-wide analysis of cancer/testis gene expression. *Proc Natl Acad Sci U S A.* 2008;**105**(51):20422–7. doi: 10.1073/pnas.0810777105. [PubMed: 19088187]
30. Atanackovic D, Arfsten J, Cao Y, Gnjatic S, Schnieders F, Bartels K, et al. Cancer-testis antigens are commonly expressed in multiple myeloma and induce systemic immunity following allogeneic stem cell transplantation. *Blood.* 2007;**109**(3):1103–12. doi: 10.1182/blood-2006-04-014480. [PubMed: 17023585]
31. Evans JP. Fertilin beta and other ADAMs as integrin ligands: insights into cell adhesion and fertilization. *Bioessays.* 2001;**23**(7):628–39. doi: 10.1002/bies.1088. [PubMed: 11462216]
32. Turner RM, Musse MP, Mandal A, Klotz K, Jayes FC, Herr JC, et al. Molecular genetic analysis of two human sperm fibrous sheath proteins, AKAP4 and AKAP3, in men with dysplasia of the fibrous sheath. *J Androl.* 2001;**22**(2):302–15. [PubMed: 11229805]
33. Chiriva-Internati M, Ferrari R, Yu Y, Hamrick C, Gagliano N, Grizzi F, et al. AKAP-4: a novel cancer testis antigen for multiple myeloma. *Br J Haematol.* 2008;**140**(4):465–8. doi: 10.1111/j.1365-2141.2007.06940.x. [PubMed: 18217892]
34. Andrade VC, Vettore AL, Felix RS, Almeida MS, Carvalho F, Oliveira JS, et al. Prognostic impact of cancer/testis antigen expression in advanced stage multiple myeloma patients. *Cancer Immun.* 2008;**8**:2. [PubMed: 18237105]
35. van Baren N, Brasseur F, Godelaine D, Hames G, Ferrant A, Lehmann F, et al. Genes encoding tumor-specific antigens are expressed in human myeloma cells. *Blood.* 1999;**94**(4):1156–64. [PubMed: 10438702]
36. Condomines M, Hose D, Raynaud P, Hundemer M, De Vos J, Baudard M, et al. Cancer/testis genes in multiple myeloma: expression patterns and prognosis value determined by microarray analysis. *J Immunol.* 2007;**178**(5):3307–15. [PubMed: 17312182]

37. Hayama S, Daigo Y, Kato T, Ishikawa N, Yamabuki T, Miyamoto M, et al. Activation of CDCA1-KNTC2, members of centromere protein complex, involved in pulmonary carcinogenesis. *Cancer Res.* 2006;**66**(21):10339–48. doi: 10.1158/0008-5472.CAN-06-2137. [PubMed: 17079454]
38. Sayer JA, Otto EA, O'Toole JF, Nurnberg G, Kennedy MA, Becker C, et al. The centrosomal protein nephrocystin-6 is mutated in Joubert syndrome and activates transcription factor ATF4. *Nat Genet.* 2006;**38**(6):674–81. doi: 10.1038/ng1786. [PubMed: 16682973]
39. Zhao WM, Seki A, Fang G. Cep55, a microtubule-bundling protein, associates with centralspindlin to control the midbody integrity and cell abscission during cytokinesis. *Mol Biol Cell.* 2006;**17**(9):3881–96. doi: 10.1091/mbc.E06-01-0015. [PubMed: 16790497]
40. Richardson P, Barlogie B, Berenson J, Singhal S, Jagannath S, Irwin D. Prognostic factors associated with response in patients with relapsed and refractory multiple myeloma (MM) treated with bortezomib. *Proc Am Soc Clin Oncol.* 2003.
41. Liggins AP, Lim SH, Soilleux EJ, Pulford K, Banham AH. A panel of cancer-testis genes exhibiting broad-spectrum expression in haematological malignancies. *Cancer Immun.* 2010;**10**:8. [PubMed: 20726502]
42. Moreaux J, Klein B, Bataille R, Descamps G, Maiga S, Hose D, et al. A high-risk signature for patients with multiple myeloma established from the molecular classification of human myeloma cell lines. *Haematologica.* 2011;**96**(4):574–82. doi: 10.3324/haematol.2010.033456. [PubMed: 21173094]
43. Condomines M, Hose D, Reme T, Requirand G, Hundemer M, Schoenhals M, et al. Gene expression profiling and real-time PCR analyses identify novel potential cancer-testis antigens in multiple myeloma. *J Immunol.* 2009;**183**(2):832–40. doi: 10.4049/jimmunol.0803298. [PubMed: 19542363]
44. Tinguely M, Jenni B, Knights A, Lopes B, Korol D, Rousson V, et al. MAGE-C1/CT-7 expression in plasma cell myeloma: subcellular localization impacts on clinical outcome. *Cancer Sci.* 2008;**99**(4):720–5. doi: 10.1111/j.1349-7006.2008.00738.x. [PubMed: 18307538]
45. clinicaltrials.gov. *Study of MAGE-A3 and NY-ESO-1 Immunotherapy in Combo With DTPACE Chemo and Auto Transplantation in Multiple Myeloma.* Available from: http://clinicaltrials.gov/show/NCT00090493.
46. Nuber N, Curioni-Fontecedro A, Matter C, Soldini D, Tiercy JM, von Boehmer L, et al. Fine analysis of spontaneous MAGE-C1/CT7-specific immunity in melanoma patients. *Proc Natl Acad Sci U S A.* 2010;**107**(34):15187–92. doi: 10.1073/pnas.1002155107. [PubMed: 20696919]
47. Lim SH, Austin S, Owen-Jones E, Robinson L. Expression of testicular genes in haematological malignancies. *Br J Cancer.* 1999;**81**(7):1162–4. doi: 10.1038/sj.bjc.6690824. [PubMed: 10584877]
48. Lethe B, Lucas S, Michaux L, De Smet C, Godelaine D, Serrano A, et al. LAGE-1, a new gene with tumor specificity. *Int J Cancer.* 1998;**76**(6):903–8. [PubMed: 9626360]
49. Chomez P, De Backer O, Bertrand M, De Plaen E, Boon T, Lucas S. An overview of the MAGE gene family with the identification of all human members of the family. *Cancer Res.* 2001;**61**(14):5544–51. [PubMed: 11454705]
50. Yang B, O'Herrin S, Wu J, Reagan-Shaw S, Ma Y, Nihal M, et al. Select cancer testes antigens of the MAGE-A, -B, and -C families are expressed in mast cell lines and promote cell viability in vitro and in vivo. *J Invest Dermatol.* 2007;**127**(2):267–75. doi: 10.1038/sj.jid.5700548. [PubMed: 16960553]
51. van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, et al. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science.* 1991;**254**(5038):1643–7. [PubMed: 1840703]
52. Atanackovic D, Hildebrandt Y, Jadczak A, Cao Y, Luetkens T, Meyer S, et al. Cancer-testis antigens MAGE-C1/CT7 and MAGE-A3 promote the survival of multiple myeloma cells. *Haematologica.* 2010;**95**(5):785–93. doi: 10.3324/haematol.2009.014464. [PubMed: 20015885]
53. Gure AO, Chua R, Williamson B, Gonen M, Ferrera CA, Gnjatic S, et al. Cancer-testis genes are coordinately expressed and are markers of poor outcome in non-small cell lung cancer. *Clin Cancer Res.* 2005;**11**(22):8055–62. doi: 10.1158/1078-0432.CCR-05-1203. [PubMed: 16299236]
54. Cho HJ, Caballero OL, Gnjatic S, Andrade VC, Colleoni GW, Vettore AL, et al. Physical interaction of two cancer-testis antigens, MAGE-C1 (CT7) and NY-ESO-1 (CT6). *Cancer Immun.* 2006;**6**:12. [PubMed: 17137291]
55. Dhodapkar MV, Osman K, Teruya-Feldstein J, Filippa D, Hedvat CV, Iversen K, et al. Expression of cancer/testis (CT) antigens MAGE-A1, MAGE-A3, MAGE-A4, CT-7, and NY-ESO-1 in malignant gammopathies is heterogeneous and correlates with site, stage and risk status of disease. *Cancer Immun.* 2003;**3**:9. [PubMed: 12875607]
56. Jungbluth AA, Ely S, DiLiberto M, Niesvizky R, Williamson B, Frosina D, et al. The cancer-testis antigens CT7 (MAGE-C1) and MAGE-A3/6 are commonly expressed in multiple myeloma and correlate with plasma-cell proliferation. *Blood.* 2005;**106**(1):167–74. doi: 10.1182/blood-2004-12-4931. [PubMed: 15761016]
57. de Carvalho F, Costa ET, Camargo AA, Gregorio JC, Masotti C, Andrade VC, et al. Targeting MAGE-C1/CT7 expression increases cell sensitivity to the proteasome inhibitor bortezomib in multiple myeloma cell lines. *PLoS One.* 2011;**6**(11):e27707. doi: 10.1371/journal.pone.0027707. [PubMed: 22110734]
58. Pabst C, Zustin J, Jacobsen F, Luetkens T, Kroger N, Schilling G, et al. Expression and prognostic relevance of MAGE-C1/CT7 and MAGE-C2/CT10 in osteolytic lesions of patients with multiple myeloma. *Exp Mol Pathol.* 2010;**89**(2):175–81. doi: 10.1016/j.yexmp.2010.06.011. [PubMed: 20621094]
59. Lendvai N, Gnjatic S, Ritter E, Mangone M, Austin W, Reyner K, et al. Cellular immune responses against CT7 (MAGE-C1) and humoral responses against other cancer-testis antigens in multiple myeloma patients. *Cancer Immun.* 2010;**10**:4. [PubMed: 20108890]
60. Anderson LJ, Cook DR, Yamamoto TN, Berger C, Maloney DG, Riddell SR. Identification of MAGE-C1 (CT-7) epitopes for T-cell therapy of multiple myeloma. *Cancer Immunol Immunother.* 2011;**60**(7):985–97. doi: 10.1007/s00262-011-1009-3. [PubMed: 21461886]
61. van Rhee F, Szmania SM, Zhan F, Gupta SK, Pomtree M, Lin P, et al. NY-ESO-1 is highly expressed in poor-prognosis multiple myeloma and induces spontaneous humoral and cellular immune responses. *Blood.* 2005;**105**(10):3939–44. doi: 10.1182/blood-2004-09-3707. [PubMed: 15671442]
62. Chiriva-Internati M, Mirandola L, Yu Y, Jenkins MR, Gornati R, Bernardini G, et al. Cancer testis antigen, ropporin, is a potential target for multiple myeloma immunotherapy. *J Immunother.* 2011;**34**(6):490–9. doi: 10.1097/CJI.0b013e31821ca76f. [PubMed: 21654522]
63. Yousef S, Heise J, Lajmi N, Bartels K, Kroger N, Luetkens T, et al. Cancer-testis antigen SLLP1 represents a promising target for the immunotherapy of multiple myeloma. *J Transl Med.* 2015;**13**:197. doi: 10.1186/s12967-015-0562-5. [PubMed: 26088750]
64. Wang Z, Zhang Y, Liu H, Salati E, Chiriva-Internati M, Lim SH. Gene expression and immunologic consequence of SPAN-Xb in myeloma and other hematologic malignancies. *Blood.* 2003;**101**(3):955–60. doi: 10.1182/blood-2002-06-1930. [PubMed: 12393489]
65. Frank C, Hundemer M, Ho AD, Goldschmidt H, Witzens-Harig M. Cellular immune responses against the cancer-testis antigen SPAN-XB in healthy donors and patients with multiple myeloma. *Leuk Lymphoma.* 2008;**49**(4):779–85. doi: 10.1080/10428190801911688. [PubMed: 18398747]
66. Taylor BJ, Reiman T, Pittman JA, Keats JJ, de Bruijn DR, Mant MJ, et al. SSX cancer testis antigens are expressed in most multiple myeloma patients: co-expression of SSX1, 2, 4, and 5 correlates with adverse prognosis and high frequencies of SSX-positive PCs. *J Immunother.* 2005;**28**(6):564–75. [PubMed: 16224274]
67. He L, Ji JN, Liu SQ, Xue E, Liang Q, Ma Z. Expression of cancer-testis antigen in multiple myeloma. *J Huazhong Univ Sci Technolog Med Sci.* 2014;**34**(2):181–5. doi: 10.1007/s11596-014-1255-7. [PubMed: 24710929]
68. Fontecedro AC, Knights A, Tinguely M, Rosa O, Lopes B, Moch H. Expression and immunogenicity of the cancer-testis antigen CT7 (MAGE-C1) in patients with multiple myeloma. *J Clin Oncol (ASCO Annual Meeting).* 2007;**25**:8112.

69. Goodyear O, Piper K, Khan N, Starczynski J, Mahendra P, Pratt G, et al. CD8+ T cells specific for cancer germline gene antigens are found in many patients with multiple myeloma, and their frequency correlates with disease burden. *Blood.* 2005;**106**(13):4217–24. doi: 10.1182/blood-2005-02-0563. [PubMed: 16144804]
70. Szmania S, Gnjatic S, Tricot G, Stone K, Zhan F, Moreno A, et al. Immunization with a recombinant MAGE-A3 protein after high-dose therapy for myeloma. *J Immunother.* 2007;**30**(8):847–54. doi: 10.1097/CJI.0b013e318158fcff. [PubMed: 18049337]
71. Esfandiary A, Ghafouri-Fard S. MAGE-A3: an immunogenic target used in clinical practice. *Immunotherapy.* 2015;**7**(6):683–704. doi: 10.2217/imt.15.29. [PubMed: 26100270]
72. Esfandiary A, Ghafouri-Fard S. New York esophageal squamous cell carcinoma-1 and cancer immunotherapy. *Immunotherapy.* 2015;**7**(4):411–39. doi: 10.2217/imt.15.3. [PubMed: 25917631]
73. Sahota SS, Goonewardena CM, Cooper CD, Liggins AP, Ait-Tahar K, Zojer N, et al. PASD1 is a potential multiple myeloma-associated antigen. *Blood.* 2006;**108**(12):3953–5. doi: 10.1182/blood-2006-04-014621. [PubMed: 17114574]
74. Gnjatic S, Szmania S, Moreno A, Cottler-Fox M, Shaughnessy J, Barlogie B, et al., editors. Vaccination with MAGE-3 Protein Can Induce a Potent Immune Response in a Healthy Donor Which Can Be Adoptively Transferred Via Stem Cell Transplant (Tx) to a Multiple Myeloma (MM) Patient.; *ASH Annual Meeting Abstracts.*; 2005; p. 2197.
75. Rapoport AP, Aqui NA, Stadtmauer EA, Vogl DT, Xu YY, Kalos M, et al. Combination immunotherapy after ASCT for multiple myeloma using MAGE-A3/Poly-ICLC immunizations followed by adoptive transfer of vaccine-primed and costimulated autologous T cells. *Clin Cancer Res.* 2014;**20**(5):1355–65. doi: 10.1158/1078-0432.CCR-13-2817. [PubMed: 24520093]
76. clinicaltrials.gov. *Combination Immunotherapy and Autologous Stem Cell Transplantation for Myeloma*. Available from: https://clinicaltrials.gov/show/NCT01245673.
77. clinicaltrials.gov. MAGE-A3 Protein + AS15 as Consolidation for Multiple Myeloma Patients Undergoing Autologous Stem Cell Transplantation.
78. clinicaltrials.gov. Redirected Auto T Cells for Advanced Myeloma.
79. clinicaltrials.gov. CT7, MAGE-A3, and WT1 mRNA-electroporated Autologous Langerhans-type Dendritic Cells as Consolidation for Multiple Myeloma Patients Undergoing Autologous Stem Cell Transplantation.
80. clinicaltrials.gov. CT Antigen TCR-Engineered T Cells for Myeloma.
81. clinicaltrials.gov. Tumor-Associated Antigen-Specific Cytotoxic T-Lymphocytes for Multiple Myeloma (TACTAM) .
82. Stauss HJ, Morris EC. Immunotherapy with gene-modified T cells: limiting side effects provides new challenges. *Gene Ther.* 2013;**20**(11):1029–32. doi: 10.1038/gt.2013.34. [PubMed: 23804078]
83. Cruz CR, Hanley PJ, Liu H, Torrano V, Lin YF, Arce JA, et al. Adverse events following infusion of T cells for adoptive immunotherapy: a 10-year experience. *Cytotherapy.* 2010;**12**(6):743–9. doi: 10.3109/14653241003709686. [PubMed: 20429793]
84. Anguille S, Smits EL, Lion E, van Tendeloo VF, Berneman ZN. Clinical use of dendritic cells for cancer therapy. *Lancet Oncol.* 2014;**15**(7):e257–67. doi: 10.1016/S1470-2045(13)70585-0. [PubMed: 24872109]
85. Hansel TT, Kropshofer H, Singer T, Mitchell JA, George AJ. The safety and side effects of monoclonal antibodies. *Nat Rev Drug Discov.* 2010;**9**(4):325–38. doi: 10.1038/nrd3003. [PubMed: 20305665]
86. Meklat F, Li Z, Wang Z, Zhang Y, Zhang J, Jewell A, et al. Cancer-testis antigens in haematological malignancies. *Br J Haematol.* 2007;**136**(6):769–76. doi: 10.1111/j.1365-2141.2006.06484.x. [PubMed: 17223912]