

## MINI-REVIEW

# Expression of Cancer-Testis Antigens in Pediatric Cancers

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### Abstract

Cancer-testis antigens (CTAs) are a group of tumor-associated antigens with more than 140 members whose expression has been shown to be limited to gametogenic tissues and placenta among normal tissues. However, malignant tissues of different origins have shown aberrant and elevated expression of these antigens. Such a pattern of expression endows beneficial properties for use as cancer biomarkers as well as immunotherapeutic targets as a result of the immune-privileged status of the testes. CTAs have been shown to be expressed in pediatric brain tumors, different types of sarcomas, leukemias, and lymphomas as well as neuroblastomas. Although data regarding their expression pattern in childhood tumors are not as comprehensive as for adult tumors, it is supposed that CTA-based immunotherapeutic approaches can also be used for pediatric cancers. However, there are limited data about the objective clinical responses following immunotherapy in such patients. Here we try to review the available information.

**Keywords:** Cancer-testis antigen - immunotherapy - pediatric cancers

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### Introduction

Cancer-testis antigens (CTAs) are expressed in a wide variety of cancers, but their expression in normal tissues is limited to testicular germ cells and placental trophoblasts (Ghafouri-Fard and Modarressi, 2009). The testis is regarded as an immune-privileged site as a result of the existence of the blood-testis barrier, reduced ability of the large testicular inhabitant macrophages to escalate an inflammatory response, the basal expression of anti-inflammatory cytokines and the role of androgens in modifying the cytokine balance toward a tolerogenic atmosphere (Fijak and Meinhardt, 2006). Consequently, expression of testis specific antigens in other tissues can elicit cellular and humoral immune responses. Such characteristic has endowed cancer-testis antigens an important quality to be used as targets for immunotherapy. The expression pattern of CTAs has been assessed in different malignancies of various origins and spontaneous cellular and humoral responses have been observed in some of them (Ghafouri-Fard et al., 2010a; Ghafouri-Fard et al., 2010b; Ghafouri-Fard et al., 2012).

### CTAs as Cancer Stem Cell Markers

The presence of a small population of cells with stem like features has been documented in various adult malignancies. Such "cancer-stem cells (CSCs)" are believed to be sources of cancer recurrences as well as metastasis (Tabarestani and Ghafouri-Fard, 2012). Contrary to adult tumors, there are rather few childhood tumors in which CSCs have been characterized. This is

possibly due to absence of mature markers in childhood cancers, which makes it difficult to isolate these cells. However, CSCs have been isolated from pediatric leukemias, neuroblastomas and brain tumors (Castelo-Branco and Tabori, 2012). The existence of these cells in brain tumors is of special importance. Brain tumor stem cells have been identified in various pediatric tumors including medulloblastoma, ependymomas, and malignant gliomas and are supposed to be partly responsible for the resistance to existing therapies. Specific eradication of such cells will result in long-term remissions with little toxicity to normal tissues (Lasky et al., 2009). Such cells have been demonstrated in common childhood acute lymphoblastic leukemia (ALL) as well with evidences supporting that they originate from a committed lymphoid progenitor (Bernt and Armstrong, 2009). Recently, CTAs have been suggested as stem cell markers and targets for interference in tumor recurrence and metastasis (Esfandiary and Ghafouri-Fard, 2015).

### Immunotherapy in Pediatric Cancers

The aim of cancer immunotherapy is to recruit the host's immune system for tumor rejection by various tools including administration of cancer vaccines, antibodies and cytokines or by activation of Toll-like receptors (TLRs) on antigen presenting cells (APCs) (Ghafouri-Fard et al., 2012). Among different immunotherapeutic approaches, two have been translated to clinical practice: allogeneic bone marrow transplantation and monoclonal antibodies that target tumor cells (Dianatpour et al., 2012). Such approaches have been successful in some cases of

adult malignancies. As revealed by the results of recent clinical trials, cancer immunotherapy has the potential to defeat chemotherapy resistance without the common toxicities associated with cytotoxic regimens. Since relapsed malignancy is a principal cause of mortality in pediatrics, immunotherapy is likely to improve both survival and quality of life in children suffering from cancer (Wayne et al., 2010). These promising results are expected to be seen in pediatric cancers as well. As the number of children afflicted with cancer is much less than adults, novel treatment modalities are rarely developed for children alone. Instead, such treatments are used in modified ways in pediatric cancers. Considering the rapid growth of pediatric sarcomas and the fact that effective immunotherapy takes place after several weeks to months, tumor vaccinations as single agents in the setting of such cancers are implausible to have significant antitumor activity. Instead, consolidative immunotherapy has been shown to be a clinically practical modality for incorporating immunotherapy into a multimodal regimen for chemoresponsive cancer (Mackall et al., 2008).

Molecules which can be selected as targets for immunotherapy fall into 2 main categories: cell surface versus intracellular antigens. The former can be targeted in an MHC independent approach while the latter must be targeted with MHC restricted T cell approaches. NY-ESO-1, the most immunogenic CTA known until now, is among the most advanced immune targets for pediatric cancers (Orentas et al., 2012). The safety and efficacy of CTA-based immunotherapies have been evaluated in different clinical trials of adult cancer patients. However, data regarding this issue in pediatric cancers are limited. A relatively recent study has assessed the capability of adoptively transferred autologous T cells transduced with a T-cell receptor (TCR) directed against NY-ESO-1 to induce tumor regression in synovial cell sarcoma. Objective clinical responses have been documented in four of six patients with such tumors (Robbins et al., 2011).

## Expression of CTAs in Pediatric Cancers

### *Brain tumors*

MAGE-1 is a CTA whose expression has been shown in 100% of anaplastic high-grade childhood astrocytomas (ASTRs) examined in a study including glioblastomas. However, its expression has not been detected in the lowest grade, pilocytic ASTRs in the same study. Consequently, its expression levels have been suggested as biomarkers for assessment of the malignant and dedifferentiation tendencies of low-grade ASTRs. In addition, its expression levels predict the possibility of genome mutations and additional dedifferentiation towards even more malignant anaplastic ASTR and glioblastoma multiforme (Bodey et al., 2002). Another study performed in childhood ASTRs and medulloblastomas (MEDs)/primitive neuroectodermal tumors (PNETs) has shown NY-ESO-1 overexpression in all 6 MED/PNET cases examined with the highest immunostaining intensity. Although in the astrocytic tumors, the level of NY-ESO-1 expression was not as strong as that in MEDs/PNETs, there was a considerable increase in expression level in high-grade anaplastic

ASTRs and glioblastomas compared to low-grade pilocytic ASTRs. Consequently, NY-ESO-1 has been suggested as an appropriate target for antigen-directed immunotherapy of primary brain tumors (Bodey et al., 2008). Another expression study of MAGE genes, NY-ESO-1 and GAGE-1, 2, 8 in pediatric brain tumors shown expression of at least one CTA in a relatively high percentage of medulloblastomas, ependymomas, choroid plexus tumors and astrocytic tumors. However, except for a minority of tumors, the overall level of CTA expression in pediatric brain tumors has been shown to be low. Consequently, CTAs have been proposed as appropriate immunotherapeutic targets for only a selected group of childhood patients with a brain tumor (Jacobs et al., 2008). Another CTA named PRAME has been shown to be overexpressed in a high percentage of medulloblastoma samples, so it has been suggested as a strong candidate for immunotherapy in medulloblastomas (Boon et al., 2003; Vulcani-Freitas et al., 2011).

### *Sarcomas*

In an expression study of MAGE genes, NY-ESO-1 and GAGE-1, 2, 8 in different sarcomas, high levels of CTA expression have been demonstrated in all of osteosarcomas and 80% of neuroblastoma samples examined. In addition, a high proportion of rhabdomyosarcomas and Ewing's sarcomas expressed at least one CTA. So it has been concluded that pediatric solid tumors express several CTAs, which could be targeted in immunotherapeutic approaches. Coexpression of several CTAs has been documented in a large proportion of osteosarcoma and neuroblastoma samples which facilitate design of polyvalent vaccines (Jacobs et al., 2007). In addition, NY-ESO-1 expression has been shown in both biphasic and monophasic variants of synovial sarcomas and both translocation types, suggesting NY-ESO-1 based immunotherapy as an appropriate approach for such tumors (Jungbluth et al., 2001). In addition, PRAME has been shown to be expressed in most osteosarcoma samples examined (Toledo et al., 2011). Another CTA named XAGE-1 has been shown to be frequently expressed in Ewing's sarcoma (Liu et al., 2000; Zendman et al., 2002).

### *Leukemia*

PRAME has been shown to be expressed in a wide variety of adult malignancies. Its expression in normal tissues is limited. Besides, it encodes an antigen identified by autologous cytolytic T lymphocytes. So it has been suggested as a suitable target for tumor immunotherapy. Overexpression of PRAME has been detected in a significant percentage of pediatric leukemia patients (Steinbach et al., 2002; Spanaki et al., 2007). Although no significant correlation was found between PRAME overexpression and prognosis in pediatric leukemia in a study (Spanaki et al., 2007), another study has shown the rate of disease-free survival to be higher in acute myeloid leukemia (AML) and ALL patients with an overexpression of PRAME with statistically significant results in the former group (Steinbach et al., 2002). PRAME has been suggested as a useful target for immunotherapy in some leukemic children (Steinbach et al., 2002; Spanaki et al.,

2007). In addition, it is among a set of seven genes whose expressions have been shown to be decreased to normal levels in pediatric AML patients who entered a constant complete remission. This set of genes has been proposed as a sensitive and specific tool for monitoring of minimal residual disease in AML (Steinbach et al., 2006).

#### *Lymphoma*

A CTA named CT45 has been shown to be expressed in more than half of pediatric and adolescent Hodgkin's lymphoma patients examined in a study. In addition, its expression has been shown to be correlated with histologic subtypes in that higher expression frequency has been detected in nodular sclerosis Hodgkin's lymphoma compared with other subtypes (Heidebrecht et al., 2006).

#### *Neuroblastoma*

PRAME expression has been detected in 93% of primary neuroblastoma and 100% of patients with advanced disease. In addition, its expression has been shown to be associated with higher tumor stage, the age of patients at diagnosis as well as outcome of patients. Consequently, it has been suggested as an appropriate target for immunotherapy in neuroblastoma (Oberthuer et al., 2004).

## **Discussion**

The most common cancers among children are ALL, brain and central nervous system (CNS) tumors, neuroblastoma, and non-Hodgkin lymphoma respectively (Ward et al., 2014). Although conventional anticancer therapies including chemotherapy and radiation have improved cure rates for many pediatric cancer types, short and long term toxicities remain important difficulties (Haworth et al., 2014). In addition, death from relapse continues to be a principal cause of mortality in pediatrics (Wayne et al., 2010). Development of novel targeted therapies is needed to improve survival rate of pediatric cancers (Orentas et al., 2012). Such molecularly targeted therapies can be used in combination with conventional therapies to enhance the efficacy of treatment. However, translation of advances in the field of cancer genetics into practice remains challenging, due to many reasons including the lack of suitable preclinical models. Unconjugated and conjugated monoclonal antibodies (mAbs) and chimeric antigen receptors (CARs) are among novel immunotherapeutic approaches which have been used in pediatric cancers with some promising results (Saletta et al., 2014). Immunotherapeutic approaches have the advantage of specific targeting of cancer cells and are appropriate modalities for defeating minimal residual disease as well as metastases. However, the most important prerequisite for such approaches is finding appropriate tumor-associated antigen with limited expression in normal tissues while high expression in cancer cells. CTAs are important targets in this regard. Expression of CTAs has been evaluated in cancers of different origins in both adults and children. Despite few studies on expression profile of CTAs in pediatric tumors, the most common malignancies of childhood have been

the subject of such studies until now. Currently, lots of clinical trials are recruiting adult patients for CTA-based immunotherapies (<https://clinicaltrials.gov/>). NY-ESO-1 and MAGE antigens are the most popular CTAs in this field. Both antigens are among CTAs with high expression frequency in pediatric cancers as well. Although tumorigenesis mechanisms are sometimes different in adult versus pediatric tumors even in the tumors of the same histopathologic subtype (like what has been seen in glioblastomas) (Suri et al., 2009), currently there is no evidence supporting any fundamental difference in CTA expression between adult and pediatric tumors. Data from limited studies of CTA expression in pediatric tumors indicate that CTAs are expressed in a significant number of pediatric tumors. Different immunotherapeutic approaches for childhood cancers are being tested in clinical trials such as genetically modified T cells directed against some cancer biomarkers as well as therapeutic autologous dendritic cells vaccines (<https://clinicaltrials.gov/>). Currently, there are few CTA-based clinical trials recruiting pediatric cancer patients. An example is a pilot study of genetically engineered NY-ESO-1 specific (c259) T cells in HLA-A2+ patients with synovial sarcoma (<https://clinicaltrials.gov/ct2/show/NCT01343043>). Most of current clinical trials have focused on adult patients.

## **Conclusion**

Unlike adult cancers, data regarding CTA expression in pediatric cancers are limited. In addition, immunotherapeutic approaches targeting these antigens have not been used in pediatric cancers widely. As the results of clinical trials using CTAs in adult cancers have been promising in some cases, future researches should focus on the possible application of CTAs in immunotherapy of pediatric cancers. Such approaches can be beneficial for management of pediatric cancers especially refractory ones.

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