



# TARP as antigen in cancer immunotherapy

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## Abstract

In recent decades, immunotherapy has become a pivotal element in cancer treatment. A remaining challenge is the identification of cancer-associated antigens suitable as targets for immunotherapeutics with potent on-target and few off-tumor effects. The T-cell receptor gamma (TCR $\gamma$ ) chain alternate reading frame protein (TARP) was first discovered in the human prostate and androgen-sensitive prostate cancer. Thereafter, TARP was also identified in breast and endometrial cancers, salivary gland tumors, and pediatric and adult acute myeloid leukemia. Interestingly, TARP promotes tumor cell proliferation and migration, which is reflected in an association with worse survival. TARP expression in malignant cells, its role in oncogenesis, and its limited expression in normal tissues raised interest in its potential utility as a therapeutic target, and led to development of immunotherapeutic targeting strategies. In this review, we provide an overview of TARP expression, its role in different cancer types, and currently investigated TARP-directed immunotherapeutic options.

**Keywords** TARP · Cancer-associated antigen · Immunotherapy

## Introduction

Cancer remains a major public health concern worldwide, causing an estimated 9.6 million deaths in 2018 [1], and leading to treatment-associated late sequels in an ever-growing survivor population [2, 3]. Contemporary treatment protocols often combine surgery, chemotherapy, radiotherapy and hormone therapy. Although this approach has improved cure rates for several cancer entities, these strategies are not specific and lead to side effects that include e.g., cardiovascular complications, neurotoxicity and an increased risk of secondary malignancies [3–5]. Moreover, metastasis,

resistance, and relapse after initial therapy pose challenges to treatment, often due to the appearance of therapy-resistant clones [6, 7]. Recently, novel therapeutic approaches have been developed to increase survival rates while avoiding excess toxicity.

Immunotherapy exploits tumor specificity and humoral or cell-mediated immunity with the intent of inducing a durable anti-cancer response [8, 9]. Although the concept of cancer immunotherapy is long-known, it has come to the forefront in oncology only during recent decades, driven primarily by an exponential growth of knowledge of tumor biology [9]. Clinical trials evaluating amongst others the benefit of rituximab in CD20+ lymphoid malignancies [10], checkpoint inhibitors such as ipilimumab (anti-CTLA-4) and nivolumab (anti-PD-1) in melanoma [11], and tisagenlecleucel CAR T-cell therapy in CD19+ relapsed and refractory acute lymphoblastic leukemia [12, 13] have illustrated the potential of immunotherapy. Although these successes have stimulated the design of similar immunotherapeutics for other cancer types, clinical responses have often been disappointing for those tumor types with a strong immunosuppressive microenvironment or low mutational burden, such as prostate cancer [14]. In addition, the identification of cancer-associated antigens (CAA) that can be used as

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therapeutic targets without off-tumor effects remains an important field of research [15].

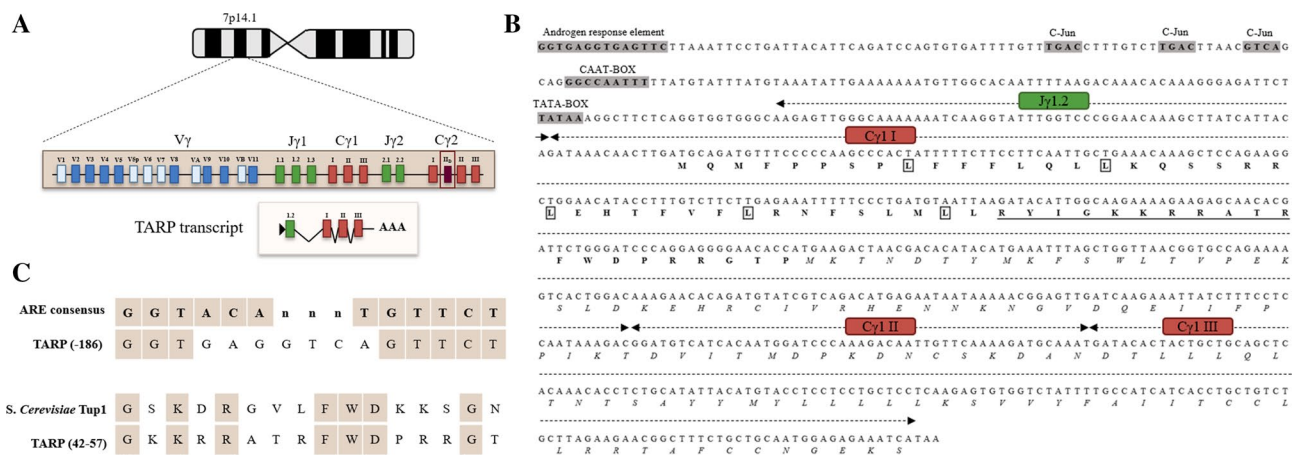
While establishing a procedure to identify prostate-specific gene expression, Vasmatazis and colleagues identified a novel prostate-specific protein encoded in the constant region of the T-cell receptor (TCR) gamma ( $\gamma$ ) chain gene, later designated as TCR alternate reading frame protein (TARP) by Essand and colleagues [16, 17]. Surprisingly, expressed sequence tags from TCR delta ( $\delta$ ), TCR alpha ( $\alpha$ ), and TCR beta ( $\beta$ ) chains were absent. This finding was unexpected, because TCR $\gamma$  and TCR $\delta$  are normally co-expressed. Essand et al. illustrated that, the identified TCR $\gamma$  transcript originates from prostate epithelial cells and not from infiltrating  $\gamma\delta$  T-lymphocytes or other prostatic cell types [17]. Although expression in healthy tissue is limited to the prostate, TARP is highly expressed in various cancers including androgen-sensitive prostate cancer [17, 18], breast [18] and endometrial cancers [19], salivary gland tumors [20], and acute myeloid leukemia (AML) [21, 22]. Upregulated TARP expression promotes tumor progression and metastasis [23], and correlates with unfavorable tumor characteristics [21]. Consequently, TARP has recently gained interest as a bona-fide CAA. In this review, currently available data regarding the expression and function of TARP in different cancer types will be discussed. In addition, the potential of TARP-targeted immunotherapeutic strategies will be highlighted, some of which are presently being evaluated in clinical trials (NCT00694551, NCT00972309).

## Structure and regulation of TARP

### TARP transcript organization

The *TARP* gene is located on chromosome 7p14.1 and encoded within the TCR $\gamma$  gene locus (Fig. 1a), which is composed of variable ( $V\gamma$ ), joining ( $J\gamma$ ), and constant ( $C\gamma$ ) gene segments. To comprise the variable domain of TCR $\gamma\delta$ , the TCR $\gamma$  locus undergoes VJ recombination, thus enabling the recognition of a wide range of antigens [17, 24]. The prostate-specific TCR $\gamma$  transcript, derived from prostate epithelial cells, begins within an intron directly upstream of the  $J\gamma 1.2$  gene segment, and contains three exons from the  $C\gamma 1$  segment and an untranslated sequence followed by a poly(A) sequence [18]. Interestingly, we recently demonstrated two different TARP transcripts with alternate use of both  $C\gamma 1$  and  $C\gamma 2$  gene segments in AML, with the only difference between the transcripts being a duplicated second exon in the  $C\gamma 2$  segments (Fig. 1a) [21].

Further analysis in androgen-dependent prostate cancer and breast cancer of *TARP* transcripts revealed two potential open reading frames (ORFs) and two protein products with molecular masses of 7 kDa and 13 kDa [18]. The second ORF encodes the 13 kDa protein corresponding to the original TCR $\gamma$  reading frame. More interestingly, the first ORF has a second in-frame ATG codon downstream of the first ATG, either of which can be used as a start site to encode the 7 kDa protein. Moreover, the protein encoded by the first ORF had no resemblance to any published protein sequences



**Fig. 1** Transcript organization of TARP. **a** TCR(JC) $\gamma$  transcript found in the prostate, adapted from Essand et al. [17]. The TCR $\gamma$  transcript found in the prostate comprises a  $J\gamma 1.2$  segment, three exons of  $C\gamma 1$ , and an untranslated sequence. A duplicated second exon in the  $C\gamma 2$  segment of the TCR $\gamma$  gene locus is indicated by a box. **b** Nucleotide and amino acid sequences of the prostate TCR $\gamma$  transcript, adapted from Wolfgang et al. [18]. A segment of the prostate-specific TCR $\gamma$  transcript is shown starting with a sequence upstream of the TARP

gene with suggested regulatory motifs in gray. The arrows indicate the exon boundaries, and the predicted amino acid sequences are indicated by bold or italic fonts. Leucines in a heptad repeat pattern are shown by boxes, and the following basic region is bold underlined. **c** Sequence homology of ARE consensus and *S. Cerevisiae* Tup1 and TARP, adapted from Chen et al. [30] and Wolfgang et al. [18]

in GenBank. Hence, the 7 kDa protein was referred to TARP. Although TARP was originally identified as a 7 kDa protein, other molecular masses have been demonstrated [20, 25]. Post-translational modifications could explain the observed differences in molecular masses [18]. Similar to findings in humans, a TCR region is highly transcribed in cats in combination with a TCR $\gamma$ J pseudogene in the feline spleen and prostate as well as in mammary neoplasm [26].

### TARP protein structure and localization

The TARP protein sequence features five leucines in heptad repeats and a typical leucine zipper motif followed by a basic region (Fig. 1b) [18]. Normally, the position of the basic region precedes the leucine repeats, yet the role of this interesting orientation needs further examination. The leucine heptad has been shown to facilitate protein–protein interactions, and is frequently found in transcription factors including the bHLH-ZIP proteins (e.g., Myc, Max) [27]. Furthermore, TARP shares homology with the fifth WD-40 repeat of *Tup1* in *Saccharomyces cerevisiae* (Fig. 1c) [18]. WD40 repeats also mediate protein–protein interactions whereby even an isolated WD repeat can confer protein binding [28]. Altogether, these observations strongly suggest that TARP interacts with other proteins. The identification of such interactors through immunoprecipitation followed by mass spectrometry analysis—an approach currently undertaken by our research team—might be a path forward to further elucidate the role of TARP in cell biology.

The TARP protein sequence does not contain any functional elements that would allow the deduction of its subcellular localization. Consequently, Maeda et al. used a specifically developed monoclonal TARP antibody (clone number: TP1) to discover TARP localization in the mitochondrial outer membrane of prostate cancer cells [29]. Recently, we conducted confocal microscopy in AML cells, and showed a perinuclear membranous-type TARP staining pattern, and only limited mitochondrial co-localization [21].

### Regulation of TARP expression

As the androgen-sensitive LNCaP prostate cell line highly expresses TARP, in contrast to the low-expressing PC3 prostate cancer line, it was hypothesized that TARP expression is tightly regulated by androgens [18] such as testosterone. Following the detailed characterization of the proximal TARP promoter [30], it became clear that it contains a fully functional androgen-response element (ARE) (Fig. 1b) [18]. Androgen-stimulated upregulation of TARP expression has been further corroborated by data illustrating that TARP mRNA is induced by 5- $\alpha$ -dihydrotestosterone, a potent testosterone metabolite, in a dose-and-time dependent manner, similar to the well-known androgen-sensitive expression

of prostate-specific antigen (PSA) [23]. Moreover, the androgen receptor can specifically bind the ARE [30].

In addition to its androgen-dependent regulation, TARP expression in prostate cancer may be induced by *HOXC6* expression, which is crucial for the development and proliferation of epithelial cells in response to hormonal signals [31, 32].

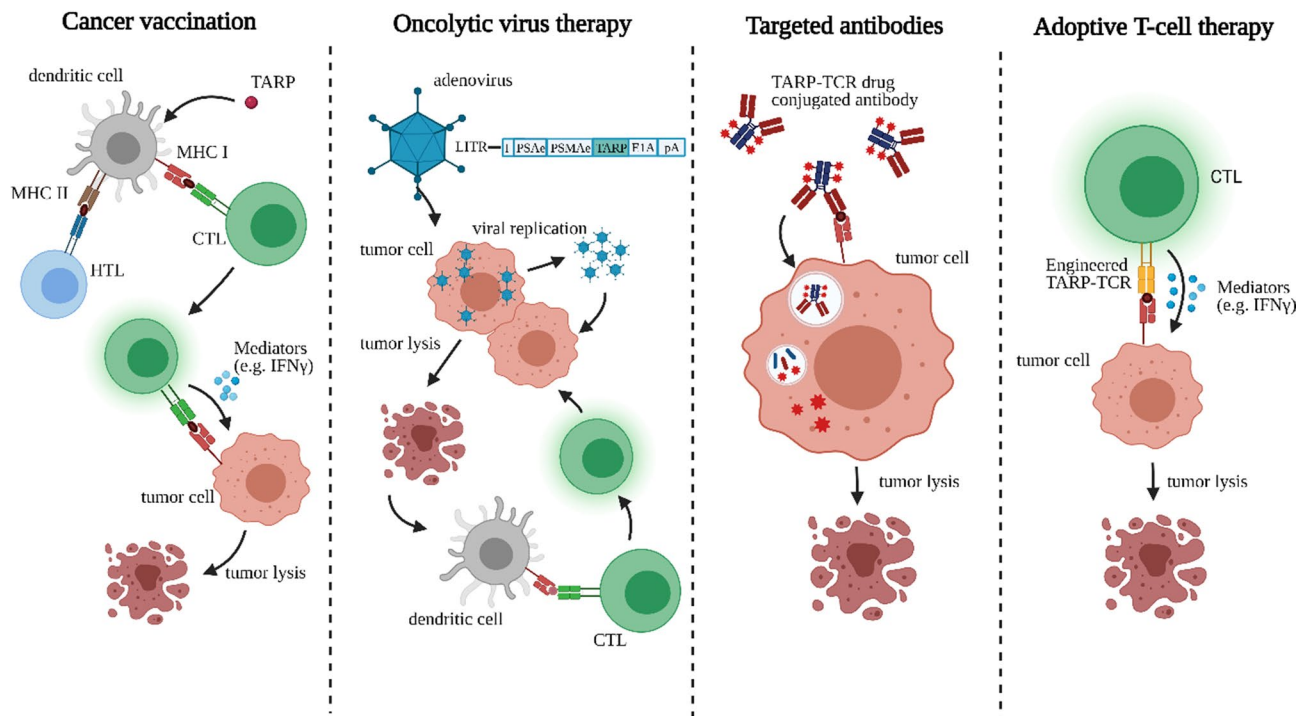
### Expression and role of TARP in normal and malignant tissues

In non-cancerous tissues, expression datasets show TARP expression in the prostate, CD4 and CD8 T-cells and NK cells. However, the oligonucleotide probes used in the microarrays do not distinguish between TCR $\gamma$  and TARP [29]. Therefore, one cannot rely on this data type to map TARP expression. Essand and colleagues have shown using Northern blot analyses, differentiating between TCR $\gamma$  and TARP transcripts, that TARP expression is limited to the prostate [17]. Also, the research group of Maeda performed a histochemical analysis of human prostate cancer tissue, which showed that only the prostate and not the infiltrating lymphocytes stained positive for the presence of TARP [29]. Nevertheless, a detailed tissue protein expression analysis is currently lacking.

TARP expression has been described in detail in human cancer cell lines and tissues. In addition to the initial observation of expression in androgen-dependent prostate cancer cell lines and tissues [17, 18], TARP transcript and protein expressions have been reported in breast cancer [18], primary and metastasized salivary adenoid cystic carcinoma (SACC) [33], malignant endometrial tissue [19] and AML cell lines and primary samples [21, 22].

To define the role of TARP in oncogenesis and cancer progression, Wolfgang et al. overexpressed TARP in an androgen-independent prostate cell line that does not express TARP [23], which resulted in a dramatic increase in the growth rate through a decreased doubling time and shortened S-phase. Analysis of gene expression profiles in a limited array representing 6538 genes showed that high TARP mRNA levels upregulated the expressions of caveolin 1 and 2 (*CAVI/2*), amphiregulin (*AREG*), and chemokine C-X-C ligand 1 (*GRO1*); and downregulated the expression of IL-1 $\beta$  [23]. Expression of *CAVI* and *AREG* have been associated with androgen-stimulated cell proliferation and metastasis of prostate cancer cells [34, 35]. *GRO1* expression has been implicated in melanocyte tumor progression, but not yet documented as a factor in prostate cancer progression [23].

As described above, TARP shares its mitochondrial location with the Bcl-2 family members that control apoptosis through the regulation of cytochrome c release [29].



**Fig. 2** Overview of therapeutic strategies targeting TARP-expressing cells. First, cancer vaccination with TARP-peptide pulsed dendritic cells (DC) that stimulate immune system effectors, the cytotoxic T-lymphocytes (CTL) and helper T-lymphocytes (HTL). Second, oncolytic virotherapy comprising a chimeric sequence including TARP to induce tumor specificity. Third, targeted antibodies directed

against TARP in complex with major histocompatibility class I. Conjugation of the antibody with a potent toxin enhances tumor targeting. Fourth, adoptive T-cell therapy with genetically engineered TCRs which express receptors specifically directed toward TARP. Figure created with BioRender (<https://biorender.com>)

Androgen is an essential anti-apoptotic factor in prostate cells. Because *TARP* expression is regulated by androgens, Maeda and colleagues hypothesized that TARP may control apoptosis through its interaction with other mitochondrial proteins.

*TARP* expression in breast cancer cell lines and its absence in normal breast cells suggest that its expression is induced after malignant transformation and is regulated by estrogen [18]. However, mutant AREs may cause expression of certain prostate-specific genes in breast cancer [36, 37]. Further study is needed to define the role of TARP in breast cancer.

Yue and colleagues showed that *TARP* expression was positively correlated with tumor size, TNM stage, perineural invasion, and distant metastases in SACC patients [20]. Moreover, TARP overexpression promoted in vitro SACC proliferation, migration, and invasion. The clinical relevance was explored in a small cohort ( $n = 15$ ) of NOPHO-DBH AML2012-treated pediatric AML patients and showed that TARP expression was significantly inversely correlated with event-free survival [22], warranting confirmation in a larger cohort. Moreover, in contrast to adult AML, high *TARP* expression in childhood AML was exclusively associated

with central nervous system involvement, high risk profiles, and FMS-like tyrosine kinase 3 (FLT3) internal tandem duplication [21], all associated with poor clinical outcome [38]. *TARP* was also highly expressed in the CD34 + CD38-leukemic stem cell fraction, which is thought to be responsible for AML relapse [21, 39].

## Therapeutic strategies

*TARP* expression in multiple cancer types and its limited expression in healthy tissue have fueled research to develop TARP-directed immunotherapeutic strategies (Fig. 2). Several clinical trials are ongoing or have been completed.

### TARP vaccination

Vaccinations targeting microbial pathogens are highly effective and safe, and provide the framework for cancer vaccinations based on cancer cell lysates or specific target proteins. Several excellent reports of preclinical findings have led to the clinical evaluation of TARP-based vaccination strategies in prostate cancer [40, 41].

A computer-based algorithm (BIMAS) and T2 binding assays have predicted TARP-peptides with expected high binding affinities to HLA-A2. These peptides include TARP<sub>4-13</sub>, TARP<sub>27-35</sub> and TARP<sub>29-37</sub> [25, 42]. Because HLA-A\*0201 is one of the most common MHC class I molecules in Caucasian humans, it is most often used in cancer vaccine development. TARP-peptide-pulsed dendritic cells (DCs) generated TARP-directed CD8 T-cells that could be expanded in vitro eliciting cytotoxic T-cell (CTL) responses in vitro and in murine models, as measured by IFN $\gamma$  production, chromium release, and flow cytometric live/dead staining [25, 42]. Because the immunogenicity of a peptide is dependent primarily on its affinity for HLA-A2, peptide enhancement is an attractive strategy to generate peptides with improved immunogenicity. Carlsson et al. increased the binding affinities of TARP<sub>27-35</sub> and TARP<sub>4-13</sub> by substituting the N-terminal anchor residues to Leu, resulting in TARP(V28L)<sub>27-35</sub> and TARP(P5L)<sub>4-13</sub> peptides [42]. Subsequently, large numbers of TARP(P5L)<sub>4-13</sub>-specific CTLs were generated by repeated ex vivo stimulation with autologous peptide-pulsed DCs followed by tetramer cell sorting and general T-cell expansion. The generated CTLs lysed both prostate and breast cancer cells [42]. Similarly, Oh and colleagues increased the binding affinity of TARP<sub>29-37</sub> by substituting either Arg at position 3 with Ala or Leu at position 9 with Val, resulting in TARP<sub>29-37-3A</sub> and TARP<sub>29-37-9V</sub> [25]. DCs pulsed with TARP<sub>29-37-9V</sub> induced the highest levels of CD8 + T cell responses that included the recognition of the wild-type peptide TARP<sub>29-37</sub>. Also, TARP<sub>29-37-9V</sub> and TARP<sub>29-37</sub> CD8 + T-cells could effectively kill human tumor cells that express TARP endogenously. Similar observations were made using TARP-mRNA pulsed DCs, demonstrating that the investigated peptides are also processed endogenously.

To determine the safety and immunogenicity of therapeutic TARP peptide vaccination, a first-in-human pilot study ( $n=41$ ) was performed in men with stage D0 prostate cancer [40]. TARP vaccination provoked a CD8 + T-cell response against the peptides present in the vaccine in 80% of the subjects and was well tolerated, with adverse events restricted to local reactions at injection sites. To validate these observations, a randomized placebo-controlled study in an identical population is currently being conducted (NCT02362451).

In conjunction with CD8-positive cytotoxic T-cells, MHC class II helper T-lymphocytes (HTLs) play a major role in antitumor responses by enhancing CTL activity and performing direct effector functions [43]. Therefore, Kobayashi et al. focused on the identification of TARP epitopes with binding affinities for MHC class II HLA-DR1, HLADR4, and HLA-DR7 [43]. TARP<sub>14-27</sub>-specific HTLs recognized TARP expressed on tumor cells and DCs. The combination of peptides that induce HTLs or CTLs may thus represent an important strategy to induce potent and durable antitumor

responses. However, further research is needed to elucidate the clinical applicability of such an approach.

### TARP promotor-based adenovirus

Oncolytic virotherapy has gained increasing attention, and is based on modification of viral genomes to ensure specific therapeutic applications [44]. One approach utilizes conditionally replicating adenoviruses (CRADs) that selectively replicate in defined target cells. Chen and colleagues investigated the potential of a prostate-specific CRAD in which they brought E1A, a viral protein required for replication, under the control of a chimeric sequence designated as PPT comprising the PSA enhancer, followed by the PSMA enhancer and the TARP promotor. This CRAD was highly prostate-specific and active in prostate cells both in the presence and absence of testosterone [45, 46]. Transduction of the Ad[I/PPT-E1A] sequence in cancer lines of multiple origins killed prostate cancer cells selectively and independently of testosterone levels. Moreover, prostate-specific cytolysis was related to selective viral replication, as replication was at least 1000-fold higher in prostate cancer cells compared to non-prostate cells. Furthermore, results were confirmed in vivo when CRAD injection in an LNCaP xenograft model resulted in reduced tumor size and tumor-free mice after 27 days. A prostate-specific CRAD utilizing PSA promotor and enhancer elements to control E1A gene expression was safe and exerted therapeutic benefits in a phase I clinical trial [46].

Yet another approach was taken by Muthana et al. who co-transduced monocyte-derived-macrophages (MDMs) with the oncolytic adenovirus described above [47]. Macrophages and their monocytic precursors have drawn attention as gene delivery vehicles as they are continuously recruited into tumors, and thus migrate into avascular, hypoxic sites. Considering that mild hypoxia also exists in healthy tissues, tumor targeting was achieved by placing viral replication under the control of prostate-specific promoters (as outlined above). Importantly, MDMs triggered sufficient adenoviral replication to induce widespread cell death in the LNCaP prostate cancer cell line. Most interestingly, mice bearing both subcutaneous and orthotopic LNCaP tumors demonstrated tumor regression after a single injection of co-transduced MDMs and showed no signs of regrowth. Moreover, human anti-adenovirus neutralizing antibodies delivered to tumor-bearing mice had no impact on the activity of macrophage-delivered oncolytic adenovirus. Taken together, macrophages represent a promising new delivery system to deliver oncolytic viruses to tumors with hypoxic microenvironments. Further research is needed to investigate the clinical applicability of this promising approach.

## TARP-T cell receptor (TCR) engineered CTLs

Although chimeric antigen receptor (CAR)-T cells have recently brought major breakthroughs in the treatment of relapsed/refractory acute lymphoblastic leukemia [12], the identification of novel specific CAR targets is limited, because CAR-T cell activity requires the expression of antigenic targets on tumor cell surfaces. Because TARP expression is intracellular, Hillerdal et al. generated CD8 T-cells expressing a TCR recognizing the TARP(5PL)<sub>4–13</sub> peptide presented by HLA-A2 molecules [48]. Peripheral blood human CD8 T-cells engineered with this unique TCR demonstrated selective and efficient killing of TARP-expressing HLA-A2 + prostate and breast cancer cells [48, 49].

Similarly, the therapeutic potential of TARP-TCR CTLs in TARP-positive AML was recently evaluated by our research team [21]. Target specificity of the TARP-TCR was demonstrated by using T2 cells pulsed with exogenous peptides, with TARP-TCR CTLs pulsed with TARP(P5L)<sub>4–13</sub> induced stronger cytokine responses and higher killing rates compared to T2 cells pulsed with non-TARP related proteins. However, we observed a weaker response against endogenous TARP-expressing cell lines and primary patient cells, compared to pulsed T2 cells. This observation might be due to dysfunctional peptide processing, transport, and/or MHC-I presentation in leukemic cells. In addition, in vitro culture of leukemic cells could diminish the transgenic expression of HLA-A2, which is vital for triggering a lytic response [21]. Further research and confirmation using in vivo studies are ongoing.

An important limitation of using TCR-engineered T-cells is the risk of mispairing between the exogenous and endogenous TCR- $\alpha$  and TCR- $\beta$  chains [50]. Mispairing may give rise to TCRs with unpredictable specificity and create TCRs reactive against self-antigens, thereby generating autoreactive T-cells. Furthermore, mispaired TCRs may compete for CD3 and thereby reduce the surface expression level of the correctly paired transferred TCR. Several strategies to avoid mispairing include the murinization of human TCRs by replacing the human constant domain with murine counterparts, thus resulting in a competitive advantage for CD3 interaction [48, 51]; and by using the OP9-DL1 in vitro coculture system and agonist selection to develop TCR-transduced hematopoietic stem and progenitor cells into mature tumor antigen-specific T-cells that express only one TCR [50].

## TARP-directed TCR-antibody

The potential role of TCR-like mAbs as an option in the growing TARP-based therapeutic arsenal has been explored by a single research team. TCR-like mAbs combine the advantages of mAbs with the additional potential

to recognize intracellular targets that can be cancer-cell specific, rather than the lineage-specific membrane markers often targeted by the conventional mAbs [52–56]. Epel et al. [57] developed a human recombinant TCR-like antibody directed against the TARP<sub>29–37</sub> epitope presented on MHC class I HLA-A2 molecules and illustrated its specific binding to peptide-pulsed RMA-S-HDD cells, RNA-pulsed HLA-A2 presenting JY cells, and TARP-expressing HLA-A2 tumor cells. Interestingly, when conjugated to the potent toxin *Pseudomonas* Exotoxin A (PE38) lacking its cell binding domain, the TARP/HLA-A2-specific TCR-like mAb demonstrated potent PE38-dependent inhibition of protein synthesis in vitro. Cytotoxicity was further enhanced by modifying the C-terminal REDLK to KDEL, the most common estrogen receptor retention signal. When preincubated with the PE38-TARP/HLA-A2-specific TCR-like mAb fusion protein, MCF-7 tumor cells were unable to generate tumors in mice, further illustrating clinical potential.

## Future perspectives

Recent advances in cancer immunotherapy have created hope to improve cure rates, with the identification of CAAs playing a major role. The identification of TARP rapidly led to multiple therapeutic opportunities, some of which have been included in clinical trials. Still, little is known regarding the molecular function of TARP and its precise role in the development of certain cancers and further research is certainly needed to address open questions. In this review the promising results and therapeutic potential of TARP-related research is summarized.

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## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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