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Novel antigens of CAR T cell therapy: New roads; old destination

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ABSTRACT

Chimeric antigen receptor T cell (CAR-T) therapy has so far proved itself as a reliable therapeutic option for the treatment of relapsed/refractory (R/R) B-cell acute lymphoblastic leukemia (B-ALL), diffuse large B-cell lymphoma (DLBCL), multiple myeloma (MM), and mantle cell lymphoma (MCL). However, this picture is not as colorful when it comes to the treatment of solid tumors mainly due to the lack of definitive tumor antigens, as well as the immunosuppressive tumor microenvironments and poor CAR-T infiltration. The recent developments in bioinformatics and cell biology, such as single-cell RNA sequencing, have offered silver linings in the subject of tumor antigen discovery. In the current review, we summarize the development of some CAR-T therapies that target novel tumor antigens, rather than the traditionally CAR-T-targeted ones, and briefly discuss the clinical antitumor achievements of those evaluated in patients, so far. Furthermore, we propose some tumor antigens that might someday be therapeutically beneficial while targeted by CAR-Ts based on the experimental evaluations of their specific monoclonal antibodies.

Introduction

More than a century after the "*magic bullet*" concept was developed by the German Nobel laureate *Paul Ehrlich* and decades after the development of the first gene-manipulated T cells expressing chimeric receptors (that could redirect their cytotoxic effects towards cancer cells of interest), these two phenomena blended to be one of the most promising anticancer therapeutics known as chimeric antigen receptor T cells (CAR T cells or CAR-Ts). In detail, the magic bullet theory proposed that it might be feasible to specifically eliminate invading elements in the body without damaging healthy organs (similar to a bullet fired from a weapon to hit a distinct target) [1]. CAR-Ts proudly stepped into the clinic arenas with *Kymriah*™ (*Tisagenlecleucel*) and *Tecartus*™ (*Brexucabtagene autoleucel*) being approved by the US Food and Drug Administration (FDA) for the treatment of relapsed/refractory (R/R) B-cell acute lymphoblastic leukemia (B-ALL) and mantle cell lymphoma (MCL), respectively, while *Yescarta*™ (*Axicabtagene ciloleucel*) and *Breyanzi*™ (*Lisocabtagene maraleucel*) are approved for diffuse large B-cell lymphoma (DLBCL) [2–6]. Furthermore, in March 2021, FDA approved *Abecma*™ (*Idecabtagene vicleucel*) for the treatment of adult patients with R/R multiple myeloma (MM) [118][117]. However, some hematologic malignancy or even solid tumor patients might have yet to benefit from the therapeutic effects of CAR-Ts. The complicated nature of the tumor microenvironments (TME) has limited the tumoricidal capacity of CAR-Ts in the case of solid tumors. One of the hurdles is associated with the antigens that have a poor density on the respective tumor cells or simply the loss of tumor-associated antigens (TAAs). Some researchers have expressed the CD19 antigen on the surface of particular CD19-negative solid tumor cells using oncolytic viruses to render these cells susceptible to being targeted by CD19-redirected CAR-Ts (CD19.CAR-Ts), and they have reported encouraging results [7]. Other strategies such as the utilization of boosting vaccines designed to overcome the issue of poor CAR-T stimulation and persistence have also proven to be rather encouraging [8,9]. In the case of tumor heterogeneity, some researchers have engineered CAR-Ts that secrete bispecific T-cell engagers (BiTEs) that engage bystander T cells against the heterogeneous tumor, thus enforcing a stronger tumor rejection [10]. Of note, not all BiTEs are secretory as *Blinatumomab* (an FDA-approved CD19/CD3-bispecific BiTE used for the treatment of R/R B-ALL) is administered intravenously [11,12]. Additionally, to fight against the immunosuppressive nature of TMEs, Osborne et al. have investigated (NCT03287817) the effect of administrating the anti-PD-1 antibody *Pembrolizumab*, whereas others have used gene-editing techniques such as transcription activator-like effector nucleases (TALEN®) or CRISPR-Cas9 to develop immunosuppression-

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resistant effector cells (such as those deficient in the expression of NR4A transcription factors) [13–15]. In a slightly different approach, Ping et al. have developed CAR-Ts that secret PD-1-neutralizing scFvs that counteract immunosuppression [16]. To address the poor CAR-T infiltration issue, researchers have demonstrated that the expression of particular receptors such as CXCR1/2, CCR4, and CCR2b, might be beneficial in helping leverage particular tumor-secreted molecules for the redirection of CAR-Ts towards the tumors themselves [17–20]. Another reasonable approach is the regional delivery of CAR-Ts, rather than their systemic administration, which has recently shown promising outcomes in the case of peritoneal carcinomatosis, brain-metastasized breast cancer, and medulloblastoma [21–23]. To amplify the antitumor effects of CAR-Ts, some investigators have evaluated the effects of preconditioning interleukins (IL), such IL-15 or IL-7, and have reported slightly positive effects such as the increased sensitivity of CAR-Ts to immune-checkpoint blockade regimens [24,25]. However, since such systemic administrations might result in the stimulation of non-transduced lymphocytes leading to unwanted toxicities, some researchers have engineered CAR-Ts that secrete particular ILs such as IL-23 or IL-2 receptor that can operate in an autocrine fashion, thus less likely to mediate toxicities [26,27]. The disappearance of CAR-Ts from the circulation of patients has also been observed in clinical trials which might be due to the presence of neutralizing antibodies reactive with the animal-originated targeting domains of CAR-Ts. To fully benefit from the therapeutic impact of CAR-Ts, humanized or fully human targeting domains have been implemented in CAR constructs, and they have exhibited promising clinical outcomes [28–30]. Some researchers have also used “AND” or “NOT” gates to limit the unfavorable effects of CAR-Ts on healthy tissues and have reported limited success in proof-of-concept studies [116][5]. However, the implementation of such gates requires definitive antigen sets restricted to only healthy or tumor cells [116][5]. Besides all these elaborate strategies [116][5,31–37], the need for introducing novel TAAs is fiercely sensed. In this review, we briefly summarize the recent clinical success of several novel antigen-redirected CAR-Ts (Tables 1 and 2) from some of which clinical evaluations have been released, so far. Furthermore, we take a peek at the experimental success of some other novel antigen-targeting CAR-Ts that are currently under laboratory investigation alongside proposing several novel antigens that have not yet been targeted by CAR-Ts.

Non-traditional CAR-T therapy target antigens

From the early days of CAR-T therapy until today, numerous antigens, including CD19, CD20, CD22, BCMA, GD2, Mesothelin, TAG-72, CEA, EGFR, B7H3, HER2, IL13Ra2, MUC1, EpCAM, PSMA, PSCA, NKG2D, and various others, have been targeted by CAR-Ts in clinical trials that can now be dubbed “traditional CAR-T targets” [5,32,35,37,38]. In contrast, the future of CAR-T therapy might be slightly leaning towards different antigens for some of which multiple clinical trials have been conducted in recent years and their outcomes are either concisely discussed in the next section or expected in the years to come. Also, in the upcoming section, we briefly discuss several other novel antigens targeted by CAR-Ts that are still under experimental development, and next we explore the suitability of possible future CAR-T targets (Fig. 1). Also, Fig. 2 details the obstacles that a given CAR-T product targeting a novel antigen should overcome on its way towards approval for medical use.

Novel antigens currently under clinical investigations

PLAP

Placental alkaline phosphatase (PLAP, or alternatively known as ALPP) is a human metalloenzyme normally expressed in the placenta and testis but overexpressed in a wide spectrum of malignancies including ovarian and cervical cancer, colon adenocarcinomas, teratomas, and seminomas [39,40]. Furthermore, PLAP has also been evident to be incorporated in the membrane of non-small cell lung cancer patients’ exosomes, branding it as a novel prognostic target [39,40]. In 2020, Li et al. generated PLAP-redirected CAR-Ts (PLAP.CAR-Ts) that harbored murine or humanized PLAP-specific scFvs as targeting domains that exhibited high selectivity towards PLAP-positive colon cancer cells [39,40]. Moreover, the humanized PLAP.CAR-Ts significantly inhibited tumor growth in PLAP-positive colon cancer cell xenograft models [39,40]. Li et al. also demonstrated that combination therapy with checkpoint inhibitors, such as PD-1, PD-L1, or LAG-3 inhibitors, can significantly increase the therapeutic force of humanized PLAP.CAR-Ts against colorectal cancer [39,40]. Of note, in December 2020, a clinical trial (NCT04627740) started with 20 participants to investigate the clinical efficacy of ALPP-redirected CAR-Ts.

CS1 (SLAMF7)

Signaling lymphocytic-activation molecule F7 (SLAMF7), also known as CS1, CD319, or CRACC, is a surface glycoprotein that, despite its low-level expression on a spectrum of immune cells including natural killer cells (NKs) and particular subsets of T cells, is highly and uniformly expressed in MM [41]. In this regard, SLAMF7 might be considered a candidate for an immunotherapy target antigen [41]. In the bone marrow, SLAMF7 has been known to contribute to the adhesion and viability of malignant plasma cells (mPCs) [42]. In 2014, Chu et al. generated second-generation CS1-redirected CAR-Ts (CS1.CAR-Ts) and reported that these cells exhibited potent tumoricidal effects against CS1-positive MM cells which coincided with CD69 upregulation and elevated levels of INF-γ and IL-2 [41]. Furthermore, upon CS1.CAR-Ts administration into MM.1S and IM9 MM human xenograft mouse models, the CAR-Ts mediated considerable tumor rejection that resulted in the induction of prolonged survival [41]. In another study, Danhof et al. generated SLAMF7-redirected CAR-Ts (SLAMF7.CAR-Ts) and reported that, besides the pronounced anti-myeloma efficacy of these cells, a single administration of them would suffice to induce prolonged survival by medullary and extramedullary myeloma eradication in xenograft MM models [42,43]. Additionally, these researchers concluded that even though SLAMF7.CAR-T-mediated fratricide of SLAMF7-positive normal lymphocyte subsets (including NK cells, CD4-positive and CD8-positive T cells, and B cells) did not impede the preparation process of SLAMF7.CAR-Ts, it might promote acute cytokine release syndrome (CRS) or contribute to viral infections (which might be clinically manageable via lymphodepleting regimens or antiviral prophylaxis, respectively, as proposed by Danhof and colleagues) [42,43]. In 2017, using TALEN® gene-editing technology, Mathur et al. developed off-the-shelf double knock-out universal CS1.CAR-Ts (UniCS1.CAR-Ts), deficient in the expression of TRAC and SLAMF7, as a strategy to tackle fratricide and graft-versus-host disease (GVHD) [44]. In detail, upon encountering primary MM tumor cells, allogeneic UniCS1.CAR-Ts expanded and secreted elevated levels of IFN-γ and GM-CSF and subsequently exerted pronounced cytolytic responses against MM tumor cells, both *in vitro* and *in vivo* [44]. In 2018, Wang et al. conducted an experiment to investigate the impact of lenalidomide on the phenotype and effector function of second-generation CS1.CAR-Ts *in vitro* and in MM tumor-bearing mice [45]. Lenalidomide is an FDA-approved immunomodulatory agent used for the treatment of MM (either with or without dexamethasone) [46]. *In vitro* findings attributed the enhanced anti-myeloma capacity, memory maintenance, and Th1 cytokine secretion to the lenalidomide treatment of CS1.CAR-Ts alongside *in vivo* data indicating that lenalidomide might amplify the tumoricidal impact and persistence of CS1.CAR-Ts [45]. Alongside highlighting the possible therapeutic importance of CS1, these findings might accentuate the benefits of using combinatorial therapy for relapsed myeloma [45]. In 2020, Amatya et al. equipped their SLAMF7.CAR-Ts with an inducible caspase 9-based suicide switch that could trigger at-will elimination of the effector cells following the introduction of the dimerizing drug AP1903 (rimiducid), upon feeling the

Table 1
Some of the CAR-T therapy target antigens that are currently or planned to be under clinical investigation. Data from Clinicaltrials.gov.

Clinical trial identifier	Antigen	Disease	Estimated enrollment (participants)	Start date	Estimated completion date	Source	Conditioning regimen	Phase	Location
NCT04627740	ALPP	Ovarian and endometrial cancer	20	December 1, 2020	December 31, 2023	Autologous	Flu / Cy	I/II	Not provided
NCT03958656	CS1	Multiple myeloma	13	June 13, 2019	January 19, 2021	Autologous	Flu / Cy	I	United States
NCT04142619	(SLAMF7)	R/R multiple myeloma	18	November 21, 2019	November 1, 2022	Allogeneic	–	I	United States
NCT03159819	CLDN18.2	Pancreatic and gastric adenocarcinoma	24	April 1, 2017	December 31, 2021	Autologous	–	Not Applicable	China
NCT04467853		Gastric cancer	34	September 21, 2020	November 2024	Autologous	Flu / Cy	I	China
NCT03393936	AXL ROR2	Renal cell carcinoma	66	March 26, 2018	March 30, 2035	Autologous	Flu / Cy	I/II	China
NCT04151186	TM4SF1	Advanced solid tumors	72	November 20, 2019	November 20, 2021	–	–	Not Applicable	China
NCT04420754	ICAM-1	Anaplastic thyroid cancer	24	September 28, 2020	June 2024	Autologous	–	I	United States
NCT02311621	L1CAM (CD171)	Neuroblastoma, ganglioneuroblastoma	40	November 25, 2014	November 2037	Autologous	–	I	United States
NCT03829540	CD4	T-cell lymphoma, T-cell leukemia	20	June 18, 2019	December 2037	Autologous	–	I	United States
NCT03081910	CD5	T-cell acute lymphoblastic lymphoma, non-Hodgkin T-cell lymphoma	42	November 1, 2017	September 1, 2039	Autologous	Flu / Cy	I	United States
NCT04599556	CD7	Acute leukemia and lymphoma	108	October 2020	December 2023	–	–	I/II	China
NCT03690011		T-cell acute lymphoblastic lymphoma, T-cell acute lymphoblastic leukemia, non-Hodgkin T-cell lymphoma	21	September 1, 2021	May 1, 2038	Autologous	Flu / Cy	I	United States
NCT04430530	CD10 CD38	CD19-negative B-cell malignancies	100	June 1, 2020	December 31, 2023	–	–	I/II	China
NCT04348643	CEA	Lung, colorectal, liver, pancreatic, gastric, and breast cancer	40	February 20, 2020	April 30, 2023	–	–	I/II	China
NCT03904069	FLT3	R/R acute myeloid leukemia	40	March 15, 2022	May 9, 2029	Autologous	–	I	United States
NCT02830724	CD70	Pancreatic, renal cell, ovarian, and breast cancer, melanoma	2	April 6, 2017	January 1, 2028	Autologous	Flu / Cy	I/II	United States
NCT04288726	CD30	Hodgkin lymphoma	18	September 16, 2020	June 1, 2037	Allogeneic	–	I	United States
NCT04136275	CD37	Leukemia and B-cell, T-cell, and Non-Hodgkin lymphoma	18	June 19, 2020	September 30, 2024	Autologous	–	I	United States
NCT04045847	CD147	Glioblastoma	31	May 30, 2019	May 30, 2022	Autologous	–	I (early phase)	China

Abbreviations: Flu, fludarabine; Cy, cyclophosphamide; R/R, relapse/refractory.

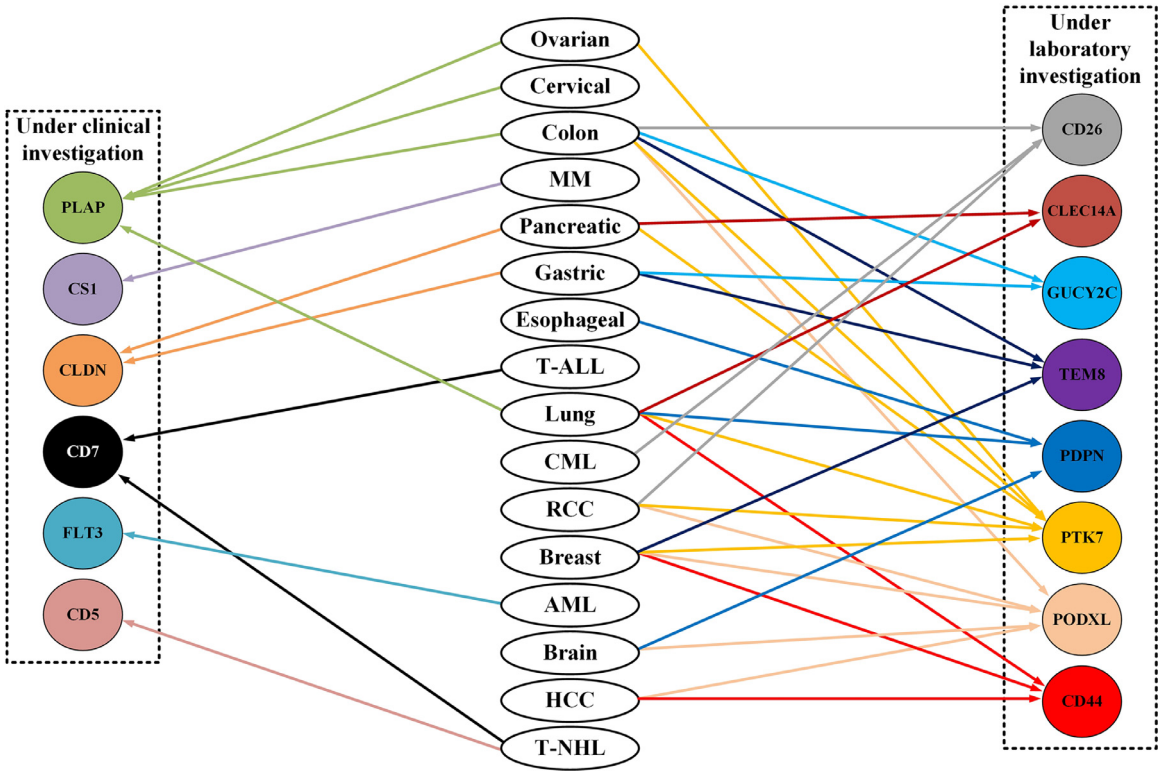

Fig. 1. An overall representation of the novel antigens discussed in this review and the overlapping involvement of some of them in different oncological indications. The ovals in the center represent different types of cancers. AML, acute myeloid leukemia; CML, chronic myeloid leukemia; HCC, hepatocellular carcinoma; MM, multiple myeloma; RCC, renal cell carcinoma; T-ALL, T-cell acute lymphoblastic leukemia; T-NHL, non-Hodgkin T-cell lymphoma.

need [47]. In detail, the adoptive transfer of these SLAMF7.CAR-Ts into mouse models resulted in the efficient eradication of SLAMF7-positive tumors, and also the rapid elimination of the effector cells was achieved via the administration of AP1903 [47]. Eventually, Zah et al. elaborately designed and generated B-cell maturation antigen (BCMA)/CS1 bispecific CAR-Ts (BCMA/CS1.CAR-Ts) as a possible option to fight against heterogeneous MM [48]. In comparison to engineered T cells individually co-expressing BCMA and CS1 CARs, BCMA/CS1.CAR-Ts exhibited superior characteristics in terms of tumoricidal activity [48]. Furthermore, even though *in vivo* complete tumor eradication and durable remission via the combination of BCMA/CS1.CAR-Ts and anti-PD-1 antibodies required a shorter timeline, compared with CAR-T therapy alone, it did not impact the overall durability of response [48].

CLDN

Claudin 18.2 (CLDN18.2), the stomach-specific claudin 18 (CLDN18) isoform, is a membrane-bound protein that has been associated with various types of cancers including gastric cancer and pancreatic adenocarcinoma [49,50]. In 2019, Jiang et al. developed the first CLDN18.2-redirected CAR-Ts (CLDN18.2.CAR-Ts), which harbored CLDN18.2-specific humanized single-chain variable fragments (scFv) as targeting domains, and investigated their tumoricidal capacity in patient-derived tumor xenograft (PDX) models and BGC-823 cell-bearing gastric cancer mouse models [50]. The findings indicated no serious signs of CAR-T-mediated adverse events on the healthy tissues of the animal models despite the CLDN18.2.CAR-T-mediated antitumor responses against the tumor cells expressing the murine form of CLDN18.2 [50]. Eventually, Zhan et al. conducted the first-in-human Phase I pilot study (NCT03159819) to assess the safety and tumoricidal capacity of autologous CLDN18.2.CAR-Ts in 12 patients with CLDN18.2-positive metastatic adenocarcinoma (7 with advanced gastric and 5 with pancreatic adenocarcinoma who had fludarabine- and cyclophosphamide-induced lymphodepletion prior to CAR-T administration) [51]. In the first report, no serious CAR-T-mediated unfavorable side effects, severe neurotoxicity, or treatment-related mortality was documented, except for a decline in lymphocytes and neutrophils, as well as grade 1/2 CRS, which could lead to the conclusion that CLDN18.2.CAR-Ts might be well-tolerated by patients [51]. With the total objective rate of 33% and 1 complete remission (CR), 3 partial remissions (PR), 5 stable diseases, and 2 disease progressions out of 11 patients eligible for response assessment, it might be moderate to assert that advanced gastric and pancreatic adenocarcinoma patients might be the beneficiaries of the therapeutic potential of CLDN18.2.CAR-Ts, as a possible treatment option [51]. Additionally, smart strategies have also been conducted to overcome the limitation of poor *in vivo* stimulation of CAR-Ts, which is mainly due to the inaccessibility of the effector cells to their target antigen. In 2020, Zhu et al. developed a nanoparticle-based RNA vaccine that acts as a carrier for the delivery of the natively-folded Claudin 6 (CLDN6), as the target antigen of CLDN6-redirected CAR-Ts (CLDN6.CAR-Ts), to the antigen-presenting cells (APCs) residing in lymphoid compartments [9]. Involved in tight-junction structure, CLDN6 is a membrane-spanning protein whose expression has been frequently associated with several cancers including uterine, ovarian, testicular, and lung adenocarcinoma [9,52]. Theoretically, the adoptively transferred CLDN6.CAR-Ts should expand more efficiently upon encountering their target antigen after they traffic into lymphoid compartments and interact with the relative APCs [9]. *In vivo* findings have indicated that using this method, lower CLDN6.CAR-T doses are required to induce tumor rejection in large-tumor-bearing animal models [9]. Additionally, alongside CAR-T modalities, other elaborate anticancer strategies such as antibody-drug conjugates (ADCs) or CLDN/CD3-bispecific antibodies might also be taken into consideration as possible options for the treatment of CLDN-positive gastric and pancreatic cancer (as experimental evidence has validated their potential in the case of CLDN18.2) [53].

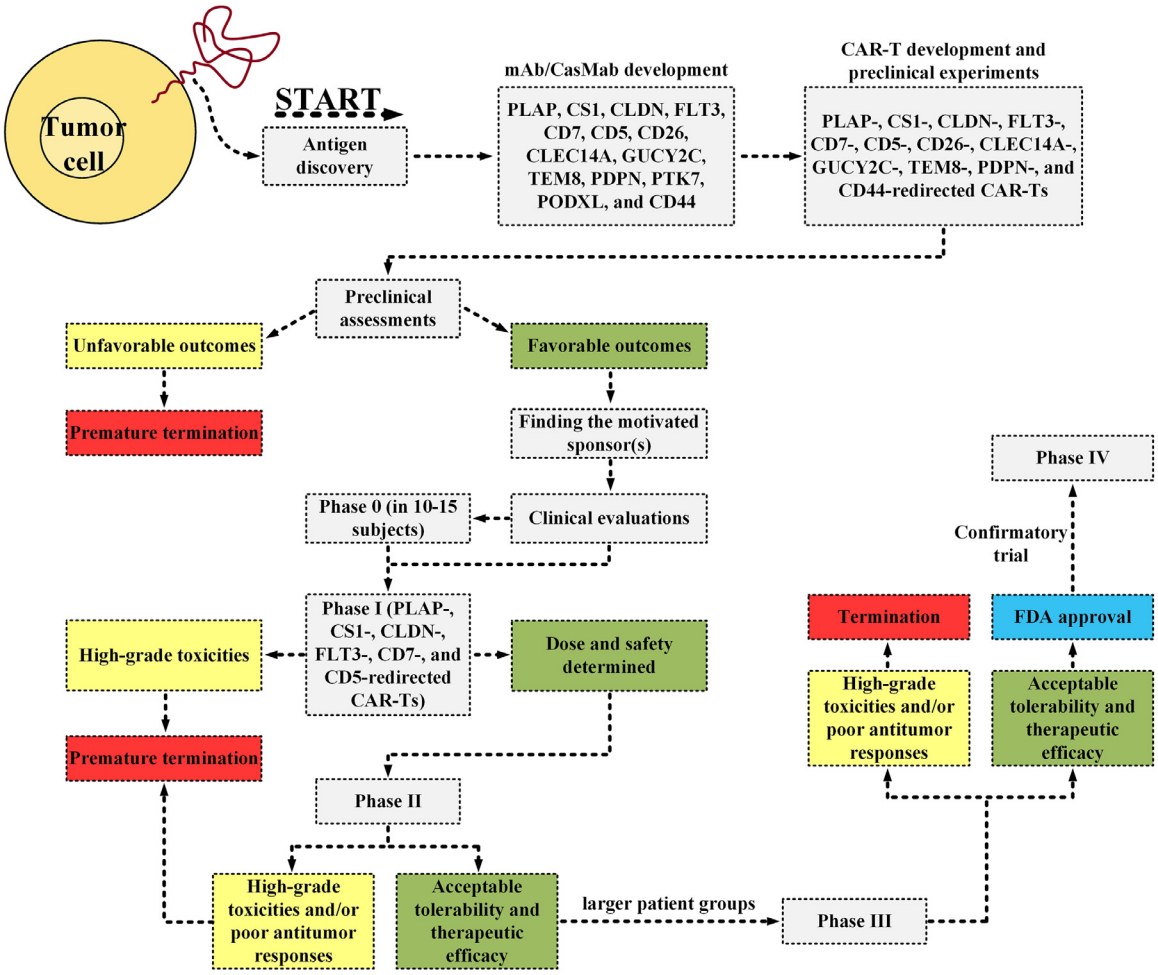

Fig. 2. The "*hard-to-survive*" journey of a proposed CAR-T product redirected against a novel antigen from antigen discovery to the approval of the product for medical use by the US FDA. The journey starts with the discovery of the novel antigen. Next, scientists should develop a high-affinity mAb or CasMab specific for that antigen (using *hybridoma, phage-display, ribosome display*, etc. techniques). In the next step, the mAb can be engineered as the targeting domain of CAR-Ts. Fully human or humanized mAbs should be preferred over murine mAbs since highly immunogenic CAR targeting domains might encounter neutralizing antibodies in the prospective recipients following CAR-T administration. This phenomenon could lead to the elimination of the infused CAR-Ts from the patient's body and consequent abrogation of all CAR-T-related antitumor effects. After achieving success in broad and meticulous experiments (both *in vitro* and *in vivo*) and finding the willing sponsor(s), the investigators can take steps towards primary clinical assessments (Phase 0) in a limited number of subjects using subtherapeutic doses. This step is designed to evaluate the pharmacokinetics of the proposed CAR-T product in human subjects (rather than preclinical animal models that provide findings that might not be that translatable in clinics). Phase 0 helps pharmaceutical companies to alleviate their doubt about similar products and go to Phase I with the best choice. Next, the proposed CAR-T product should be evaluated in a Phase I study (with 20–100 participants) in terms of its adverse events, safety index, and tolerable doses. After dose determination and demonstration of safety, the proposed CAR-T product can be investigated in a Phase II trial (with a larger population of enrolled patients). This step is conducted for a broader determination of safety alongside investigating therapeutic efficacy. After a successful Phase II, a Phase III trial could be conducted to gauge the definitive therapeutic efficacy of the CAR-T product and, if successful, it can be granted FDA approval for the intended oncological indication. After obtaining permission for medical use, a Phase IV trial might be conducted for monitoring long-term or uncommon side effects of the product. As represented in the figure, any given proposed CAR-T product faces numerous serious challenges that can prematurely terminate its journey towards final approval upon the slightest lapse (such as toxicity or therapeutic inefficacy). mAb, monoclonal antibody; CasMab, cancer-specific monoclonal antibody; CAR-T, chimeric antigen receptor T cell; FDA, Food and Drug Administration.

FLT3

FMS-like tyrosine kinase 3 (FLT3, also known as CD135) is a cytokine receptor of the receptor tyrosine kinase class III which is expressed on the surface of malignant blasts in acute myeloid leukemia (AML), as well as healthy hematopoietic stem cells (HSCs) and progenitor cells [54]. In 2018, Jetani et al. demonstrated that FLT3- or FLT3 with internal tandem duplication (FLT3-ITD)-expressing cell lines of MOLM-13, THP-1, and MV-4-11 and primary AML blasts can be selectively eliminated using FLT3-redirected CAR-Ts (FLT3.CAR-Ts) [54]. Moreover, they elucidated that the FLT3-inhibitor crenolanib can promote the expression of FLT3 by AML cells which consequently facilitates their targeting by FLT3.CAR-Ts [54]. One of the potential explanations for this phenomenon might be that AML blasts elevate FLT3 expression as a resistance mechanism to tackle the inhibitory impacts of FLT3 inhibitors [55–57]. Numerous distinct mechanisms have been proposed to contribute to the emergence of resistance to different FLT3 inhibitors including mutations (such as the tyrosine kinase domain-related K429E and F691L mutations that mediate resistance to crenolanib), elevated FLT3 ligand expression, intracellular pH decline, or even amplified expression of oncogenic kinases [58]. Regardless of the underlying mechanism, since CAR-Ts require the establishment of a high number of immunological synapses (approximately 150 antigen engagements for each CAR-T) to be cytotoxically activated against their target cells, the crenolanib-

Table 2
Some of the completed clinical trials that investigated novel CAR-T therapy target antigens. Some data from clinicaltrials.gov.

Clinical trial identifier	Antigen	Disease	Estimated Enrollment	Start / completion date	Notes	Phase	Location	Ref.
NCT01837602	c-MET	Metastatic breast cancer, TNBC	6 participants	April 2013 / August 13, 2018	• Autologous mRNA-electroporated CAR-Ts well-tolerated • no CRS reported • No measurable clinical responses	0	United States	[113]
NCT03060356		Malignant melanoma, breast cancer	77 participants (4 TNBC and 3 M patients received infusions)	December 21, 2016 / March 27, 2020	• Autologous mRNA-electroporated CAR-Ts • Grade 1/2 toxicities in 5 patients • No CRS nor higher grade toxicities • SD in 4 patients (2 TNBC, 2 M) • PD in 3 patients (2 TNBC, 1 M)	I (early phase)	United States	[114]
NCT02862704	MG7	Liver metastases	20 participants	June 2016 / December 2017	• Autologous CAR-Ts	I/II	China	
NCT01886976	CD138	Multiple myeloma	10 participants	June 2013 / June 2016	• Autologous CAR-Ts induced no intolerable toxicities • 4 out of 5 patients achieved SD longer than 3 months • 1 out of 5 patients with PD experienced a reduction in peripheral myeloma cells	I/II	China	[115]
NCT01722149	FAP	Malignant pleural mesothelioma	4 participants	February 19, 2015 / July 18, 2019	• Autologous CAR-Ts	I (early phase)	Switzerland	

Abbreviations: TNBC, triple negative breast cancer; M, melanoma; CRS, cytokine release syndrome; SD, stable disease; PD, progressive disease.

induced elevation in the surface expression of FLT3 could be exploited for antitumor purposes [59]. One of the twists of using FLT3.CAR-Ts is the requirement of eliminating the adoptively transferred FLT3.CAR-Ts after treatment completion and reconstituting the hematopoietic compartment of the patients using allogeneic HSC transplantation (since FLT3.CAR-Ts are incapable of discriminating between healthy HSCs and malignant AML blasts) [54].

Selective elimination of CAR-Ts can be achieved using caspase-, herpes simplex virus thymidine kinase (HSV-TK)-, and monoclonal antibody (mAb)-based safety switches [116][5]. Numerous clinical trials (NCT02146924, NCT01865617, NCT02159495, NCT01953900, NCT02414269, etc.) are currently evaluating the clinical applicability of these safety switches which might be completed in the upcoming years. According to a report focusing on 5 patients from a clinical trial with 10 enrolled participants (NCT00710892), 5 patients with relapsed acute leukemia that had received stem-cell transplantation underwent a treatment of inducible caspase 9 (iCasp9)-equipped T cells for immune restoration [60]. In detail, 4 out of 5 patients (80%) that had developed GVHD received a single dose of AP1903 administration that led to the elimination of a high proportion (>90%) of the infused T cells in half an hour [60]. Also, the signs of GVHD commenced disappearing one day after AP1903 administration without re-emergence [60]. According to a long-term follow-up of the same trial in all of the 10 enrolled patients, the effects of this safety switch on GVHD happens to be in a permanent fashion [61]. Moreover, there have not yet been any reports regarding the possible immunogenicity of this safety switch which might be a result of this switch being based on a gene encoding human caspase [62]. Also, the dimerizing agent used in this setting has been considered to be non-toxic, so far [62]. In regards to mAb-based switches, despite their acceptable capacity in managing CAR-T-mediated toxicities, the unhinged biodistribution of the administered mAbs might be one of their downsides as it might result in mild to severe toxicities towards healthy tissues [63–65]. Such mentioned switches also lack a preventive strategy that could be used against them in the cases of unforeseen toxicities caused by the switches themselves. Moreover, the HSV-TK switch has encountered immune responses in patients due to its immunogenicity (owing to its viral origin) [66]. Such immune responses correlated with the rapid elimination of the infused T cells harboring this safety switch [66]. As briefed, safety switches have their advantages and disadvantages, all of which might be elucidated as the related clinical trials are completed.

In comparison with the study mentioned earlier in which the investigators had derived their CAR targeting domain from the FLT3-specific mAb 4G8, Wang et al. developed slightly different CAR-Ts (named FLT3L.CAR-Ts) that harbored the FLT3 binding domain of FLT3L, known as FLT3L-BD, as their targeting domains [54,67]. *In vitro* findings indicated that FLT3L.CAR-Ts exhibited more pronounced tumoricidal effects against FLT3-ITD-expressing malignant blasts, rather than FLT3-positive ones, and negligible cytolytic effects against healthy CD34-positive umbilical cord blood stem cells [67]. Moreover, the induction of long-term survival achieved in xenograft AML mice might further validate the potential antitumor capacity of ligand-based FLT3-specific CAR-Ts [67]. To expand the applicability of FLT3.CAR-Ts and abrogate their toxicities towards HSCs and progenitor cells, Sommer et al. equipped their off-the-shelf scFv-based FLT3.CAR-Ts with a rituximab-dependent safety switch, and indicated that not only the selective depletion of these CAR-Ts does not impede AML remission, but it also facilitates the hematopoietic system recovery [68]. These findings might pave the way for the possible clinical assessment of off-the-shelf FLT3.CAR-Ts in FLT3-positive hematologic malignancies, inclusive of AML [68]. Other treatment strategies based on the FLT3 antigen such as FLT3/CD3 bispecific antibodies might shine more light on the importance and suitability of this antigen in cancer immunotherapy [69]. Of note, FLT3/CD3 bispecific antibodies have also exhibited therapeutic potential in cynomolgus monkeys by inducing the complete eradication of FLT3-positive dendritic cells (DCs) [69].

CD7

CD7 might be considered as a potential target for the immunotherapy of patients with T-cell lymphoblastic lymphoma (T-LBL) and T-cell acute lymphoblastic leukemia (T-ALL). To our knowledge, Zhang et al. conducted the first-in-human clinical trial (NCT04004637) that investigated the therapeutic efficacy of autologous nanobody-based CD7-redirected CAR-Ts (CD7.CAR-Ts) in patients with R/R T-LBL/T-ALL [70]. Moreover, since the administration of such CAR-Ts might result in CAR-T fratricide, they developed an elaborate strategy that anchors CD7 in the ER and/or Golgi of these cells as a strategy to tackle the issue of fratricide [70]. In detail, CRS with elevated levels of IL-6 was observed in all three CD4- and CD8-negative T-LBL/T-ALL patients (who had undergone lymphodepletion with fludarabine/cyclophosphamide prior to CD7.CAR-T infusion) which might indicate that the tolerability index of CD7.CAR-Ts is acceptable [70]. Moreover, the therapeutic efficacy of CD7.CAR-Ts was later confirmed as two of the patients achieved minimal residual disease (MDR)-negative CR and the other experienced a considerable decline in the number of abnormal T cells after the treatment [70]. In a different approach, Gehrke et al. generated allogeneic second-generation CD7.CAR-Ts that are almost resistant to GVHD and/or fratricide or do not encounter immune rejection and/or immunosuppression [71]. In detail, they used novel gene-editing reagents, called "*base editors* (*BE*)", that create single-base DNA changes without causing hazardous genomic rearrangements [71]. The mentioned CD7.CAR-Ts were tumoricidal in animal tumor models in a dose-dependent fashion alongside exhibiting pronounced *in vitro* antitumor effects and cytokine secretion in response to tumor cell antigen-engagement [71]. Taken together, alongside enabling simultaneous base editing at desired genomic loci, the use of such flexible multiplexed base editing methods might expedite advancing towards clinical evaluation with CD7.CAR-Ts that are universally patient-compatible for the treatment of CD7-positive malignancies [71]. According to another report from two open-labeled clinical trials (ChiCTR190002531 and ISRCTN19144142) evaluating the safety index and antitumor potential of a single infusion of "off-the-shelf" allogeneic CD7.CAR-Ts, called GC027, in two lymphodepleted patients with R/R T-ALL, the patients achieved MRD-negative CR, with one of them remaining disease-free even one year after the treatment [72]. Even though the report indicated no signs of GVHD, grade 3 CRS was observed in both of the patients which was subsequently resolved using ruxolitinib [72]. All of the mentioned reports point out the fact that it might require further clinical evaluations with broader R/R T-LBL or T-ALL patient populations to determine the safety index and therapeutic potential of CD7.CAR-Ts.

CD5

CD5 is abundantly present in several T-cell malignancies, alongside being expressed by normal T cells, therefore, it might be considered as a likely candidate for a CAR-T therapy target antigen [73,74]. In this regard, Hill et al. conducted a Phase I dose-escalation study (NCT03081910) to investigate the safety and applicability of autologous second-generation CD5-redirected CAR-Ts (CD5.CAR-Ts) in 21 patients with R/R non-Hodgkin T-cell lymphoma (T-NHL) [73]. According to a 2020 report presenting the findings from only 5 of the patients of the mentioned trial (who had undergone lymphodepleting chemotherapy prior to CD5.CAR-T infusion, which consisted of fludarabine/cyclophosphamide), 3 out of 5 patients (60%) achieved CR [73]. Furthermore, CRS was reported to be observed in 3 of the patients (60%) for the resolution of which tocilizumab and other related clinical considerations were taken into account [73]. Since the complete clinical evaluations of CD5.CAR-T trials have not yet been publicly released, in the upcoming years, it will be elucidated whether this type of therapy might be able to offer acceptable clinical outcomes (with rather manageable toxicities) to T-cell malignancy patients [73]. However, CD5-based immunotherapies could also be intertwined with off-tumor toxicities due to the expression of CD5 by a subclass of B cells called B-1a cells [75]. Since B-1a cells play key roles in the fight against opportunistic bacterial (such as streptococcal infections) and viral infections, their unwanted elimination renders patients susceptible to life-threatening conditions [75,76]. To overcome this limitation, patients undergoing CD5-based immunotherapies might require to be under meticulous clinical care in a sterile environment alongside undergoing immunoglobulin replacement (obtained from healthy donors) after treatment completion [5,77].

Novel antigens under laboratory development

CD26

Despite the clinical success of tyrosine kinase inhibitors (TKIs) in chronic myeloid leukemia (CML) patients, complete disease regression has been out of reach due to the incapability of TKIs in the elimination of the quiescent leukemia stem cells (LSCs) [78]. Moreover, despite the targeting of IL-1RAP, CD33, CD44, and CD123 using CAR-Ts, only IL-1RAP-redirected CAR-Ts have been able to target CML LSCs [79]. In addition to the mentioned antigens, experimental evidence has confirmed the expression of CD26 (DPP IV) to be restricted to LSCs, basophils, and activated T cells [79]. Moreover, alongside TKI-insensitive CML LSCs and anaplastic large T-cell lymphoma, CD26-redirected CAR-Ts (CD26.CAR-Ts) might also be beneficial in the cases of renal cell carcinoma, malignant pleural mesothelioma, and colorectal cancer [79]. In 2019, Zhou et al. developed CD26.CAR-Ts to investigate their antitumor capacity against CD26-positive cancer cells, but besides their poor viability, these cells were cytotoxic against themselves, due to the self-expression of CD26 [78]. In 2020, Zhou et al. stated that besides some off-tumor toxicity towards activated lymphocytes, CD26.CAR-Ts enhanced tumor rejection in a mouse model and they enforced tumoricidal effects against primary CML LSCs and the CD26-positive cell lines of K562 and Karpas 299 [79]. Furthermore, it was elucidated that the accelerated expansion of CD26.CAR-Ts, after fratricide-induced delayed expansion, correlated with the elevated expression of cathepsin B and SERPINB9 and reduced expression of CD26 [79].

CLEC14A

One of the alternative strategies for overcoming the limitations of CAR-T therapy in solid tumors is their redirection against the tumor vasculature, rather than the tumor cells [80]. In this regard, CLEC14A is recognized as an overexpressed tumor endothelial marker (TEM) whose physiological expression in normal endothelial cells is rather negligible [81]. It is worth mentioning that low shear stress might act as a contributing factor in elevating the expression level of CLEC14A by the tumor endothelial cells [82,83]. In 2020, Zhuang et al. indicated that CLEC14A-redirected CAR-Ts (CLEC14A.CAR-Ts) sufficiently proliferated, released IFN-γ, and enforced tumoricidal effects upon encountering their specific antigen, *in vitro* [80]. The intelligence behind the targeting of CLEC14A is that it is a glycoprotein whose expression is elevated in various human solid tumors [80]. Furthermore, the mouse models of mPDAC, Lewis lung carcinoma, and Rip-Tag2 exhibited considerable tumor rejection under the treatment of CLEC14A.CAR-Ts that coincided with tremendous downregulation of CLEC14A and significant disruption of the tumor vasculature system alongside showing "no signs of toxicity" [80]. These results might validate the feasibility of targeting the tumor vasculature, as an alternative approach, as well as highlight the applicability of CLEC14A as a likely immunotherapy target.

Additionally, other investigators have also developed CAR-Ts that target different TEMs such as $\alpha v\beta 3$ integrin, VEGFR1, and VEGFR2 [84-86]. Besides reporting potential CAR-T-mediated tumor elimination in the mentioned studies, Chinnasamy and colleagues reported severe toxicities following the administration of VEGF-2-redirected CAR-Ts (2×10^7 T cells) in BALB/c mouse tumor models [85]. However,

equivalent tumoricidal responses, but without the occurrence of toxicities, were achieved by simply lowering the administered dose of the CAR-Ts (down to 5×10^6 T cells) [85]. Regarding VEGFR2, in a clinical trial completed in 2015 (NCT01218867), only 1 out of 24 patients (about 4%) achieved a partial response [80]. Such poor clinical responses alongside the possibility of toxicities against the normal endothelium could raise serious concerns about the applicability of the alternative approach of targeting TEMs. Of note, CAR-Ts need to be safe and capable of inducing high rates of disease remission in patients to meet clinical standards for clinical approval.

GUCY2C

In the context of minimizing the unwanted damages of CAR-T therapy of epithelial cancers, delivered to healthy tissues as a result of targeting non-tumor-specific antigens, targeting of novel antigens is of paramount importance. Guanylyl cyclase C (GUCY2C), a regulator of intestinal homeostasis, is considered a cancer mucosa antigen overexpressed in around 90% of colorectal cancer , alongside other gastrointestinal malignancies [87,88]. The bright side of targeting GUCY2C is its inaccessibility in polarized epithelial tissue cells, due to its restriction to the apical membrane tight junctions, which minimizes the risks of intestinal toxicity while providing the opportunity of targeting metastatic colorectal lesions with disrupted apical-basolateral architecture [88,89]. In 2016, Magee et al. developed GUCY2C-redirected CAR-Ts (GUCY2C.CAR-Ts) targeting the murine homolog of GUCY2C, and they reported that GUCY2C.CAR-Ts successfully eliminated only GUCY2C-expressing mouse colorectal cancer cells while they spared GUCY2C-deficient cells [88]. As *in vivo* findings indicated, besides mediating no secondary adverse events towards healthy tissues, GUCY2C.CAR-Ts were able to enforce tumor rejection and increase the survival rates of colorectal cancer metastasis mouse models [88]. Additionally, in 2018 and as an attempt to target human GUCY2C-expressing metastases, Magee et al. developed GUCY2C.CAR-Ts equipped with a murine GUCY2C-specific targeting domain [89]. Alongside inducing prolonged survival in a syngeneic mouse model with lung metastases of human GUCY2C-expressing murine colorectal cancer cells, GUCY2C.CAR-Ts also induced durable survival in a human xenograft model by enforcing tumoricidal effects against GUCY2C-positive human colorectal cancer cells [89]. Also, in 2020, Baybutt et al. further confirmed the antitumor responses of third-generation GUCY2C.CAR-Ts, as indicated by elevated levels of TNF-α and INF-γ, against the metastatic colorectal cancer cell line T84 [90]. All these findings, alongside other therapeutic strategies, such as the application of anti-GUCY2C/CD3 BiTEs to recruit endogenous T cells against GUCY2C-expressing tumor cells, might highlight the importance of GUCY2C as a likely target of cancer immunotherapy [87]. However, the actual safety index and therapeutic efficacy of GUCY2C.CAR-Ts can only be determined in clinical trials with a broad population of patients with GUCY2C-positive oncological indications.

TEM8/ANTXR1

In 2018, Byrd et al. asserted that TEM8-redirected CAR-Ts (TEM8.CAR-Ts) are capable of releasing immunostimulatory cytokines and enforcing cytotoxic effects that result in the elimination of TME8-positive triple-negative breast cancer (TNBC) cells alongside tumor endothelial cells [91]. Furthermore, tumor rejection by the elimination of TEM8-positive TNBC tumor cells and disruption of tumor vascularization were also observed in patient-derived and TNBC cell line-derived xenograft tumor models that received TEM8.CAR-T treatment [91]. However, Petrovic et al. reported considerably contrasting findings as the adoptive transfer of their TEM8.CAR-T panel, one of which harbored the same targeting domain as that of the Byrd et al. study, caused rapid toxicity and resulted in the absence of the TEM8.CAR-Ts from the circulation of healthy C57BL6 and NSG mice [92]. Moreover, further analysis of the animal models presented evidence of spleen and lung inflammation alongside attributing the loss of the TEM8.CAR-Ts from the circulation to the healthy-tissue targeting of TEM8 by TEM8.CAR-Ts [92]. Such contradictory findings accentuate the necessity for further meticulous preclinical experiments for assessing the suitability of TME8 as a likely immunotherapy target. Also, such reports demonstrate that preclinical studies should be interpreted cautiously. Furthermore, in 2019, Sotoudeh et al. conducted an *in silico* experiment to find suitable target antigens for the CAR-T therapy of gastric adenocarcinoma, with overexpression only in tumor tissues as a strategy to minimize off-tumor toxicities [93]. According to the results, alongside MSLN and MUC3A, TEM8 was also selected as a possible antigen for this aim, and it was suggested by the authors that simultaneous targeting of these antigens might result in the circumvention of resistance and improvement of CAR-T therapy outcomes [93]. However, since simultaneous targeting of multiple antigens might increase the risks of off-tumor toxicities, the practicality of such proposals might sometimes be questionable.

PDPN

Podoplanin (PDNP) is a mucin-like glycoprotein expressed in the renal, alveolar, and lymphatic endothelium, as well as in the basal skin cells [94]. Additionally, PDNP overexpression has been associated with various malignancies such as mesothelioma, esophageal cancer, lung cancer, and aggressive brain tumors such as mesenchymal glioblastoma [94]. In 2016, Shiina et al. developed third-generation PDPN-redirected CAR-Ts (PDPN.CAR-Ts), equipped with a PDPN-specific scFv called NZ-1, and reported that these cells mediated efficient tumoricidal responses against PDPN-positive glioblastoma cells, *in vitro* [95]. Moreover, the systemic administration of PDPN.CAR-Ts into a glioma mouse xenograft model managed to inhibit the growth of intracranial tumors [95]. Despite the success of PDPN.CAR-Ts in inducing tumor rejections in preclinical models, normal tissue expression of PDPN can lead to severe cases of off-tumor toxicities that might overshadow the applicability of PDPN.CAR-Ts in clinics. In this regard, Kato and Kaneko have developed a cancer-specific monoclonal antibody (CasMab), called LpMab-2, that can only react with the aberrantly glycosylated PDPN expressed by tumor tissues while being unreactive towards healthy-tissue PDPN [96]. The development of CAR constructs using LpMab-2, as the targeting tool, and genetically engineering T cells to express them will broaden the therapeutic applicability of PDPN.CAR-Ts since the adoptive transfer of such CAR-Ts might result in less immune-mediated toxicities. In our opinion, the utilization of CasMabs in the targeting domain of CAR constructs might be the most applicable and close-to-reality strategy for minimizing the off-tumor toxicities of CAR-Ts without creating complicated molecular twists that can only make it harder to meet clinical standards in the future.

PTK7

Protein Tyrosine Kinase 7 (PTK7) is a tyrosine kinase of the *Wnt* signaling pathway with possible activities in various cellular processes including proliferation, migration, adhesion, and programmed cell death [97]. High expression levels of PTK7, which is also known as colon cancer carcinoma kinase 4 (CCK-4), have been correlated with several solid tumors including pancreatic, lung, renal, breast, ovarian, and colon cancer [97]. A Phase I clinical trial (NCT02222922) has already been conducted to investigate the safety and tolerability of a PTK7-specific antibody-drug conjugate (ADC), named PF-06647020, in non-small cell lung cancer (NSCLC), TNBC, and platinum-resistant ovarian cancer (OVCA) patients [98]. PF-06647020 is linked with a cleavable linker to auristatin-0101, which itself is an auristatin microtubule inhibitor [98]. So far, clinically favorable responses have been observed in the OVCA patients with reasonable safety indications which warrant further clinical evaluations [98]. In 2020, Levitsky et al. generated allogeneic PTK7-redirected CAR-Ts (PTK7.CAR-Ts), and, after evaluating

their antitumor capacity *in vitro* and in lung, pancreatic, colon, ovarian, and breast cancer xenograft mouse models, reported encouraging outcomes [98]. However, since some healthy tissues, including the stromal cells of the lung, uterus, and ovary, maintain physiological-level expression of PTK7, it is reasonable to anticipate mild to serious toxicities in the respective clinical settings, which might determine the suitability of PTK7 as an immunotherapy target [98].

PODXL

Podocalyxin (PODXL), alternatively known as TRA-1–60, is a type I membrane-bound glycoprotein that plays critical roles in several organs including the pancreas, kidney, and heart [99]. Substantial evidence indicates that alongside improving the metastatic potential and invasiveness of renal cell carcinoma, hepatocellular carcinoma, and breast tumors, PODXL has also been associated with various other malignancies such as human oral squamous cell carcinoma, astrocytic tumors, and colorectal cancer [100]. Even though there have not yet been any CAR-Ts generated to specifically target PODXL-expressing tumor cells, in 2020, Kaneko et al. developed a murine PODXL-specific CasMab (hereafter referred to as PcMab-60) which successfully reacted with the PODXL-overexpressing glioblastoma cell line of LN229 and pancreatic cell line of MIA PaCa-2, while being unreactive towards healthy control cells [100]. Next, they conferred augmented antibody-dependent cellular cytotoxicity (ADCC) to the mentioned CasMab by first engineering it into a mouse IgG2a-type mAb and then developing a core fucose-deficient mAb [100]. The resultant product, named 60-mG2a-f, exhibited pronounced tumoricidal capacity in MIA PaCa-2 xenograft mouse models suggesting that targeted immunotherapy of pancreatic cancer, as well as other PODXL-overexpressing tumors, with PODXL as the targeted antigen, might be a promising approach that requires future experimental and clinical evaluations [100].

CD44

CD44 is a transmembrane glycoprotein that plays an important role in mediating cell adhesion, interaction, and migration, alongside promoting the expression and tumor-intrinsic functions of PD-L1 in TNBC and NSCLC patients [101]. Recognized as a cancer stem cell (CSC) antigen, CD44 has also been correlated with the hematogenous metastasis of hepatocellular carcinoma which might make it a likey immunotherapy target, especially in the field of CAR-T therapy [102]. In this regard, Wang et al. generated scFv-based CD44-redirected CAR-Ts (CD44.CAR-Ts) and reported that these cells are potentially cytotoxic towards the Hep3B2, MHCC97H, and SMMC-7721 cell lines and that CD44.CAR-Ts secreted elevated levels of IL-2, IFN-γ, and TNF-α upon encountering them [102]. Moreover, as CD44.CAR-Ts induced remarkable tumor growth inhibition in CD44-positive hepatocellular carcinoma xenograft mice, it is worth noting that there were no signs of CD44.CAR-T-mediated toxicities towards healthy tissues, as reported by Wang and colleagues [102]. However, such findings should be interpreted as cautiously as possible since xenograft mouse models do not perfectly mirror the multidimensional nature of the human immune system (in which CD44 is also expressed by healthy cells, including T cells and macrophages, and its targeting using CAR-Ts might mediate mild to serious off-tumor toxicities in cancer patients) [103, 104]. So, to avoid being farfetched, findings achieved in preclinical mouse models should not be relied on too confidently or used as a trustworthy basis for predicting future clinical safety.

Conclusion and future perspectives

Today, to tell the story of clinical success in cancer treatment and not include CAR-T therapy in it is just unfair. Even though CAR-T therapy has only been clinically approved for the treatment of B-ALL, DLBCL, MM, and MCL, it might still have a lot to offer in various other hematologic malignancies and even solid tumors [118][117][2–4,6]. The discovery of novel antigens, whose targeting using antibody-based therapy holds promising antitumor responses, can both offer fresher opportunities to and accelerate the success rate of CAR-T therapies. Moreover, since tumor cells undertake sophisticated antigen-dependent tricks to evade being recognized by a particular type of therapy, there is a growing need for finding substitute antigens that could be leveraged for therapeutic purposes in times of disease relapse. One of such resistance mechanisms is the loss of the targeted antigen [105]. Moreover, antigen downregulation can also deteriorate the antitumor efficacy of CAR-Ts towards malignant cells since, in sharp contrast with endogenous T cells, CAR-Ts need to interact with a high number of antigens only to produce the necessary downstream signals that trigger their cytolytic reactions [105–107]. For example, according to a recent report (NCT03185494) by Dai and colleagues, 1 out of 6 R/R B-ALL patients (that had achieved MDR-negative CR following treatment with CD19/22-redirected bispecific CAR-Ts) experienced relapse with CD19-negative leukemic blasts that had downregulated CD22 expression [108]. Also, there is a case of resistance to CAR-T therapy in which alternative splicing gives rise to the expression of isoforms of antigen molecules to which CAR-T targeting domains cannot bind [105,109]. However, in such cases, replacing the targeting domain of the CAR-T product with one that is able to recognize an epitope on the alternatively-spliced antigen would simply suffice to reinvigorate the desired tumoricidal efficacy.

The majority of clinical trials currently investigating CAR-T therapies for hematologic malignancies focus on CD19, CD20, CD22, and BCMA as their selected antigens. Amongst the novel antigens discussed in this review, CS1, FLT3, CD7, and CD26 have often been regarded as non-solid tumor antigens. CAR-Ts targeting these novel antigens might have a stroke of better luck, compared with CAR-Ts targeting solid tumor antigens, in inducing therapeutic benefits. The basis for such mere expectation lies behind the substantial differences between solid and non-solid malignancies. In the context of solid tumors, CAR-Ts face multiple difficulties accessing the tumor cells they are redirected against [110]. The tumor-associated vasculature and the enriched stroma are considered tenacious obstacles that CAR-Ts need to overcome only to be able to interact with the tumor cells expressing their target antigen [110]. Moreover, the hostile TME also imposes harsh conditions on CAR-Ts resulting in their exhaustion [110].

HER2 has been the most famous antigen targeted by CAR-Ts in clinical trials related to solid tumors. HER2 overexpression has been associated with breast, gastric, uterine, ovarian, and lung cancer [111]. Out of the novel antigens presented in this paper, ALPP, CLDN, CD44, PODXL, PTK7, TEM8, GUCY2C, and CLEC14A might be suitable antigens that could be targeted in relapse cases with HER2 expression loss (for instance, in TNBC) because they have been associated with the same oncological indications in which HER2 overexpression is observed. In regards to TNBC, TEM8.CAR-Ts have demonstrated tumor rejection in preclinical animal models [91]. Moreover, PTK7.CAR-Ts have also been able to induce favorable results in xenograft models of breast, ovarian, and lung tumors [98]. However, findings obtained in preclinical animal models of human cancers might not be as translatable in the clinics. An outstanding example in this regard is that the toxicities (mainly CRS and neurotoxicity) observed during the early days of CAR-T therapy investigations in clinical trials came as unforeseen adverse events since they were not anticipated by preclinical experiments [112]. Nevertheless, such preclinical data are necessary key points that have to be taken before advancing into clinical investigations since they play important roles in attracting sponsors to a particular CAR-T product. Also, as discussed throughout this article, some of these novel target antigens are currently under clinical investigation. In the upcoming years and after the related clinical trials have been completed, it can be said more confidently that how promising any of these antigens are in the CAR-T therapy of a particular type of cancer.

Besides the introduction of novel antigens, the utilization of these antigens for the implementation of various intelligent strategies such as bispecific CAR-Ts, multi-CAR-expressing T cells, pooled CAR-Ts, BiTE-secreting CAR-Ts, combinatorial antigen targeting strategies using two- or three-antigen dependent AND or NOT gates, boosting vaccines, and various other recently introduced twists might also be able to amplify the therapeutic efficacy of this type of immunotherapy [116]. Furthermore, some of such tactics might also endow CAR-Ts with powerful discriminatory abilities that can consequently minimize “on-target off-tumor” toxicities delivered to healthy tissues [116]. Moreover, the utilization of various novel gene-editing techniques, such as CRISPR-Cas9, TALEN®, and BE, or using ER and/or Golgi antigen anchoring methods can both facilitate the production of fratricide-resistant CAR-Ts, in the case of targeting antigens that are expressed by healthy T cells, and minimize the deleterious impacts of tumor-mediated immunosuppression. Furthermore, the discovery of novel TAAs via single-cell RNA sequencing or whole genome sequencing and the development and equipping of CARs with CasMabs that can only react with the cancer-specific forms of such antigens (that are aberrantly glycosylated and expressed only by the relative tumor cells) might bring new blood to the veins of CAR-T therapy. Aside from CAR-Ts, other treatment modalities such as ADCs, BiTEs, or even naked mAb therapies can also benefit from the discovery of novel antigens. At the end of the day, it is redeeming to conclude that it might not be too unreasonable to hope that years of infallible science behind cancer immunotherapy, and in particular CAR-T therapy, might eventually penetrate through the stubborn dome of cancer.

Author contributions

Pooria Safarzadeh Kozani: Conceptualization, Investigation, Writing - original draft, Writing - review & editing, Validation, Supervision. Pouya Safarzadeh Kozani: Conceptualization, Investigation, Writing - original draft, Writing - review & editing, Validation, Supervision. Fatemeh Rahbarizadeh: Writing - review & editing, Validation, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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