Summary  Chimeric antigen receptor (CAR) T cell therapy has been established in the treatment of hematological malignancies. However, in solid tumors its efficacy remains limited. The aim of this article is to give an overview of the field of cell therapy itself, to introduce the underlying concepts of CAR T cell-based treatment approaches and to address its limitations in advancing the treatment for solid malignancies.

Keywords  Adoptive T cell therapy · CAR T cells · Solid tumors · Immunotherapy · Tumor immunology

Background

Over the last decade, treatment of cancer has undergone a radical paradigm shift. Targeted therapies, either utilizing tyrosine kinase inhibitors (TKI) or therapeutic antibodies, have developed into integral elements of oncological treatment regimes. In contrast, cellular therapies are merely starting to enter clinical routine [1]. Of these, T cell-based methods, also known as adoptive T cell therapy (ACT), are the most advanced. ACT aims to combine the extraordinary specificity of the adaptive immune system and the natural antitumor response of T cells in the fight against cancer. To date, depending on the source of the T cells and the subsequent genetic or nongenetic manipulation, three main forms of T cell-based therapies can be distinguished:

- Tumor-infiltrating lymphocytes (TIL),
- T cell receptor (TCR)-engineered T cells and
- Chimeric-antigen receptor (CAR) T cells [2].

TIL are T cells, found in the tumor tissue, which in most cases are equipped with endogenous TCR specific for tumor-associated antigens. In TIL-based ACT, these T cells are isolated from surgical tumor specimens, are expanded in vitro and re-infused into the patients [3]. However, one major limitation to this approach is the often low number of antigen-specific T cells found in tumor explants and the inability to retrieve and expand T cells from all patients. To overcome these limitations, in vitro engineering methods have been developed to create antigen-specific T cells without needing to isolate them from tumor tissues. As such, naïve, unspecific T cells are isolated from the peripheral blood of the patients via leukapheresis, are then genetically modified with a tumor-specific recognition construct (e.g., tumor-specific TCR, CAR), expanded and finally re-infused into the patient [4].

TCR-engineered T cells and CAR T cells

As described, TCR T cells are genetically modified to express an antigen-specific TCR. In the treatment of neoplastic diseases, the target is usually a tumor-specific antigen (TSA) or tumor-associated antigen (TAA). Ideally, these would be uniquely expressed in malignant cancer cells, but not in healthy cells. Peptides derived from TSA are generated through intracellular proteasome-mediated processing mechanisms and
subsequently presented on the MHC-I complex of the tumor cells [2]. TCR-engineered T cells are able to recognize the MHC-TSA-peptide complex, which leads to an activation of T cells and subsequent lysis of neoplastic cells. In contrast, CAR T cells are engineered through introduction of an artificial synthetic construct, which ultimately also leads to the activation of the T cells and tumor cell lysis [2]. Both strategies have certain advantages and disadvantages, which have been extensively reviewed elsewhere [2, 5].

The artificial CAR construct usually contains an antibody-derived single-chain variable fragment (scFv) as an extracellular domain, a hinge domain and a transmembrane domain, anchoring the receptor in the cell membrane. T cells expressing the construct are able to bind the respective TSA via the extracellular antibody-derived scFv domain of the CAR receptor. Activation of T cells is subsequently induced by an intracellularly located signaling domain, consisting of a CD3ζ chain and one or more co-stimulatory domains (e.g., CD28, 4-1BB) [2]. The CD3ζ chain is physiologically part of the TCR-CD3 complex and is the major inducer of T cell activation following antigen recognition. Co-stimulatory domains were included in the second and third generations of CAR constructs, as augmented antitumor efficacy [6] and increased persistence of the transferred T cells [7] has been observed. Depending on the CAR receptor used, CAR T cells are classified in different generations, as depicted in Fig. 1.

In recent years, further improvements of the CAR structures have been employed in order to improve efficacy of CAR T cells, especially in solid malignancies (see “CAR T cells in solid tumors” section). These innovative approaches have been extensively reviewed by us and other groups and interested readers are referred to the following literature for a more detailed overview [5, 8]. In short, fourth-generation CAR constructs incorporate a cassette into the intracellular domain to induce the secretion of pro-inflammatory cytokines. This strategy enables the innate immune system to contribute to the antitumor effect (Table 1; [9]). In contrast, fifth-generation CAR T cells followed a completely different approach and aimed to activate the Janus kinase-signal transducers and activators of transcription (JAK-STAT) signaling pathway to promote T cell proliferation. Fifth-generation CAR T cells were shown to have superior antitumor effect and persistence compared to second- and third-generations [10]. However, these developments are merely at the beginning and not part of the clinical routine. All U.S. Food & Drug Administration (FDA)- or European Medicines Agency (EMA)-approved ACT strategies are second-generation CAR-based approaches and only approved for the treatment of certain hematological malignancies.

**CAR T cells in hematological malignancies**

Axicabtagene ciloleucel and Tisagenlecleucel, both approved in 2017 by the FDA and in 2018 by the EMA, are CAR T cells engineered to target the B cell lineage antigen CD19. CD19 is exclusively expressed on both healthy and malignant B cells. Consequently, these CAR T cells can be used to treat B cell malignancies such as diffuse large B cell lymphomas (DLBCL) and B cell acute lymphoblastic leukemia (B-ALL, only Tisagenlecleucel). Approval was granted after astonishing initial response rates of up to 93% in ALL and 54% in DLBCL were observed [5]. Importantly, these response rates were reached in extensively pretreated patients with chemotherapy-refractory or relapsed malignant disease and many were durable [2]. A third T cell product was just recently approved by both the FDA and the EMA for the treatment of mantle cell lymphoma (MCL; Tecartus, brexucabtagene autoleucel) (NCT02601313) [11]. Recent long-term follow-up studies revealed sustained response rates in patients. However, disease relapse is seen in up to 41% of patients suffering from ALL [12]. In contrast, response rates in DLBCL seems to be more durable as the majority of responding patients do not experience relapse during the 12-month follow-up period [5, 13]. In summary, CAR T cell therapy has emerged as an important therapeutic option for hematological malignancies. However, in nonhematological malignancies CAR T cell therapy has so far failed to demonstrate comparable treatment responses.

**CAR T cells in solid tumors**

Encouraged by the striking results seen in DLBCL and ALL, new CAR T cells targeting different epithelial antigens were developed and clinically tested. As such, CAR-based ACT was evaluated in differ-

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**Fig. 1** Structure and classification of CAR T cells. CAR T cells are grouped into different generations depending on the structure of the CAR. Recent advancements have added new CAR structures, which are extensively reviewed in [5, 8]. scFv single chain variable fragment, CD3ζ CD3 zeta chain, IL-2R β chain IL-2 receptor β chain
ent gastrointestinal malignancies (pancreatic cancer, NCT01869166; colorectal cancer, NCT02349724) [14, 15], glioblastoma (NCT00730613; NCT02209376) [16, 17] and non-small cell lung cancer (NSCLC, NCT01869166) [18]. Table 1 gives a summary of already conducted clinical trials in solid malignancies.

| Trial number | Cancer entity | Published | CAR Target molecule | Trial Phase | Patients | Outcome | Reference |
|-------------|---------------|-----------|---------------------|-------------|----------|---------|-----------|
| NCT01869166 | Bilary tract cancer | 2018 | EGFR | I | 19 | 1/17 complete remission | [36] |
| NCT02349724 | CRC | 2017 | CEA | I | 10 | 7/10 stable disease | [15] |
| NCT01212887 | GI tumors | 2017 | CEACAM5 | I | 14 | No objective clinical response | [37] |
| NCT02541370 | GI tumors | 2018 | CD133 | I | 23 | 3/23 partial response | [38] |
| NCT00730613 | Glioblastoma | 2015 | IL13Ra2 | I | 3 | No objective clinical response | [16] |
| NCT02209376 | Glioblastoma | 2017 | EGFRvIII | I | 10 | Not available due to surgical intervention | [17] |
| NCT01109095 | Glioblastoma | 2017 | HER-2/neu | I | 17 | 1/17 partial response | [39] |
| NCT01454596 | Glioblastoma | 2019 | EGFRvIII | I | 18 | No objective clinical response | [21] |
| NCT02395250, NCT03146224 | Solid tumors | 2020 | GPC3 | I | 13 | 2/13 partial response | [40] |
| Park et al. | Neuroblastoma | 2007 | L1-CAM | I | 6 | 1/6 stable disease then partial response | [41] |
| Pule et al. | Neuroblastoma | 2008 | GD2 | I | 11 | 4/8 evidence of regression | [42] |
| NCT00085930 | Neuroblastoma | 2011 | GD2 | I | 19 | 3/19 complete remission | [43] |
| NCT0218650 | Neuroblastoma | 2017 | GD2 | I | 11 | 5/11 stable disease | [44] |
| NCT01869166 | NSCLC | 2016 | EGFR | I | 11 | 2/11 partial response | [18] |
| Kereshaw et al. | Ovarian Carcinoma | 2006 | FRα | I | 14 | No objective clinical response | [45] |
| NCT01897415 | PDAC | 2018 | MSLN | I | 6 | 2/6 stable disease | [46] |
| NCT01869166 | PDAC | 2020 | EGFR | I | 14 | 4/14 partial response | [14] |
| Junghans et al. | Prostate cancer | 2016 | PSMA | I | 5 (6) | 2/5 partial response | [47] |
| Lamers et al. | RCC | 2016 | CAIX | I | 12 | No objective clinical response | [48] |
| NCT00902044 | Sarcomas | 2015 | HER-2/neu | I | 17 (19) | 4/17 stable disease | [49] |
| NCT02159716 | Solid tumors | 2019 | MSLN | I | 15 | 11/15 stable disease | [50] |

CRC Colorectal Carcinoma, GI tumor, Gastrointestinal Tumor, HCC Hepatocellular Carcinoma, NSCLC Non-Small Cell Lung Cancer, PDAC Pancreatic Ductal Adenocarcinoma, RCC Renal Cell Carcinoma, IL13 Ra2 Interleukin-13 receptor subunit alpha 2, L1-CAM L1 Cell Adhesion Molecule, HER2/neu Human epidermal growth factor receptor 2, EGFRvIII Epidermal Growth Factor Receptor (variant III), CEACAM5 Carcinoembryonic antigen-related cell adhesion molecule 5, MSLN Mesothe- lin, CEA Carcino-Embryonic Antigen, GPC3 Glypican-3, CAIX Carboxyanhydrase-IX, FRα α-folate Receptor, PSMA Prostate-specific membrane antigen

As a consequence, several newly initiated clinical trials have primarily focused on the safety of the newly developed CAR T cells, directed against various target antigens of solid tumors (e.g., EGFRvIII, MUC-1, MAGE, CEA, GD2, CA125, MSLN; Table 1; [22]). These studies were most often conducted in malignancies with poor overall survival such as glioblastoma and pancreatic ductal adenocarcinoma; however, as depicted in Table 1, therapies are assessed in a wide range of different solid malignancies. While most treatments were shown to be safe, the overall response rates observed in these trials, especially compared to the impressive clinical benefit obtained in ALL and DLBCL, were rather disappointing. Overall mortality remained approximately the same and the patients usually only benefited from the treatment temporarily [8].

The congestion of pulmonary vasculature and lethal respiratory failure (NCT01454596) [21].
Hurdles of CAR T cell therapy in solid tumors

As described above the responsiveness of solid malignancies to CAR T cell therapy is bleak at best.

Over the last years, researchers have identified several underlying mechanisms responsible for the lack of treatment efficacy in solid tumors and have identified three major hurdles: (1) trafficking of T cells as the first key limiting step, (2) the choice of target antigen and antigen loss (tumor cell recognition) and (3) the hostile tumor microenvironment [8].

Trafficking of the transferred T cells into solid tumors is a limiting factor dampening therapeutic efficacy. As such, different strategies have been applied to improve T cell trafficking into the tumors. Direct application (intratumoral injection) of T cells has been employed to directly deliver the CAR T cells to the tumor site (NCT00730613) [16]. The need for invasive interventional procedures as well as the often inaccessible tumors however limit these approaches. Alternative strategies make use of physiological processes of immune cell trafficking: Immune cell recruitment to the site of inflammation is mediated by the chemokine–chemokine receptor axis. High levels of chemokine ligands secreted at the site of inflammation lead to the recruitment of immune cells expressing matching receptors. As solid tumors tend to show enhanced levels of chemokine ligands, co-transduction of chemokine receptors commonly not present on T cells, and CAR receptors into T-cells, has been employed by us and another groups [23, 24]. This has been shown to enhance both T cell infiltration and therapeutic efficacy in preclinical models. This concept is currently under investigation in clinical trials (NCT03602157).

Loss of the target antigen on tumor cells is a problem common in treatment of both hematological and nonhematological malignancies. Relapse with CD19-negative disease, for example, is frequently observed after treatment with CD19 CAR T cells. In solid tumors, down-regulation of the target antigen following CAR T cell therapy has also been reported in different clinical trials [17]. Targeting of multiple antigens (e.g. CD19 plus CD20; CD19 plus CD22) or alternatively sequential targeting strategies have shown benefit in different clinical trials [25–27].

Finally, solid tumors exhibit a complex, often hostile tumor microenvironment (TME). Besides cancer cells, the TME of solid tumors comprises infiltrating and resident immune cells, stromal cells as well as many pro- and anti-inflammatory mediators [28]. The interactions between the different components of the TME are complex and cannot be described in detail here. For further information please see the following literature [28–30]. In general, the components of the TME suppress an appropriate immune response against cancer cells, thus, creating a conducive environment for the tumor cells to proliferate.

The nowadays commonly used checkpoint inhibitors (e.g., pembrolizumab, nivolumab, ipilimumab) boost the activation and function of T cells through blockade of inhibitory receptors on T cells (e.g., PD-1, CTLA-4). As such, combining CAR T cells with immune checkpoint inhibitors or other drugs influencing the immunosuppressive nature of the TME are currently being investigated [8]. In addition, genetic engineering can be employed to lift immunosuppressive effects on the transferred T cells. Our group has developed a fusion receptor, switching the inhibitory signal of PD-1 into a T cell activating signal [31]. Alternatively, CRISPR-Cas9-mediated disruption of the PD-1 locus in CAR T cells has been shown to increase therapeutic efficacy of CAR T cells in vitro and in vivo. Several clinical trials are currently investigating these strategies (NCT03081715, NCT02867332, NCT02867345, NCT02793856, NCT03044743) [32].

Lastly, preclinical research has demonstrated the feasibility of redirecting CAR T cells not against tumor cells, but at immunosuppressive cells in the tumor microenvironment. Thus, CAR T cells targeting cancer-associated fibroblasts or tumor-associated macrophages have been shown to delay cancer progression in preclinical mouse models [33, 34]. However, to date no clinical data on these strategies are available, so the value remains uncertain.

Conclusion

The clinical transition of CAR T cell therapy has started a new era in oncology. Although these approaches have already given hope to incurable cancer patients suffering from hematological malignancies, it still remains to be proven in the comprehensive field of solid malignancies. As one might infer from our short overview, glioblastoma was often targeted in clinical studies. Due to limitations arising from its anatomical location and quick progression rate, glioblastoma remains clinically challenging to this date. Even a tumor as aggressive as glioblastoma was reported to be fully regressed in a case collection by Brown et al. [35]. The overall results of clinical studies might seem disappointing, but such case reports highlight the potential of CAR T cell therapy in solid cancers and maybe give a glimpse into what can be achieved in the future. Consequent advancement of promising preclinical strategies into clinical testing is now crucial to broaden the scope of cellular therapies and to increase the efficacy in solid tumors, with the hope that these therapies will not only be effective in single patients, but present a real clinical alternative for so many incurable cancer patients in daily oncological routine.
Take home message

- Adoptive T cell therapy has emerged as an important treatment option in relapsed and chemotherapy-refractory hematological malignancies.
- Clinical trials in solid tumors have primarily focused on establishing the safety of CAR T cell therapy; however, secondary endpoint analyses have so far only revealed modest efficacy.
- Preclinical research has been able to identify major caveats of CAR T cell therapy in solid tumors. Clinical trials will now have to determine whether this can be translated into clinically relevant improvements in patient outcome.

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