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Commentary

Recent perspective on CAR and Fcγ-CR T cell immunotherapy for cancers: Preclinical evidence versus clinical outcomes

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ABSTRACT

The chimeric antigen receptor T cell (CAR-T cell) immunotherapy currently represents a hot research trend and it is expected to revolutionize the field of cancer therapy. Promising outcomes have been achieved using CAR-T cell therapy for haematological malignancies. Despite encouraging results, several challenges still pose eminent hurdles before being fully recognized. Directing CAR-T cells to target a single tumour associated antigen (TAA) as the case in haematological malignancies might be much simpler than targeting the extensive inhibitory microenvironments associated with solid tumours. This review focuses on the basic principles involved in development of CAR-T cells, emphasizing the differences between humoral IgG, T-cell receptors, CAR and Fcγ-CR constructs. It also highlights the complex inhibitory network that is usually associated with solid tumours, and tackles recent advances in the clinical studies that have provided great hope for the future use of CAR-T cell immunotherapy. While current Fcγ-CR T cell immunotherapy is in pre-clinical stage, is expected to provide a sound therapeutic approach to add to existing classical chemo- and radio-therapeutic modalities.

Abbreviations: CAR-T, Chimeric Antigen Receptor T cells; TAA, Tumor Associated Antigen; Fcγ-CR, Fragment constant gamma-Chimeric Receptor; ATCT, Adoptive T Cell Transfer; TIL, Tumor-Infiltrating T Lymphocytes; scFv, single chain Fragment variable; MHC, Major Histocompatibility Complex; mAb, monoclonal Antibody; TNFR, Tumor Necrosis Factor Receptor; TCR, T Cell Receptor; PD-1, Programmed Death1; PD-L1, Programmed Death -Ligand 1; CTLA-4, Cytotoxic T-Lymphocyte Antigen 4; TIM-3, T cell Immunoglobulin- and Mucin domain-containing molecule; LAG-3, Lymphocyte Activating Gene 3; CRISPR/Cas9, Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated protein 9; GVHD, Graft-Versus-Host-Disease; HLA, Human Leukocyte Antigen; APO-1, Apolipoproteina A-1; ALL, Acute Lymphoblastic Leukemia; CLL, Chronic Lymphocytic Leukemia; CR, Complete Remission; RFS, Relapsed-Free survival; OS, Overall Survival; R/R, Refractory/Relapsed; FDA, Food and Drug Administration; MRD, Minimal Residual Disease; NHL, Non-Hodgkin Lymphoma; ORR, Overall Response Rate; HL, Hodgkin's Lymphoma; BCMA, B Cell Maturation Antigen; MM, Multiple Mieloma; CRS, Cytokines Release Syndrome; NKG2D, Natural Killer Gene 2D; ErbB-1/EGFR, Epidermal Growth Factor Receptor; EGFRvIII, Epidermal Growth Gactor Receptor variant III; ErbB2/HER2, Human Epidermal Growth Factor Receptor 2; CEA, Carcinoembryonic Antigen; GD2, Disialganglioside; PSMA, Prostate-Specific Membrane Antigen; IFNγ, Interferon γ; KIR, Killer-cell Immunoglobulin-like Receptor; DAP12, DNAX Activation Protein 12; LeY, Lewis-Y antigen; FRα, Folate Receptor α; MUC-16, Mucin 16; SCID, Severe Combined Immunodeficiency; CCL-2, Chemokine (C-C) ligand 2; CSF-1, Colony-Stimulating Factor-1; VEGF, Vascular Endothelial Growth Factor; CA-IX, Carboxy-Anhydrase-IX; NK, Natural Killer; ADCC, Antibody-Dependent Cell-Mediated Cytotoxicity; MICA/B, MHC Class I-Related Chain A/B; RCC, Renal Cell Carcinoma; HCC, Hepatocellular Carcinoma; CRC, Colorectal Carcinoma; DC, Dendritic Cells; FcεRIγ, CD16γ; CD64, Fc gamma RI; CD32, Fc gamma RII; CD16, Fc gamma RIII; Kv1.3, Voltage-gated Potassium channels 1.3; KCa3.1, Voltage-gated Potassium Calcium-channels; FAP-a, Fibroblast Activation Protein-a; TNFα, Tumor Necrosis Factor alpha; MIP-1a, Macrophage Inflammatory Protein 1 alpha; RANTES/CCL5, Chemokine (C-C motif) ligand 5; TGFβ, Transforming Growth Factor beta; APC, Antigen Presenting Cells; IDO, indoleamine-pyrrole 2,3-dioxygenase; Tregs, Regulatory T cells; Foxp3, Forkhead box P3; TMB, Tumor Mutational Burden

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1. Adoptive T-cell transfer for cancer immunotherapy

The adoptive T-cell transfer (ATCT) aims at utilizing peripheral blood or tumour-infiltrating T lymphocytes (TILs) to target and destroy tumour cells [1]. Effective *in vivo* or *ex vivo* increase in the numbers of T cells is critical for generating adequate amounts of T cells to be used for ATCT. Several cytokines are known to enhance the proliferation and activity of T cells such as IL-2 which has been demonstrated to play a crucial role in induction of T cells expansion and proliferation [2,3]. Interestingly, the process of isolation of TILs has been challenging but TILs-based immunotherapy has been proven to be an effective therapeutic strategy both in mouse model [4], and in human metastatic melanoma [5]. An effective strategy for ATCT has been demonstrated where TILs are co-administered with IL-2, and the tumour bearing recipient is subjected to lymphodepletion either by chemical modality (cyclophosphamide) or irradiation. Such multimodal ATCT therapeutic strategy has been reported to effectively enhance the anti-tumour activity of TILs; although, the latter could be reproducibly grown only from melanoma. The CAR-T cell technology has significantly expanded the range of tumours that can be treated by ATCT [6].

2. Anti-cancer MHC-independent strategies and the path toward CAR-T cell therapy

The design of CARs constructs has been clearly improved. Currently, three generations of CARs are available. The first generation of CARs was described by Eshhar et al. It resulted from the fusion of a single-chain variable fragment (scFv) of an antibody, linked to a flexible hinge, with the T cell receptor, CD3 zeta chain (CD3ζ) [7–9]. This design combined the targeting element from a well-characterized monoclonal antibody (mAb) with a signaling domain (Fig. 1). This approach enables specific tumour epitope recognition and T-cells activation without dependence on the major histocompatibility complex molecules (MHC). The latter aspect is particularly important, given the ability of many tumour cell types to downregulate the MHC class I molecules.

Further enhancement of CAR methodology was achieved by the implementation of a second generation of CAR through the integration of a costimulatory molecule of T cell, such as CD28, into the endodomain of the first generation of CAR construct. The integration of a T cell co-stimulatory molecule led to a higher level of T cell activation compared to that of the first generation of CAR T cells, (Fig. 1). Thus, CD28 acts as the second activation event in the pathway, leading to heightened T cells proliferation, along with a marked increase in cytokine expression [10]. Studies have indicated that the use of a co-stimulatory domain such as CD28 correlates with a higher production of cytokines and to extend persistence in comparison to the CD3ζ alone (Fig. 1) [11–14].

The most recent generation (3rd generation) of CAR design incorporates an additional co-stimulatory domain to enhance CAR function (Fig. 1). In most cases, the co-stimulatory domain is represented by one of the members of the tumour necrosis factor receptor (TNFR) family: CD134 (OX40) or CD137 (4-1BB). Pule et al. [15] provided an analysis of this addition by comparing three different CARs constructs: CD28-f, OX40-f, and CD28-OX40-f. They found better results in the third combination, which demonstrated higher NF-κB activity, increased IL-2 and IFNγ secretion, and sustained proliferation. This promotes the T cells cytotoxic ability and release of cytotoxic granules containing perforin and granzymes leading to the killing of target cells (Fig. 2A) [16].

The extracellular domain of CAR-T cells is the equivalent of the single chain variable fragment (scFv) found in immunoglobulins (IgG) specific to certain antigens, usually a tumour associated antigen (TAA). It is mainly responsible for redirect CAR-T cells to specific TAA. The IgG molecules eliminate target antigens by direct capture, neutralization, opsonization, inactivation, and phagocytosis. When compared to IgG

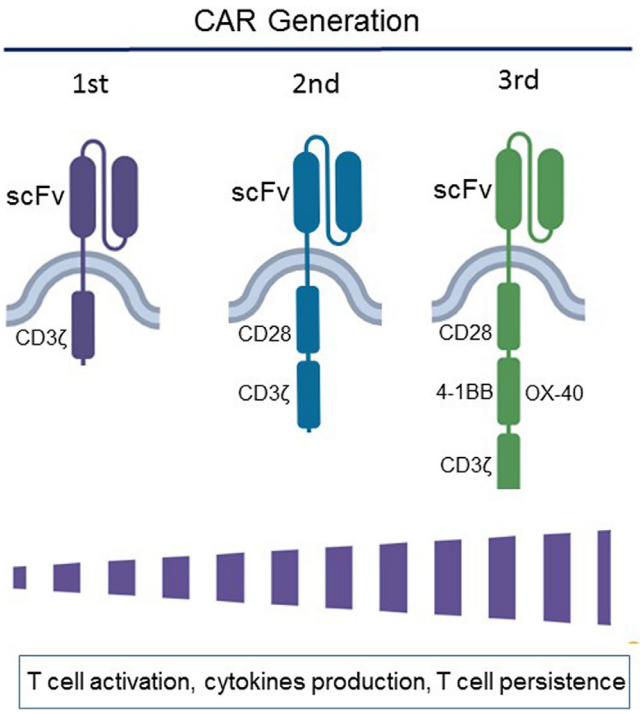

Fig. 1. The figure represents the evolution of the CAR constructs. First generation CARs contain the scFv against a specific TAA linked to the ζ-chain of TCR/CD3 complex. The 1st generation was limited in T cell activation, cytokines release and T cells persistence. The 2nd generation CARs, including the co-stimulatory domain CD28 fused to CD3ζ, have improved the antitumour activity of the engineered T cells against the target tumour cells. The best results were obtained with the third generation CARs, which added to the previous construct a second co-stimulatory domain such as 4-1BB or OX40. This resulted in higher cytotoxicity and long-term persistence of T cells in vivo.

molecules, the TCR is equipped with two additional structures: the transmembrane, and intracellular domains. There are also some major differences between the antigen binding capacity of the immunoglobulins and TCR wherein binding by TCR is dependent on antigen presentation in the context of the MHC class I or class II [17]. This is not the case for circulating antibodies. Interaction of TCR or CAR with a specific antigen triggers the CD3 multimeric protein complex to initiate a signaling cascade [18]. Introduction of CAR into allogeneic T cells promotes the ability of T cells to proliferate, produces a storm of cytokines, and induces target cell cytolysis as is seen with CD19-directed CAR-T cells used to treat CD19-expressing B-cell malignancy [19].

3. Genome editing and universal allogeneic CAR-T cells

CD19 CAR-T cell therapy has proven to be efficient mainly in the treatment of B cell malignancies [20–23]. The use of CAR-T cells to target a single antigen in B cell malignancies poses some challenges compared to solid tumours. The inhibitory environment of the solid tumours and the release of different inhibitory molecules might compromise the antitumour activity of CAR T cells. Interaction of PD-1 [24,25], and CTLA-4 on T cells [26] with their ligands triggers inhibitory pathways, T cell exhaustion and impairment of T cell function in chronic infections and cancers, suggesting another challenge for effective elimination of cancer cells. PD-1, CTLA-4, TIM-3 [27,28], and LAG-3 [29] have been reported to be involved in T cell exhaustion.

To enhance the efficacy of CAR-T cells in treatment of solid tumours several strategies have been suggested. Elimination of endogenous TCR and inhibitory molecules on the surface of CAR-T cells, using genome editing (such as CRISPR/Cas9 system) was expected to lead to an

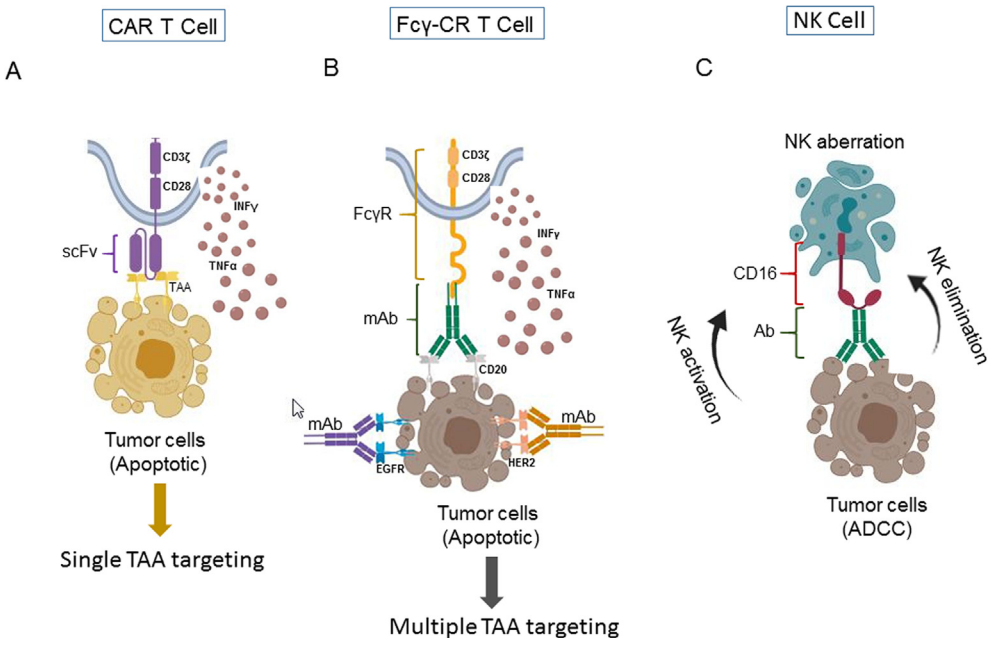

Fig. 2. The picture describes the major differences among CAR-T cells, Fcγ-CR T cells and NK cells. (A). Representation of the second generation CAR. The engagement of the scFv on T cells and a specific TAA on target tumour cells induces the release of inflammatory cytokines and cytotoxic granules containing perforin and granzymes leading to tumour lysis. An important limit of this technology is the single TAA targeting on the tumour cells by the effector cells. The risk of loss of the antigen on the tumour cells could make the therapy ineffective. (B). Cross-linking between the second generation of Fcγ-CR expressed on T cells and IgG opsonized-tumour cells induce T cell activation and cytokines release which results in tumour apoptosis. This technology holds more advantages compared with a typical CAR: i) multiple TAAs targeting exerted by the same Fcγ-CR T cells and ii) the withdrawal of mAbs reduces the risk of cytokines release storm (C). Representation of NK cell anti-tumour activity. The killing activity of NK cells in the tumour microenvironment is poor and tumour cells might induce NK cell elimination.

enhancement of CAR-T cells function without adverse effects on the function of primary T cells for adoptive immunotherapy [30].

Most ongoing CAR-T cell-based immunotherapy uses autologous T cells. While the use of autologous cells would normally be expected to overcome the problem of graft-versus-host disease (GVHD), widespread clinical application of autologous CAR-T cell immunotherapy could potentially be hindered by the high cost of a large scale production, poor quality, and insufficient numbers of collected autologous cells, especially in elderly and immunocompromised patients. One possible solution to overcome such obstacles is to generate high quality active

Table 1
In vitro, in vivo and clinical trials of CAR T immunotherapy.

Antigen targeted	Disease	CAR	Fcgamma CAR	Clinical phase	References
Hematological malignancies					
CD19	Acute lymphoblastic leukaemia (ALL), and chronic lymphocytic leukaemia (CLL) were	+	–	I	[34–38]
CD116	Myelomonocytic leukaemia	+	–	I	[39]
CD22	ALL showing CD19-negative antigen escape	+	–	I	[43]
					[47]
CD19/CD28	CLL	+	–	I	
					[48,49]
CD30	Hodgkin's lymphoma	+	–	I	
BCMA	Multiple myeloma (MM) and myeloid malignancies	+	–	I	[50]
LCAR-B38M	Multiple myeloma (MM) and myeloid malignancies	+	–	I	[51]
		+	–	I	[52]
NKG2D	Acute myeloid leukaemia				
Solid tumours					
HER2 and IL-13Rα2	Glioblastoma	+	–	Mouse	[57]
IL-13Rα2 and EGFRvIII	Glioblastoma	+	–	Mouse	[63,64]
IL13Rα2	Glioblastoma	+	–	I	[65]
EGFRvIII		+	–	Mouse	[49,67–69]
GD2 and CD171	Neuroblastoma	–	–	Mouse	[70,71]
GD2	Neuroblastoma	+	–	I	[72]
ErbB	ErbB-positive tumour cell lines	–	–	Cell line	[80]
HER2	Breast cancer	–	–	Mouse	[84,85]
Mesothelin	Breast cancer	–	–	Cell line	[86,87]
EGFR	Non-small cell lung cancer	+	–	I	[93]
Mesothelin	Advanced mesothelioma or pancreatic cancer	+	–	I	[95]
KIR-CAR/DAP12	Mesothelioma	–	–	Mouse	[96]
NKG2D	Ovarian cancer cell	–	–	Cell line	[97]
HER2/neu	Ovarian cancer cell	–	–	Mouse	[98]
PSMA	Prostate cancers	–	–	Mouse	[100]
CA-IX	Renal carcinoma cells	–	–	Mouse	[108]
CA-IX	Renal carcinoma cells	+	–	I	[109]
HER2	Metastatic osteosarcoma	–	–	Mouse	[110]
NKG2D	Ewing sarcoma	–	–	Cell line	[111]
IL-11Rα	Osteosarcoma, prostate cancer, breast cancer	–	–	Mouse	[112,113,114]
IL-11Rα	Primary tumours and pulmonary metastasis	–	–	Mouse	[115]
HER2	HER2-positive sarcoma	+	–	I/II	[116]

allogeneic T cells derived from young healthy individuals. However, the presence of endogenous TCR and the expression of HLA on the surface of allogeneic T cells is expected to trigger GVHD leading to rapid rejection by the host immune system [31].

Besides the existence of allogenic HLA antigen and inhibitory receptors, other molecules such as the Fas receptor (also known as CD95 and APO-1) might play a crucial role in CAR-T cells reactivity. Interaction of Fas with its ligand (FasL) is known to induce cell death and T cell apoptosis leading to attenuation of CAR-T cells activity [32].

Disruption of allogeneic and HLA antigens along with ablation of inhibitory TCR appears to be a promising strategy to overcome not only GVHD, but also potential exhaustion that might occur. Exhaustion would be due to interaction of inhibitory molecules released from the tumour microenvironment with their specific inhibitory receptors located at the surface of CAR-T cells. In a recent study, Ren et al. [30] succeeded in generating allogeneic universal CAR-T cells via the quadruple knockout of endogenous TCR, HLA class I (HLA-I), Fas, PD1 and CTLA-4. The generated universal CAR-T cells are currently being assessed against different types of solid tumours.

4. CAR-T cells and haematological malignancies (Table 1)

Currently, anti-CD19 CAR-T cells [33] were demonstrated to be effective in the treatment of B cell non-Hodgkin lymphoma (NHL), acute lymphoblastic leukaemia (ALL), and chronic lymphocytic leukaemia (CLL) [34–38]. Anti-CD116 CAR-T cell therapy has been developed for treating myelomonocytic leukaemia. In a recent clinical trial (ELIANA trial), anti-CD19 CARs was used to treat 68 paediatric patients with acute lymphoblastic leukaemia (ALL). Complete Remission (CR) was observed in 83% of these patients, and after 6 months, the estimated Relapse-Free Survival (RFS) and Overall Survival (OS) probabilities were reported to be 75% and 89%, respectively [39]. In another trial that was conducted at the Children's Hospital of Philadelphia, the 12-month RFS and OS of an anti-CD19 CAR T-cell-treated cohort of 53 children with refractory/relapsed (R/R) ALL were 45% and 78%, respectively [40]. Based on the promising outcomes of these two studies, the FDA has approved use of the anti-CD19 CAR-T cell product for B-cell ALL.

Despite encouraging outcomes for anti-CD19 CAR-T cell immunotherapy, 45% of the patients who achieved a minimal residual disease (MRD)-negative CR reported a relapse following anti-CD19 CAR-T cell therapy. In ALL relapsed patients, 39% CD19-negative leukemic blasts were observed. The CD19 negative leukaemia cells appear to be one of the main reasons underpinning the relapse that was observed following the anti-CD19 CAR-T cells [41]. Other claimed that expression of alternatively spliced isomers of CD19 might create resistance to CAR-T cell immunotherapy [42]. To overcome the CD19-negative antigen escape, a phase I clinical trial was conducted on nine children or young adults with ALL showing CD19-negative antigen escape using anti-CD22 CAR. MRD-negative CR was observed in 44% (four patients) indicating the potential of anti-CD22 CAR-T cell therapy to overcome the CD19 antigen escape [43].

Two main clinical trials were devoted to use anti-CD19 CAR-T cells for aggressive R/R Non-Hodgkin lymphomas (NHL) [44,45]. In a phase II clinical trial, 85 patients with R/R diffuse large B-cell lymphoma were treated with 4-1BB CAR-T cells after lymphodepleting chemotherapy. At a 3 month follow up, the Overall Response Rate (ORR) was 59% and the CR rate was 43% [44]. In a multicenter Phase 1 trial, 68 patients with R/R aggressive NHL had been treated with 4-1BB CAR-T cells. The six-month ORR was 40% and CR rate was 37% [45]. These findings are very encouraging but necessitate conducting a longer-term follow up to assess the efficacy of such immunotherapeutic modality against aggressive NHL.

For chronic lymphocytic leukaemia (CLL) few clinical trials have been reported on the use of anti-CD19 CAR-T cells as therapy. In 24 patients with ibrutinib-resistant CLL, the ORR at 1 month was 71%, and 8% of patients have shown cytokines releasing syndrome (CRS) [46]. In comparison to other CD19+ malignancies, the CAR-T cell therapy in CLL seems to be less effective. Currently, a phase I clinical trial is ongoing with the aim of employing CAR-T cells co-expressing anti-CD19/CD28 CAR along the co-stimulatory ligand 4-1BB [47].

Hodgkin's lymphoma (HL) is characterized by the expression of CD30. The CAR CD30-4-1BBζ T cells were used to treat 18 patients with heavily pretreated R/R HL. Remission was observed in 39% of the patients (seven patients) and 33% of the patients showed stable disease. CRS was observed in 11% of the treated patients [48,49].

Anti-BCMA (B cell maturation antigen) CAR-T cells product was utilized to treat multiple myeloma (MM) and myeloid malignancies. Out of 11 patients with R/R, seven valuable patients showed 100% ORR, and 2 patients showed strong CRs and two MRD-negative response [50]. In another clinical trial, 57 patients were treated with a CAR T therapy directed against to BCMA epitopes, LCAR-B38M. The ORR was 88% and 68% of patients showed MRD-negative CR at a median follow-up of eight months [51]. Anti-NKG2D-CD3ζ CAR T cells were used in a phase I trial to treat 12 subjects, seven with AML (acute myeloid leukaemia)/MDS (myelodysplastic syndrome) and five with MM. Consistent with preclinical studies, NKG2D-ζ-CAR T cell-expansion and persistence were limited. Neither tumour response nor toxicity after anti-NKG2D-ζ CAR-T cell therapy was noted [52]

5. CAR-T cell immunotherapy for solid tumours (Table 1)

In comparison to B-cell malignancies, fewer specific TAAs are demonstrated in solid tumours. A list of solid tumours surface antigens has been targeted using CAR-T cells. These include, but are not limited to, epidermal growth factor receptor (EGFR), EGFR type III variant (EGFRvIII), human epidermal growth factor receptor 2 (HER2), carcinoembryonic antigen (CEA), disialoganglioside 2 (GD2), mesothelin, prostate-specific membrane antigen (PSMA), and interleukin-13Rα2 (IL13Rα2) [53]. Some of the solid tumour specific antigens are expressed not only on tumour tissues but also on different normal tissues. CAR-T cells targeting bystander tissues might induce a deleterious immune reaction that are life-threatening.

The question of how to increase the specificity of CAR-T cell immunotherapy has been raised by several research groups. Dual specificity CAR-T cells have been designed [54–56] as a means to precisely recognize and destroy tumour cells in a more specific way. The use of dual CAR-T cells to target glioblastoma TAA (HER2 and IL-13Rα2) in a mouse glioblastoma xenograft model was associated with enhanced antitumor activity, decreased tumour antigen escape, and increased survival time of treated animals [57].

CAR-T cells were engineered to recognize two ligands that are specific to tumour cells, namely the Notch receptors and another second TAA. The extracellular domain of the Notch receptor was directed to recognize the first TAA (antigen A). Binding of the Notch extracellular domain with its ligand transforms the intracellular domain into a transcription factor fragment that triggers the expression of a CAR molecule specific to a second TAA (antigen B) [58–60]. Given the heterogeneity of tumour cells, the bispecific CAR-T cells would overcome the potential escape of single antigen tumours expressing antigen A or B. In a similar way, CD19 and CD20 bispecific CAR was designed, and have proven to be more efficacious against malignant B cells that harbour both TAAs [61].

For nervous system tumours, glioblastoma and neuroblastoma have been targets for CAR-T cell immunotherapy [49]. In the case of glioblastoma, two TAAs were characterized: IL-13Rα2 and EGFRvIII. IL13Rα2 is overexpressed in more than 50% of glioblastomas, and has been demonstrated to be expressed not only in glioblastoma cells, but also glioblastoma cancer stem cells [62]. First generation IL-13Rα2–specific CAR T cells has proven to be effective in eradication of glioblastoma cells and glioblastoma cancer stem cells in an orthotopic xenograft model [63,64]. In a phase I trial, the first generation

IL13Rα2–specific CAR-T cells was infused in three glioblastoma patients and limited anti-glioma responses were recorded. This was attributed principally to poor proliferation and persistence of first generation CAR-T cells [65]. Multiple intraventricular infusions of second-generation IL13Rα2-BBζ–specific CAR-T cells in a glioblastoma patient showed effective tumour regression [66].

A mutated form of wild type EGFRvIII is expressed in glioblastoma. At the preclinical level, multiple studies have demonstrated the ability of third generation EGFRvIII-specific CAR-T (CD28-4-1BB-ζ) cells to recognize and destroy EGFRvIII-positive glioblastoma without affecting the wild type EGFR that exist in normal tissues [49,67–69].

In neuroblastoma, which originates from neural crest cells, GD2 and CD171 have been demonstrated to be targets for CAR-T cells. At the preclinical level, GD2-specific CAR-T cells were reported to exert acytotoxic response against neuroblastoma [70,71]. Using a first-generation of GD2-specifc CAR-T cells, 3/11 patients with active disease, at the time of GD2-T cells infusion, achieved complete response in a phase I clinical trial, and the infused CAR-T cells were present for up to 192 weeks [72]. Different strategies have been utilized to improve the trafficking and localization of CAR-T cells in glioblastoma and other neuro-oncological malignancies. These include their modification to express the chemokine receptor [73] and tumour necrosis factor α (TNF-α) [74]. Such strategies and others (like the use of combining CAR-T cells and lenalidomide) have been demonstrated to improve trafficking and persistence of CAR-T cells in vivo [75].

Biological significance of the ErbB receptor family (EGFR or ErbB-1, ErbB-2 (HER2 or neu), ErbB-3, and ErbB-4) have been demonstrated in head and neck squamous cell carcinoma, breast and lung cancers [76–79]. In vitro and in vivo studies have demonstrated the ability of ErbB-specific CAR-T cells to recognize and lyse ErbB-positive tumour cell lines [80]. Due to the wide expression of all four ErbB receptors in normal tissues, intratumoral delivery has been proposed to be a more efficacious strategy in clinical trials [81]. Multiple T cell inhibitory mechanisms are exploited by squamous cell carcinoma cells to escape immune surveillance, such as the expression of PD-L1 [82], the presence of infiltrating regulatory T cells [83] which induce T cell inhibition due to secretion of IL-10 and transforming growth factor.

For breast cancer, HER2 and mesothelin are recognized as specific TAAs. Overexpression of HER2 oncogene is associated with uncontrolled cell proliferation and development in 20% of breast cancer [84]. In an in vivo mouse model of mammary tumours, a HER2-specific, second-generation CAR induced cytokine secretion, and exhibited potent cytotoxic reaction [85]. Triple-negative breast cancer is known to be unresponsive to targeted and hormone therapy. Mesothelin expression is associated with poor outcomes in breast cancer. CAR T cells-directed to mesothelin have been demonstrated to induce a cytolytic effects against primary breast tumour cells in vitro [86,87]. The use of a dual-targeting CAR system has been suggested to as a possibly effective strategy against antigen escape and acquired resistance, something that represents a major challenge in breast cancer therapy [88]. Moreover CAR-T cells can be engineered to overexpress and secrete IL-12, or costimulatory ligands, such as 4-1BB. Such inflammatory cytokines would induce epitopes spreading and induction of an effective endogenous immune response against cancer cells [89,90].

Multiple TAAs have been identified for non-small cell lung cancer of which EGFR, mesothelin [91] and CEA [92] are the most important targets. A second generation EGFR-directed CAR-T cell induced potent cytotoxic effects via induction of interferon γ (IFN-γ) and IL-2 secretion in EGFR-positive lung carcinoma cell [93]. In a phase I clinical trial, the use of a second-generation EGFR-specific CAR-T cells (after lymphodepletion) was associated with a partial response in two of the eleven patients with refractory non-small cell lung cancer [93].

The major TAA that is known to be overexpressed in mesothelioma is the mesothilin. A second-generation mesothelin-directed CAR-T cell therapy was associated with tumour elimination in vitro and in vivo [94]. In a phase I clinical study, the use of second generation mesothelin-specific CAR-T cells induced moderate cytotoxic responses against advanced mesothelioma or pancreatic cancer [95]. Wang et al. reported a CAR construct using the killer immunoglobulin-like receptor (KIR) and DAP12, a multichain immunoreceptor complex. They showed that the KIR-CAR/DAP12 can potently activate T cells and show anti-tumor activity in vivo on mesothelioma xenografts in mice resistant to typical CAR-T cells with 4-1BB-ζ or CD28-ζ [96].

Several TAAs have been identified in ovarian cancers of which NKG2D, HER2/neu, Lewis-Y (LeY +) antigen, MUC-16-CD, and Folate receptor α (FRα) are the most common. A first-generation NKG2D receptor-directed CAR induced tumour cell lysis in an ovarian cancer cell line [97]. A second-generation HER2/neu-directed CAR-T cells repressed flank-implanted ovarian cancer cells in a xenogeneic model [98]. In the OVCAR-3 tumour model, a second-generation CAR directed against LeY + tumours induced potent cytotoxicity and enhanced the production of IFN-γ [99]. A phase I clinical study was conducted based on the preclinical efficacy of folate receptor–directed CAR T cells. Two important TAAs have been identified in prostate cancers; the prostate stem-cell antigen (PSMA). A PSMA-directed third-generation CAR showed robust proliferation and cytotoxicity in vitro, and in a tumour-bearing SCID/beige mouse model [100]. It was also able to inhibit subcutaneous tumour growth in mice [101]. A 40% response rate was recorded for a phase I clinical study using PSMA-directed CAR, IL-2 administration, and myeloablative preconditioning [102]. PSMA-directed CAR-T cells showed systemic persistence for up to 2 weeks [103]. Prostate cancer has been reported to have a marked resistance to hormonal therapy mainly attributable to the presence of tumours associated macrophages (TAMs) which are recruited into the tumour stroma [104]. The most important chemokines for monocyte recruitment to tumours are the chemokine (C-C) ligand 2 (CCL-2), colony-stimulating factor 1 (CSF1) and VEGF. The inhibition of CCL2 resulted in reduction of macrophage infiltration, vascular and tumour growth [105].

In renal cell carcinoma, carboxy-anhydrase-IX (CA-IX), a metalloprotease that reversibly catalyses the hydration of carbon dioxide [106], has been demonstrated to be a specific TAA. Besides being expressed in renal cell carcinoma, CA-IX is expressed in many normal tissues such as the gastric mucosa, small intestine epithelium, duodenum, and the biliary tree [106]. Moreover, under hypoxic conditions, the expression of CA-IX is induced in many other tissues [107]. At the preclinical level, first-generation CA-IX–directed engineered T cells against renal carcinoma cells induced a marked cytokine production and cytotoxic activity [108]. In a phase I clinical trial, first-generation CA-IX–specific CAR-T cells and exogenous IL-2 administration without non-myeloablative preconditioning were used to treat 3 metastatic renal cell carcinoma patients. Two of the patients developed toxic symptoms that emerged as cholangitis and formation of antibodies against the murine-derived scFv [109].

In sarcoma, several TAAs have been identified such as HER2, NKG2D, and IL-11 receptor α chain (IL-11Rα). Second-generation HER2-directed CAR-T cells have been demonstrated to be effective for treatment of both localized and metastatic osteosarcoma in SCID mice [110]. In a similar fashion, second-generation NKG2D ligand-directed CAR-T cells induced a marked cytotoxicity in in-vitro models of Ewing sarcoma [111]. IL-11Rα-directed CAR-T cells have been reportedly used in cases of osteosarcoma [112], prostate cancer [113], and breast cancer [114]. In a nude mouse model of osteosarcoma, IL-11Rα–specific CAR-T cells were effective against both primary tumours and pulmonary metastasis [115]. In a phase I/II clinical study, second-generation HER2-specific CAR-T cells were used to treat 19 patients with HER2-positive sarcoma. Four patients had stable disease for 12–14 months, and three patients that underwent metastatectomy after CAR-T cell therapy remained in remission for up to 16 months [116].

6. NK cells anti-tumour activity pave the way toward Fcγ-CR T cell immunotherapy

Previous studies demonstrated promising anticancer activities for NK cells [117,118]. The active anticancer role of NK cells is mediated by several active cell surface receptors, i.e. CD16, the FcγRIIIA that enables the NK cells to identify and destroy tumour cells through ADCC (Fig. 2C) [117]. Yeap and colleagues reported that human CD16-expressing monocytes have ADCC capacity and can kill cancer cell lines in the presence of specific antibodies [117,119]. Unfortunately, the NK cell immune surveillance might be evaded by several mechanisms including the inhibitory milieu of solid tumours, NK cells elimination, downregulation of NK cells activating receptors, and blocking their infiltration ability (Fig. 2C) [120–122]. Clinical studies showed that NK cells infiltration of the solid tumour microenvironment is not associated with survival [122]. Sconocchia et al. showed that the NK cells infiltration in the colorectal carcinoma (CRC) microenvironment was insignificant, and the high levels of CD16+ cell infiltration in CRC are preferentially associated with CD3+ and CD8+ T cell infiltration. The same authors added that CD16+ cell infiltration represents a favourable prognostic factor in CRC. Moreover, the inherent nature of NK cells makes them difficult to expand in vitro, and NK cell tissue infiltration is often poor in various types of human cancer [123]. In comparison to NK cells, T cells easily expand in vitro, easily infiltrate the tumour microenvironment, and this infiltration is usually associated with a favourable prognosis [124,125]. Based on the aforementioned knowledges, Fcγ-CR T cell immunotherapy was designed to transfer the active ADCC function of NK cell to T cells by engineering T cells to express the Fcγ-CR (CD16) against immunoglobulin-G opsonized tumour cell lines (Fig. 1B) [118,126]. Such strategy would allow the induction of ADCC against tumour cells following a combined administration of Fcγ-CR T cells and a specific TAA-directed mAb.

Tumours expressing MHC class I-related chain molecule A/B (MICA/B) are potentially excellent targets for NK cell [122,123]. Despite the observation that more than 90% of renal cell carcinomas (RCC), hepatocellular carcinomas (HCC), CRC, and melanoma cells express high amounts of MICA/B, we and others demonstrated the absence of CD56+ NK cells infiltration in 71.4%, 92% and 92% of melanoma, HCC, and RCC respectively [127,128]. Moreover, low expression of CD56 and NKp46 were detected in CRC infiltrating cells [122]. These observations might suggest that the poor tumour infiltration ability of NK cells could be attributed to other factors than merely the downregulation of CD56 expression. The mechanism(s) by which cancer cells inhibit the cytolytic activity of allogeneic NK cells are not exactly known. Several factors might contribute to the functional impairments of NK cells activities within the solid tumour environments. Pietra et al. demonstrated that the melanoma cells inhibited the cytolytic activity of NK cells and downregulated the expression of NK receptors, such as NKp30, NKp44, and NKG2D, involved in recognition of leukaemia cells and solid tumour [129]. Other studies clarified that in advanced breast cancers soluble factors secreted by tumour cells, including TGF-β, might contribute to NK cells functional impairment [130,131]. These data might question the role of NK cells in the control of solid tumour progression in humans.

The promising anticancer role of a series of mAbs has been described [132,133]. Elucidation of the mechanistic insights underlining favourable anticancer response of mAbs is pivotal for their clinical applications. The effects of mAbs on cancer cells might involve an active mAb-mediated ADCC. These effects are expected to be efficacious in the presence of high numbers of tumours infiltrating FcγR+ cells [134].

7. Humoral immune response, Fcγ-CR T cells and TAA-specific monoclonal antibodies

The role of humoral immune response against cancer cells has been studied for more than fifty years [135]. Indications for interdependence and interaction between both cellular and humoral anticancer immune response has been explored [136]. Antibodies against specific TAA might be effective to capture cancer cells, and with the help of effector cells (e.g., NK cells, macrophage, DCs, other myeloid cells) they may be able to induce an effective ADCC against cancer cells [136]. The effector cells interact with tumour-specific antibodies through Fcγ receptors (FcγRs) expressed on the surface of NK and other effector cells. Interaction of FcγRs on the surface of NK cells with the Fc region of tumour-bound antibodies promotes tumour cell cytotoxicity but inhibitory Fc receptors may modulate in vivo cytotoxicity against tumour [137], and based on this, several therapeutic antibodies have been developed to target various tumour types [138]. Polymorphic variants of the FcγRs can alter the binding affinity of Fc portion of tumour specific antibodies, and the FcγRs on the surface of effectors cells. It's one of the main factors that led to multiple conflicting outcomes and varying clinical success for the use of mAbs to target several tumour types [139,140]. One possible strategy to enhance the binding affinity of Fcγ receptors to the Fc moiety of tumour specific mAb is to re-engineer/manipulate the Fc glycosylation state. This manipulation strategy would enhance ADCC antitumor activity [141].

Despite the promising early outcome of CAR-T cell immunotherapy, some obstacles still exist that may hinder its clinical application such as the off-target toxicity and cancer immune evasion. To overcome these limitations, an improved version of CAR-T cells has been developed. In this improved version, T cells are engineered to express the Fc gamma RI (CD64) or RIIa (CD32) or RIIIa (CD16) instead of the ScFv specific to TAA. CD64 is the only high-affinity receptor able to bind monomeric IgG molecules, in contrast CD32 and CD16 are low-affinity receptors, for which polymorphic variants 131R/H and 158F/V have been reported respectively [142,143]. CD16 and CD32 polymorphisms influence their binding to IgG Fc fragments [144]. The first generation of CD16-chimeric receptors (CR) developed by Clémenceau and colleagues, it is composed of a fusion protein of the extracellular domain of CD16 ligated to the transmembrane (TM) and the intracellular domain of FcεRIγ (referred to as CD16/γ) [145]. Ochi et al. reported a successful inhibition of CD20+ lymphoma cells by CD16V-CD3ζ-CR (cCD16z) construct in combination with rituximab in mouse xenograft tumours model [146]. The second generation of CD16-CR was described by Kudo et al. who generated the CD16V-BB-ζ-CR by introducing the TM portion of CD8α and the co-stimulatory endodomain of the 4-1BB fused to CD3ζ chain signalling domain [147]. Kudo et al. compared the CD16V-BB-ζ T cells function with T cells transduced with a typical CD19-CAR (CD19-BB-ζ) and found that the former was more effective in eliminating target cells than CAR anti-CD19-BB-ζ. Fcγ-CR T cells given in combination with specific mAbs, utilize ADCC to target and eliminate cancer cells (Fig. 2B). Interaction of the antibody with the Fcγ-CR on T cells triggers the occurrence of perforin/granzyme-dependent tumour target cell lysis. In the presence of the appropriate TAA-specific mAbs, the Fcγ-CR T cells can be utilized to target multiple cancer types (Fig. 2B). Withdrawal of the mAb can control the off-target effect of engineered T cells which is crucial to control potential cytokines release syndrome (CRS) [143].

8. Immunosuppressive network within solid tumour microenvironment

The use of CAR-T cell base-immunotherapy against different solid tumours is still challenging. Solid tumour microenvironments are equipped with a complex inhibitory network that might compromise the action of CAR-T cells. Understanding the different key factors involved in induction of such immunosuppressive tumour microenvironment is vital for development of an efficacious CAR T-cell based-therapy. Modulation of inhibitory tumour microenvironment to enhance antitumor immune response, and to increase access of immune cells able to infiltrate the tumour is still in need of further work at both

the preclinical and clinical trial levels. Such highly immunosuppressive microenvironment in solid tumours is formed of a complex interaction between different immune cells and cytokines.

One of the main modulators of T cells in the tumour environment is extracellular adenosine. Elevation of adenosine under tumour hypoxic conditions is inhibitory to T cells [148]. The effects of adenosine are mediated by adenosine receptors of which the A2a is expressed in T and B lymphocytes [149]. Ablation of A2a receptors [150] and blocking of the adenosine forming enzymes ectonucleotidase CD73 and CD39 on $CD4^+$ Treg cells, enhanced eradication of a lymphoma and improved efficacy of an anti-lymphoma tumour vaccine [150].

The role of ion channels in regulation of T cells function, including T cell motility, and cytokine and granzyme production has been described [151]. The Kv1.3 and KCa3.1 channels co-localize with the TCR in human T cells [152,153], and Ca^{2+} influx is important for Ca^{2+}-mediated inhibition of tumour growth.

The expression of fibroblast activation protein-a (FAP-a) on the surface of tumour-associated stromal cells contributes to the tumour immunosuppressive microenvironment [154]. Tumour cell growth is inhibited by targeting FAP expressed in stromal cells [155]. Similarly, the elimination of FAP expressed in a murine model enhanced the survivability and tumour cell activity of $CD8^+$ T cells [156]. Thus, modulating the multiple immunosuppressive hurdles in the tumour microenvironment would enhance tumour eradication and potentiate the use of CAR-T cells modalities as a promising therapeutic approach.

The natural killer (NK) cells are crucial for regulation of solid tumour microenvironments. When NK cells meet their specific antigen they release a plethora of interleukins and chemokines (IFNγ, TNFα, MIP-1a, MIP-1b, and RANTES) which are central in regulation of DCs, T cells, and B cells [157,158]. IL-12 produced by dendritic cells triggers expansion and activation of $CD8^+$ T cells [159–161]. IFNγ secreted by NK and other immune effector cells such as tumours associated macrophages (TAM) (which are known to release VEGF and TGFβ) have been demonstrated to inhibit the activities of the $CD8^+$ T cells [162]. Cytokines and chemokines released by the activated cytotoxic T cells are known to enhance the cytotoxic function of the NK cells functions located within the tumour microenvironment [163].

The role of regulatory $CD4^+$ T cells (Tregs) in solid tumour microenvironments is remarkable. Tregs have been reported to exert an inhibitory or immunosuppressive effects on effector immune cells targeting solid tumours by interfering with the function of antigen presenting cells (APCs) [164,165], and the cytotoxic $CD8^+$ T cells. Interaction of Tregs with APCs inhibits the expression of CD80 and CD86 on the APC cell surface leading to impairment of cytotoxic T cells function [166]. Of note, the inhibitory effects of Tregs on $CD8^+$ T cells are ameliorated/removed by blocking of CTLA-4 [167,168]. In melanoma, the $CD8^+$ T cells might induce immunosuppression of effector immune cells by different mechanisms, including overexpression of PD-L1; and increase the level of indoleamine-pyrrole 2, 3- dioxygenase (IDO), which has tolerogenic function [168]. Increased expression level of PD-1 is associated with activation of Tregs, which triggers an immunosuppressive influence on the cytotoxic $CD8^+$ T cells. This impairs their ability to release cytokines and granzyme [162,166]. The inhibitory effect of Tregs on $CD8^+$ T cells was also attributed to their ability to release IL-10 and TGFβ which are known to inhibit $CD8^+$ T cells [169]. TGFβ has been reported as essential to the maturation of naïve T cells into mature Tregs mainly due to induction of Foxp3, the transcription factor crucial for Treg maturation [170].

In an attempt to alleviate the inhibitory role of Tregs upon $CD8^+$ T cells, it has been demonstrated that transient depletion of Tregs in a mouse model was associated with inhibition of metastatic activities, and increased the tumour's sensitivity to radiotherapy [171]. Interestingly, manipulation of Treg signalling as in case of inhibition of PI(3)K isoform p110σ, led to activation of the $CD8^+$ T cells function, and regression of several types of cancer [172].

In contrast to the inhibitory influences induced by Tregs, successful recruitment of CD103+ (mouse)/CD141+ (humans) dendritic cells (DC) can activate $CD8^+$ T cells. Under the inhibitory condition of a solid tumour microenvironment, the recruitment of these cells appears to be impaired [173]. Activation and expansion of the $CD141^+$ cell subpopulation by overexpression of IL-12 [174] enhanced the anti-tumor therapeutic potential of the $CD8^+$ T cells. Such an approach, might offer an effective therapeutic modality against a refractory solid tumour.

9. Challenges, adverse effects and patient safety of CAR-T cancer immunotherapy

Despite the promising results of CAR-T cell based-immunotherapy for B cell malignancies, and the ongoing clinical trial for several other types of solid tumours, several challenges are still in need to be solved before moving toward safe and efficient clinical applications.

It is possible that interaction of CAR with its specific TAA, might induce the release of a huge number of cytokines leading to "cytokines release syndrome" which may be fatal. Of note also, the use of PD-1 or CTLA-4 inhibitors might trigger the risk of autoimmune disease following treatment [175,176].

Patient safety concerns over CAR-T cells immunotherapy are of utmost importance. A phase II clinical trial on the use of CD19 CAR-T cells infusion to treat ALL, has been temporarily halted by FDA because 3 patients less than 25 years old died due to development of cerebral edema. After intense investigation, the death was attributed mainly to the preconditioning procedures in which they received fludarabine plus cyclophosphamide. Later, the trial was continued after modulation of the preconditioning protocol with removal of fludarabine.

The cost of CAR-T cell based-immunotherapy still represents one of the biggest challenges. The major cost comes from the personalized nature of CAR-T cells wherein the process includes multiple successive steps such as collection of autologous (leukopheresis) T cells, ex-vivo proliferation, genetic modifications with a retrovirus or lentivirus encoding the CAR construct, and patient infusion. To alleviate the high costs of such multiple steps, "Universal" engineered T cells (UCART) have been generated [156]. The UCART were used to treat an 11-month girl with relapsed CD19+ B-ALL, and led to complete molecular and clinical remission [177].

The tumor mutational burden is the one of the main underpinnings mechanism of cancer formation. Gene mutations might activate proto-oncogenes [178,179] leading to disruption of global genomic stability. The efficacy of immunotherapy might be enhanced by the high mutational load, which is a characteristic of certain types of tumours [180–182]. The use of retrovirus or lentivirus viral vectors to generate CAR- T cells is another potential risk that might occur due to random integration within the CAR-T cells genome. Using the engineered self-inactivating lentiviral vectors (which appear to have more restricted integration sites) has a minimal risk for disruptive insertional mutations [183].

The ex vivo expansion of T cells might impose an additional risk factor. Culturing cells at ambient oxygen versus culture at 3% oxygen, significantly increases mutation rate [184,185]. Other concerns exist with respect to the overcoming the problem of immune checkpoints. Disruption of immune checkpoint proteins such CTLA-4, PD-1, or PD-1L might interfere with the mechanism of detection of "self" and "non-self" antigen, and this imposes a great risk for development of autoimmunity against "self" antigens [186]. The most dangerous and life-threatening side effect of CAR-T cell immunotherapy is the potential development of cytokines release syndrome (CRS) or a "cytokines storm" which may be fatal (186–188). Several strategies have been suggested/executed to overcome the CRS such as the use of corticosteroids, including prednisone [187]; or elevation of IL-6 levels through the use of tocilizumab, a mAb directed to the IL-6 receptor [188]. Finally, care it should be taken in the selection of TAAs to avoid on-target (correct antigen target) off-target/off tumour (incorrect cell type target)

toxicities, which are life-threatening.

Despite the value and promising outcomes of CAR T immunotherapy against hematological and solid tumours, utmost care must be taken in consideration to avoid potential fatal CRS. In this regards, implementation of rigorous RECIST (Response Evaluation Criteria In Solid Tumors) is crucial to guard against such potential deleterious effects of CAR T immunotherapy. RECIST is a set of published rules that define when cancer patients improve ("respond"), stay the same ("stable") or worsen ("progression") during treatments. Assessment of the change in tumour burden is an important feature of the clinical evaluation of cancer therapeutics. Since RECIST was published in 2000, many investigators, cooperative groups, industry and government authorities have adopted these criteria in the assessment of treatment outcomes. However, a number of questions and issues have arisen which have led to the development of a revised RECIST guideline (version 1.1) [189].

Currently, the chemo- and radiotherapeutics modalities constitutes the standard treatment against hematological and solid cancers in the hands of pharmacologists. However, such protocols might fail to provide effective regression for aggressive malignant tumours. This necessitates the introduction of new therapeutic modalities such as CAR T and Fcγ-CR T cell immunotherapy to be added to existing therapeutic modalities available for pharmacologists.

10. Summary

The above review summarize the potential of CAR T and Fcγ-CR T immunotherapy for hematological and solid tumours. Although this is a crucial and vital goal against fatal cancers, there are still several hurdles that need to be extensively analyses before such goal be realized. Targeting a single TAA in hematological malignancies might be much simpler than targeting the complex inhibitory environment of solid tumours. Identification of new TAA would help to design precise and multitargeted cellular therapeutic strategies that is more specific and safe. The interaction between the multiple TCR with the TAA and complex antigenic arrays existing within the inhibitory solid tumor microenvironment might complicate the design of an effective CAR T immunotherapeutic modality. Although a great advance has been made in dissecting the different components of mutanome that seems to be specific at the individual/patient level, much work is still needed to achieve a complete and long lasting remission of different cancer using this novel cellular modality. The combination of mAb and the Fcγ-CR T cells is an important step in alleviating many of the risk associated with the use of CAR T modality alone. Such design would provide control over the fatal CRS which represents the main risk for the wide-spread application of CAR T immunotherapy. The development of universal CAR T cells would also provide a great advance to overcome the inappropriate quality of patients PBMC, and would also help to overcome the high cost issue of such novel immunotherapeutic modalities that still not affordable by the health insurance system of USA and EU countries.

11. Conclusion

The CAR-T cell and its upgraded version Fcγ-CR T immunotherapy exploit the ability of CAR T active domains and the recognition ability of specific mAb to recognize and destroy different types of tumor cells. Previous preclinical and clinical studies have revealed the ability of CAR-T cell immunotherapeutic modalities to inhibit growth and proliferation of hematological and solid tumours. While the presence of a single tumor specific antigen as in the case of hematological tumors might induce an effective antitumor response, the complex inhibitory environments for the solid tumors and the presence of multiple tumor associated antigens are still representing major challenges against the clinical applications of such new immunotherapeutic therapeutic modalities.

Declarations

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