REVIEW

Chimeric Antigen Receptor (CAR) T-Cell Therapy for Patients with Lung Cancer: Current Perspectives

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Abstract: Immunotherapy using chimeric antigen receptor (CAR)-engineered T-cells has achieved unprecedented efficacy in selected hematological cancers. However, solid tumors such as lung cancer impose several additional challenges to the attainment of clinical success using this emerging therapeutic modality. Lung cancer is the biggest cause of cancer-related mortality worldwide, accounting for approximately 1.8 million deaths worldwide each year. Obstacles to the development of CAR T-cell immunotherapy for lung cancer include the selection of safe tumor-selective targets, accounting for the large number of candidates that have been evaluated thus far. Tumor heterogeneity is also a key hurdle, meaning that single target-based approaches are susceptible to therapeutic failure through the emergence of antigen null cancers. There is also a need to enable CAR T-cells to traffic efficiently to sites of disease, to infiltrate tumor deposits and to operate within the hostile tumor microenvironment formed by solid tumors, resisting the onset of exhaustion. Multiple immune, metabolic, physical and chemical barriers operate at the core of malignant lesions, with potential for further heterogeneity and evolution in the face of selective therapeutic pressures. Although the extraordinarily adaptable nature of lung cancers has recently been unmasked, immunotherapy using immune checkpoint blockade can achieve long-term disease control in a small number of patients, establishing clinical proof of concept that immunotherapies can control advanced lung carcinomas. This review summarizes pre-clinical CAR T-cell research that is specifically focused on lung cancer in addition to published and ongoing clinical trial activity. A number of advanced engineering strategies are also described which are designed to bridge the gap to the attainment of meaningful efficacy using genetically engineered T-cells.

Keywords: immunotherapy, malignancy, engineered T-cell, pulmonary

Introduction

Lung cancer is the biggest cause of cancer deaths worldwide, accounting for over 18% of mortality attributable to malignant disease.[1] It is estimated that almost 237,000 people were diagnosed with lung cancer in the United States (US) in 2022.[2] Histological subtypes include (i) non-small cell lung cancer (NSCLC – approximately 85% of tumors), which includes adenocarcinoma, squamous cell, large cell and adenosquamous variants, and (ii) small cell lung cancer (SCLC – approximately 15% of tumors). Most patients present with advanced disease, significantly limiting the use of therapeutic options with curative intent. The main modalities used to treat these cancers include surgery, radiotherapy, chemotherapy, targeted therapies using small molecules, tumor-targeted monoclonal antibodies, and immune checkpoint inhibition. However, 130,000 deaths resulted from lung cancer in 2022, making this the leading cause of cancer death in the US.[2] These data highlight a pressing unmet need for new treatment options for these patients.

Chimeric antigen receptors (CARs) are synthetic fusion molecules which direct the specificity of immune cells against native cell surface target molecules found on tumors and other cell types.[3] In general, antigen engagement by CARs is direct rather than human leukocyte antigen (HLA)-restricted, akin to the recognition process used by an antibody. As a result, CAR T-cell immunotherapy obviates the need for HLA matching of the receptor to the target. Moreover, HLA downregulation by tumor cells (a common immune evasion mechanism in lung cancer)[4–6] affords no

protection to CAR T-cell recognition. CAR technology has been in development since 1987 when T-cell receptor/antibody chimeric receptors were first described.[7] More than 20 years later, several large clinical centers independently described compelling clinical efficacy of CD19-targeted CAR T-cells in the treatment of patients with relapsed refractory B-cell malignancy.[8] More recently, CAR T-cells specific for B-cell maturation antigen (BCMA) have proven to be highly effective in relapsed refractory multiple myeloma.[9] However, solid tumors such as lung cancer remain largely refractory to this approach. Hurdles to the successful transition of this technology for common solid tumors have recently been reviewed, together with potential approaches that may be taken to mitigate these issues.[10] This review sets out to consider pre-clinical efforts to develop effective CAR T-cell solutions specifically for lung cancer, in addition to ongoing and published clinical trial activity in this arena. Only primary lung cancer has been included in this review, meaning that metastatic lung cancer and malignant pleural mesothelioma are not discussed. Other T-cell-based immunotherapies for lung cancer have been considered in recent reviews and are not further covered here.[11]

Structurally, CARs consist of a targeting moiety, spacer domain, transmembrane region and one or more signaling units. Most commonly the targeting moiety consists of a single-chain antibody fragment (scFv) in which variable heavy and variable light chain domains are joined using a short linker. Generations of CARs that have been evaluated thus far are summarized in Figure 1. Successful application of CAR T-cell immunotherapy for blood cancers relies upon the use of so-called second generation (2G) designs in which the signaling domain consists of a co-stimulatory unit (generally either CD28 or 4–1BB), placed upstream of an activating unit (most commonly CD3 zeta). However, a range of CAR generations have been evaluated in lung cancer models as well as in the clinic.

Evaluation of CAR T-Cell Immunotherapy in Models of Lung Cancer

Several pre-clinical investigations of CAR T-cell immunotherapy have been undertaken in models of lung cancer. These involve a diverse range of target molecules and are summarized in the section that follows.

Receptor Tyrosine Kinases
Epidermal Growth Factor Receptor

Many early studies focused on CAR T-cell targeting of receptor tyrosine kinases. The epidermal grown factor receptor (EGFR) is highly expressed in about 60% of NSCLC tumors.[12] Zhou et al found that 2G (CD28) CAR T-cells directed against EGFR could delay disease progression in an A549 NSCLC tumor xenograft model.[13] No toxicity was observed, although ability of the targeting moiety to engage murine EGFR was unclear.
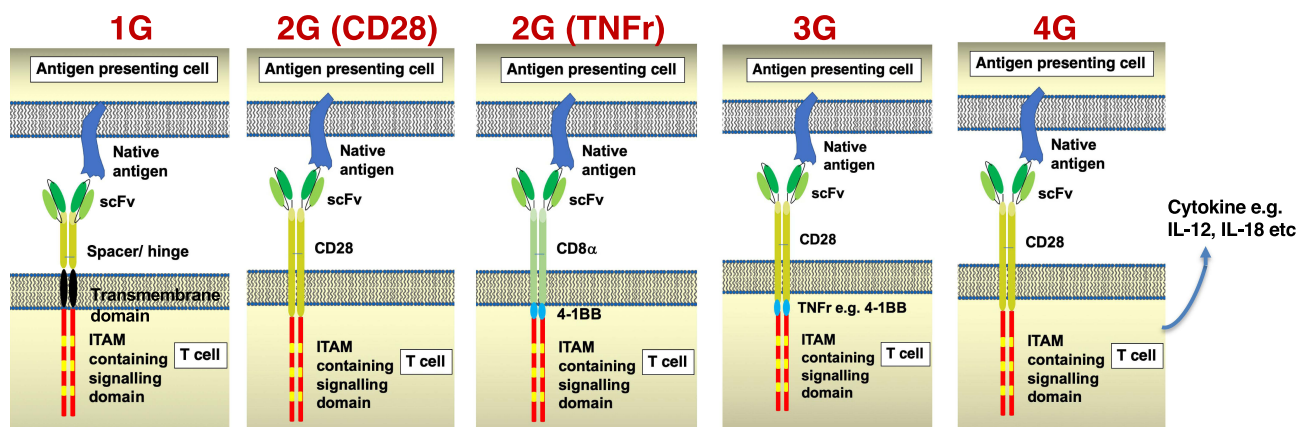

Figure 1 Generations of chimeric antigen receptors. Chimeric antigen receptors consist of a targeting moiety (most commonly an scFv as illustrated here), a spacer/hinge, transmembrane domain and a signaling endodomain. Antigen recognition is direct provided that a target cell (here designated as antigen-presenting cell) expresses the target antigen on the cell surface. In first generation (1G) CARs, the endodomain contains an ITAM (immunoreceptor tyrosine-based activation motif)-containing activating module. Most commonly CD3 zeta is used for this purpose since each monomer contains 3 ITAMs. Second generation (2G) CARs contain a co-stimulatory domain placed upstream of CD3 zeta and most commonly derived from CD28 or 4–1BB. Third generation (3G) CARs contain two complementary co-stimulatory units while armored CARs produce a cytokine such as IL-12 or IL-18.

A key difficulty with targeting of EGFR (like many solid tumor targets) is the fact that it is expressed at low levels in many normal tissues, most notably in the basal layers of skin epidermis.[14] One approach that has been used to improve therapeutic index involves the co-expression of an appropriate chemokine receptor alongside the CAR. Using this approach, tumor cell homing of CAR T-cells is enhanced while on-target off-tumor toxicity is minimised due to reduced transit time in normal tissues.[15] NSCLC tumors produce high levels of CXCL13 which has been exploited through the co-expression of CXCR5 alongside an EGFR-specific 2G (4–1BB) CAR.[16] As a result, enhanced CAR T-cell migration to and destruction of A549 tumors was demonstrable, albeit only when tumor cells were engineered to over-produce CXCL13. In a related approach, 2G (4–1BB) EGFR CAR T-cells have been engineered to co-express CCR6, in combination with a PD1 blocking antibody.[17] Although significant anti-tumor activity was observed, the A549 tumor model used in this study was once again engineered to overproduce the relevant chemokine ligand (in this case, CCL20).

While most CARs are targeted using an scFv, an adnectin (derived from the 10th type III domain in fibronectin) with specificity for EGFR has also been used to direct the specificity of a 2G (4–1BB) containing CAR.[18] When compared to a matched cetuximab-derived scFv-based CAR, comparable anti-tumor activity was observed in a H292 lung cancer xenograft model.

Lentiviral and retroviral vectors are most commonly employed to generate CAR T-cells. In an alternative strategy, Li et al have used the *piggyBac* transposon system to engineer EGFR-specific 2G (4–1BB) CAR T-cells.[12] Using a H460 NSCLC xenograft model, they found that co-administration of intra-tumoral and intravenous CAR T-cells was effective in mediating disease control without toxicity. Once again, however, reactivity of the scFv with mouse EGFR was not shown.

In up to 10% of NSCLC tumors, a splice variant of EGFR known as EGFRvIII is found.[19] As a result, extracellular amino acids 6–273 are deleted, leading to constitutive receptor activity. A 3G (CD28 + 4–1BB) CAR with specificity for EGFRvIII demonstrated therapeutic efficacy against an established metastatic A549 xenograft in which EGFRvIII expression had been enforced.[20] A difficulty with this target is the fact that expression is often heterogeneous in tumors, meaning that antigen null tumors are likely to emerge in the face of CAR T-cell-mediated selective pressure.[21]

c-Met Receptor

The c-Met receptor tyrosine kinase is expressed in 60–100% of NSCLC and commonly provides a mechanism by which tumors acquire EGFR inhibitor resistance.[22] CARs have been engineered to recognize MET using derivatives of its natural ligand, hepatocyte growth factor[23] or using an scFv.[22] A 2G (4–1BB) CAR targeted against c-Met demonstrated anti-tumor activity against A549 NSCLC tumor xenografts without toxicity, although ability of this scFv-based CAR to engage the mouse ortholog of this target was unclear.[22] Given the widespread expression of c-Met in normal tissues,[24] clinical evaluation of CAR T-cells directed against this target was first performed using mRNA transfected T-cells, injected intratumorally in six patients with metastatic breast cancer.[25] However, safety of systemically delivered CAR T-cells with specificity for c-Met remains to be determined.

Receptor Tyrosine Kinase-Like Orphan Receptor (ROR) 1

Receptor tyrosine kinase-like orphan receptor family member (ROR)1 is an onco-fetal receptor tyrosine kinase which is expressed in 93% of SCLC,[26] up to 90% of lung adenocarcinomas, 12% of lung squamous cell carcinomas as well as a subset of rarer lung cancer subtypes.[27,28] By contrast, normal tissue expression of ROR1 is minimal with low levels found in regenerating B-cells, parathyroid gland, pancreatic islets, gastrointestinal tract and adipose tissue.[29] Moreover, 2G (4–1BB) ROR1-specific CAR T-cells have proven to be safe in primate studies.[30] In vitro testing of the same CAR using 3D culture systems has revealed that they can control 3D basement membrane-supported cultures of A549 NSCLC cells,[31] prompting clinical studies which are described later in this review.

Other Receptor Tyrosine Kinases

AXL is expressed in 69% of NSCLC tumors although low-level expression in a number of normal tissues has been highlighted.[32] 3G (CD28 + 4–1BB) AXL-specific CAR T-cells elicit anti-tumor activity against A549 NSCLC tumor xenografts.[33]

Vascular endothelial growth factor receptor (VEGFR)2 is expressed in tumor endothelial cells that support lung and other tumor types. VEGFR2 has been successfully targeted using a 2G (CD28) CAR in mice bearing A549 NSCLC xenografts although VEGFR2 expression was enforced in this model.[34]

Over 90% of NSCLC tumors are reported to express erythropoietin-producing human hepatocellular carcinoma type A receptor 2 (EphA2) with negligible expression in normal lung.[35] A 2G 4–1BB containing CAR with specificity for EphA2 demonstrated anti-tumor activity in an A549 lung cancer xenograft model.[35]

Carcinoembryonic Antigen (CEA)

Carcinoembryonic antigen (CEA) is expressed in about 70% of NSCLC tumors.[36] When 2G (CD28) CEA-specific CAR T-cells were administered to mice with an established A549 NSCLC tumor, transient disease control was noted.[37] 3G (CD28 + 4–1BB) CEA-specific CAR T-cells have also demonstrated anti-tumor activity in NSCLC models that were resistant to antibody-drug conjugates directed against the same target.[38] Regarding healthy tissue biodistribution, CEA is found in the gastrointestinal and respiratory tract. However, in contrast to tumor cell expression, CEA is polarized at the luminal surface in normal epithelia meaning that access to CAR T-cells should theoretically be restricted at these sites. Nonetheless, Phase 1 clinical testing revealed pulmonary toxicity in subjects who received a 1G CAR with specificity for CEA, highlighting the risks associated with this and related targets that exhibit polarized epithelial cell expression.[39]

Glypican 3

Glypican 3 is an oncofetal heparan sulfate glycoprotein that is found in about 63% of squamous cell lung carcinomas.[40] By contrast, expression in normal tissue, including lung, is negligible.[40,41] Anti-tumor activity of 3G (CD28 + 4–1BB) glypican-3 CAR T-cells has been demonstrated against two squamous cell lung carcinoma tumor xenografts, although glypican-3 expression was enforced in tumor cells by lentiviral transduction in each case.[40]

Prostate Stem Cell Antigen (PSCA)

PSCA is also expressed in non-small cell lung cancer.[42] Exploiting this, 2G (CD28) CAR T-cells have proven efficacious against patient-derived xenograft (PDX) models of NSCLC.[43] When PDX co-express both PSCA and MUC1 (another potential target of interest in NSCLC), synergistic therapeutic efficacy was reported in a second PDX model.[43]

CD44v6 Splice Variant

The CD44v6 adhesion receptor is commonly expressed in a range of tumor types, including lung cancer. It is estimated that about 90% of squamous cell carcinomas express this splice variant while up to 50% of lung adenocarcinomas are positive.[44] In pre-clinical testing, T-cells that were engineered to co-express a CD44v6-specific 2G (CD28) CAR co-expressed with a suicide gene mediated anti-tumor activity in mice engrafted with MR232 lung adenocarcinoma tumor xenografts.[45] However, low-level expression of CD44v6 in skin has been documented.[46] Potential significance of this finding for CAR T-cell immunotherapy is highlighted by the fact that the CD44v6-specific antibody–drug conjugate, bivatuzumab, caused fatal toxic epidermal necrolysis in one subject.[47]

CD56

The CD56 antigen (neural cell adhesion molecule) is expressed in many cancers that exhibit neuronal or neuroendocrine differentiation, including SCLC.[48] Crossland et al used the *Sleeping Beauty* transposon system to generate 2G (CD28) CAR T-cells with specificity for this target.[48] Therapeutic efficacy against CD56-expressing H526 SCLC xenografts was demonstrated. Although CD56 is expressed on NK cells and neurones, CAR T-cells directed against this target have been safely administered as consolidation therapy to a child with embryonal rhabdomyosarcoma following repeated treatment with surgery, radiotherapy and chemotherapy.[49]

Epithelial Cell Adhesion Molecule (EpCAM)

EpCAM is expressed in about 50% of NSCLC tumors.[50] Similar to CEA, expression by normal cells is polarized to the luminal surface, whereas such polarity is not evident in transformed cells. Once again, however, pulmonary immuno-pathology has been noted in some pre-clinical models when EpCAM has been targeted using CAR T-cells.[51] Using an orthotopic Lewis lung carcinoma tumor model, Xu et al demonstrated that a 2G (CD28) CAR T-cells targeted against EpCAM could delay intracranial disease progression following local but not systemic delivery, although persistence of cells was insufficient to achieve disease eradication.[50] One strategy that may be used to dial down risk of toxicity associated with the targeting of self-antigens such as EpCAM entails the use of low affinity targeting moieties that can discriminate between healthy (EpCAM low) and malignant cells (EpCAM) high.[52] Nonetheless, risk of antigen down-regulation leading to therapeutic failure needs to be considered with this approach.

B7 Family Members

Two members of the B7 family are commonly expressed on lung cancers, namely PD-L1 and B7-H3.

Programmed Death Receptor Ligand 1 (PD-L1)

PD-L1 is widely expressed on a range of solid tumors including lung cancer. 2G (4–1BB) CAR T-cells with specificity for B7-H3 delayed disease progression in an A549 NSCLC xenograft model[53] while 3G (4–1BB + Toll-like receptor 2) PD-L1-specific CAR T-cells demonstrated efficacy against an NSCLC PDX tumor. Surprisingly however, co-treatment with this and a second population of CAR T-cells targeting mesothelin (MSLN) yielded inferior anti-tumor activity to PD-L1-specific CAR T-cells alone.[54] Nonetheless, PD-L1-specific CAR T-cells have been evaluated clinically in NSCLC as summarized later in this review.

B7-H3

B7-H3 (CD276) shares 30% amino acid homology with PD-L1 although its cognate receptor(s) remains unclear. Expression of B7-H3 in normal tissue is negligible while it is found in about three quarters of NSCLC tumors,[53] both on tumor and vascular/stromal elements, and expression is correlated with poor prognosis.[55] Clinical experience with B7-H3 targeted CAR T-cells in the treatment of other solid tumor types is summarized elsewhere.[24] In pre-clinical studies, 2G (4–1BB) CAR T-cells delayed tumor progression in an A549 NSCLC xenograft model.[53] B7-H3 CAR T-cell trafficking has also been directed by the co-expression of the CCR2b chemokine receptor to facilitate traversal of the blood–brain barrier in order to tackle central nervous system metastases.[56]

Delta-Like Ligand (DLL)3

DLL3 is an inhibitory Notch ligand that is predominantly expressed on intracellular membranes. However, due to substantial over-expression in SCLC, moderate cell surface expression is common in this tumor, of the order of 10,000 molecules per cell.[57] 3G (CD28 + 4–1BB) DLL3-specific CAR T-cells have been developed for application against SCLC, in which this target is selectively expressed in about 70% of tumors.[58] Transient toxicity was observed in treated mice, which may have been due to cytokine release syndrome and authors found that a bispecific antibody co-targeting DLL and CD3 was more effective in similar pre-clinical models. More recently, Jaspers et al developed a panel of DLL3-specific CARs which proved efficacious in immunodeficient and immune competent mouse models of SCLC.[59] On a cautionary note, however, the DLL3-specific antibody–drug conjugate rovalpituzumab tesirine (Rova)-T was unsuccessful from an efficacy perspective in Phase 3 testing in patients with SLCL.[60]

CD47

CD47 is commonly expressed on solid tumors including lung cancer and represents a macrophage immune checkpoint. It imparts a "don't eat me" signal via interaction with signal regulatory protein a on macrophages.[61] A 3G (CD28 + 4–1BB) CAR has demonstrated in vitro anti-tumor activity against A549 NSCLC cells, although in vivo testing was not undertaken.[61]

Gangliosides

Disialoganglioside D2

Disialoganglioside D2 (GD2) has been studied quite extensively as a CAR T-cell target in neuroblastoma. A recent clinical trial undertaken in Italy using a 3G (CD28 + 4–1BB) GD2-specific CAR has yielded the most compelling solid tumor CAR T-cell efficacy data yet described worldwide, with a complete response rate of 33% (9/27) and a partial response rate of 30% (8/27).[62] GD2 is also expressed in 39% SCLC, 56% of squamous cell lung carcinomas and 72% of lung adenocarcinomas.[63] Using both orthotopic and metastatic lung cancer models, anti-tumor activity of 2G (CD28) CAR T-cells with specificity for GD2 was demonstrated.[63]

Ganglioside M2

Ganglioside M2 (GM2) is also expressed in a range of solid tumors including lung cancer. Third generation (CD28 + 4–1BB) CAR T-cells directed against GM2 have been armored to co-express IL-7 and CCL19 and demonstrated compelling efficacy in a xenograft models of SCLC, contrasting with lack of efficacy of non-armored CAR T-cells in these models.[64] This was accompanied by significant intra-tumoral infiltration by CAR T-cells arising from autocrine IL-7 and CCL19 stimulation, which promote increased proliferation and migration of the CAR T-cells, respectively.

Mesothelin

Mesothelin (MSLN) is a target of interest in lung cancer, in addition to mesothelioma, pancreatic and ovarian cancers. A 2G (CD28) CAR with specificity for MSLN delayed tumor progression in a Lewis lung carcinoma model in a manner that was further potentiated by delivery of a glutamine antagonist to the tumor microenvironment (TME) using anti-PD-L1 targeted nanovesicles.[65] Alternatively, a novel CAR design known as a TRuC in which a MSLN-specific scFv is fused to CD3 epsilon achieved potent anti-tumor activity in a range of solid tumor models, including an A549-MSLN lung cancer xenograft.[66] In the TRuC design, the scFv CD3 epsilon fusion becomes incorporated into the endogenous TCR CD3 complex, thereby providing T-cell receptor-like signaling to the host cell. When compared to a matched 2G (4–1BB) CAT, the TRuC CAR T-cells achieved more rapid tumor accumulation and disease regression.[66]

Intensity of co-stimulation may also influence anti-tumor activity in lung cancer models. Positioning of Dap10 downstream of CD3 zeta in a 2G (CD28) CAR with specificity for MSLN enhanced cytolytic activity, cytokine secretion, serial killing activity and therapeutic efficacy against A549 NSCLC tumor xenografts and a lung cancer PDX when compared to unmodified 2G CAR T-cells.[67]

Miscellaneous Targets

Several additional candidate CAR T-cell targets have been pursued pre-clinically in lung cancer models. Lung-specific (Lun)X is selectively expressed in approximately 80% of NSCLC but not healthy tissues.[68,69] A 3G (CD28+4-1BB) CAR has been developed to target LunX and demonstrated efficacy in an A549 NSCLC xenograft and a patient-derived xenograft model.[69]

PTK7 is a non-canonical signalling Wnt family pseudokinase that is expressed in a range of solid tumors including NSCLC whereas normal tissue expression is low level in some epithelia.[70] Anti-tumor activity of a 2G (4–1BB) CAR has been demonstrated in xenograft models of both NSCLC and SCLC.[70] By contrast, screening of reactivity of these CAR T-cells with a limited panel of human tissues did not uncover any evidence of on-target off-tumor toxicity.

Olfactory receptor OR2H1 has recently been identified as a common solid tumor cell surface target for which normal tissue expression is restricted to testis.[71] Expression has been identified in 13% of lung carcinomas and a 2G (4–1BB) CAR directed against this target demonstrated anti-tumor activity in a H2009 lung adenocarcinoma tumor xenograft model.[71]

Mutation of EGFR is common in NSCLC and leads to resistance to EGFR tyrosine kinase inhibitors. In this setting, CD70 has been identified as commonly expressed on these tumor cells, most notably in those that have undergone epithelial-to-mesenchymal transition.[72] Moreover, expression of CD70 in NSCLC was associated with poorer survival. CAR T-cells and NK cells directed against CD70 demonstrated anti-tumor activity in a number of NSCLC models.[72]

Although clinical trials of CD70-targeted CAR T-cells in lung cancer were not identified, one patient with renal cell carcinoma has achieved a complete response following the infusion of CD70-targeted CAR T-cells.[73]

A major advantage of CAR technology is the HLA-independent nature of antigen recognition, as alluded to above. Nonetheless, this denies access to intracellular antigens, many of which are more tumor selective. One such example is MAGE-A1, a cancer testis antigen that is expressed in 44% of lung adenocarcinomas.[74] CAR T-cells with anti-tumor activity against this target have been developed, although the precise HLA-restriction of the CAR is unclear.[74]

Multi-Targeted and Combinatorial Approaches

Several advanced CAR T-cell approaches have been described for experimental treatment of lung cancer. The first of these entails the co-administration of CAR T-cells targeted against tumor and tumor-supportive stroma. Karakla et al showed that growth of an A549 lung tumor xenograft was delayed following treatment with CAR T-cells directed against fibroblast activation protein α (FAP), which targets stromal fibroblasts, but not malignant cells.[75] However, the combined administration of FAP- and EphA2-specific CAR T-cells (which target tumor cells) led to a further enhancement of therapeutic activity and survival.

Dual antigen targeted CAR T-cells represent an alternative strategy to enhance potency, mitigate risk of therapeutic failure due to antigen loss and maximize discrimination between malignant and normal cell types. Tandem CARs that co-target B7-H3 and CD70 have been shown to exert superior anti-tumor activity against a large cell lung cancer xenograft (NCI-H460) when compared to single antigen controls, meaning that lower dosing levels were sufficient for efficacy.[76] Chu et al employed universal CAR T-cells with specificity for fluorescein isothiocyanate (FITC) for combined use with an NSCLC-specific FITC-conjugated ligand.[77] This approach offers the potential for combined use with one or more targeting moieties, administered either simultaneously or sequentially. Improved discrimination between malignant and healthy cells may be achieved using logic gating strategies that exploit dual antigen recognition[78] or use of inhibitory CARs that recognise targets on normal tissue.[79,80] Multi-target recognition may also be used to boost potency within the tumor microenvironment with the use of so-called parallel CARs.[81] This solution entails the co-expression of a 2G CAR (eg containing CD28) with a chimeric co-stimulatory domain that contains a complementary co-stimulatory unit (eg 4–1BB). Using this parallel approach, two or more targets may be simultaneously engaged and optimized dual co-stimulation is delivered since both signaling units are located in their natural juxtamembrane position. To address the distinct co-stimulatory requirements of different T-cell subsets, Guedan et al co-administered MSLN-specific CD4$^+$ CAR T-cells that contained an ICOS + CD3 zeta endodomain with MSLN-specific CD8$^+$ CAR T-cells containing a fused 4–1BB + CD3 zeta endodomain.[82] Sustained persistence and anti-tumor immunity was demonstrated against subcutaneous L55 NSCLC xenografts.

A number of combinatorial strategies involving CAR T-cells and other pharmaceuticals have also been described. For example, oncolytic viruses may be used in combination with CAR T-cells since they not only exert a direct anti-tumor effect but also create a more favorable immune environment within the tumor. In the context of lung cancer, this approach was exemplified using both EGFR and HER2 CAR T-cells in A549 NSCLC xenograft models.[83] Alternatively, it has been shown that that N-linked glycans hinder recognition of solid tumor cells (including lung cancer) by CAR T-cells.[84] This inhibitory effect may be overcome by deletion of mannoside acetyl-glucosaminlytransferase 5 (MGAT5) in tumor cells or treatment with the N-glycan inhibitor, 2-deoxy-D-glucose.

Clinical Experience of CAR T-Cell Immunotherapy of Lung Cancer

There have been limited reports of CAR T-cell clinical trials targeted against lung cancer, although there is extensive ongoing clinical trial activity in this arena (summarized in Table 1). A summary of clinical reports published this far is presented below.

Feng et al described a clinical study in which 11 subjects with EGFR$^+$ NSCLC were treated with 2G (4–1BB)-engineered CAR T-cells.[89] Two subjects achieved a partial response, although the contributory effect of conditioning chemotherapy with cisplatin, cyclophosphamide and either pemetrexed or docetaxel remains uncertain. Five additional patients achieved stable disease in this study. Despite issues with toxicity in other EGFR-targeted clinical trials,[24] therapy was well tolerated with the exception of one grade 3–4 increase in serum lipase. A more recent study in NSCLC was

Table 1 Clinical Trials Evaluating CAR T-Cell Immunotherapy of Lung Cancer

Ref	Year	Institution/ Sponsor	Clinical Trial Register	Target	CAR Gen.	Disease	No. Treated	Conditioning	Cell Product and Dose	Clinical Outcome	Toxicity (>Grade 3)	Survival
[85]	2011	UPenn	NCT01355965	MSLN	2G: 41BB	MSLN -expressing cancer	2	Nil	1 to 10×10^{8} cells x 3 infusions (mRNA transfected)	1PR	1 anaphylaxis	Not known
[86]	2012	NCI	NCT01583686	MSLN	Not known	MSLN -expressing tumors including lung cancer	Not known	Cy/Flu + IL-2	Not known	Terminated due to slow accrual 1/15 SD; 14/15 PD	Not known	Not known
[87]	2015	MSKCC	NCT02414269	MSLN	2G: CD28	Pleural cancers	20 14 in cohort 3 evaluable for efficacy	Nil (3) Cy (3) Cy + anti-PD-1 (14)	3×10^{5}-1×10 [7]/Kg	14% CR 36% PR 29% SD	0% CRS 0% ICANS	Not known
N/A	2015	PersonGen Bio-Therapeutics (Suzhou) Co. Ltd.	NCT02587689	MUC1	Not known	Multiple including NSCLC	Not known	Not known	Not known	Trial status unknown	Not known	Not known
N/A	2015	Southwest Hospital China	NCT02349724	CEA	2G: CD28	Multiple including lung cancer Colorectal cancer data published[88]	Not known	Not known	Not known	Trial status unknown	Not known	Not known
N/A	2015	Chinese PLA General Hospital	NCT02580747	MSLN	Not known	Multiple including lung cancer	Not known	Not known	Not known	Trial status unknown	Not known	Not known
[89]	2016	Chinese PLA General Hospital	NCT01869166	EGFR	2G: 41BB	NSCLC	11	Nil, Cy alone or Cy with additional cytotoxic drugs	up to 2.5×10 [7] CAR T-cells/ kg (median dose 0.97×10^{7}/ kg)	18% PR 45% SD	0% CRS 0% ICANS	Responses lasted 2–8m
N/A	2016	Zhi Yang	NCT02713984	HER2	Not known	Multiple, including lung cancer	Not known	Not known	Not known	Study withdrawn due grade 4 CRS and interstitial pneumonia	Not known	Not known

[90]	2016	Fred Hutchinson Cancer Center	NCT02706392	ROR1	2G: 41BB	Multiple, including NSCLC	4	Cy/Flu	3.3 x 10^{5} to 1 x10^{6}/kg	2SD Study terminated due to slow accrual	Not known	Not known
N/A	2016	Shanghai International Medical Center	NCT02862028	ErbB family	Armored with PD1 antibodies	Multiple including lung cancer	Not known	Not known	1–5 x 10^{7}/kg x 2 cycles	Trial status unknown	Not known	Not known
[88]	2017	Third Military Medical University	NCT02349724	CEA	2G: CD28	Lung cancer and other CEA+ cancers	10	Cy	1 x 10^{5} to 1 x10^{8}/kg	70% SD	0% CRS 0% ICANS	20% SD at 30 week follow up
[91]	2017	Shanghai Cell Therapy Research Institute	NCT03182816	EGFR	2G: 41BB PiggyBac transposon mediated gene transfer	Multiple including lung cancer	9 lung cancer	Cy	1 x 10^{6} to 3 x 10^{6}/kg	1/9 PR 6/9 SD	1/9 grade 3 fever. No other grade 3 toxicities	
N/A	2017	Second Affiliated Hospital of Guangzhou Medical University	NCT03198052	Multiple	Not known	Multiple including lung cancer	Not known	Not known	1×10^6 to 1×10^7/kg x 3 cycles	Not known	Not known	Not known
N/A	2017	Second Affiliated Hospital of Guangzhou Medical University	NCT03198546	Glypican-3	TGF-b targeting CAR which secretes IL-7 and CCL19	Hepatocellular cancer and NSCLC	Not known	Not known	Not known	Not known	Not known	Not known
[92]	2017	Sun Yat-sen University	NCT03330834	PD-L1	2G: 41BB	NSCLC	Not Known	Cy/Flu	1 x10^{6} to 6 x10^{5}/kg (3 doses over 7 days)	Study terminated due to toxicity (see text)	Not known	Not known
N/A	2017	UPenn	NCT03054298	MSLN	2G: 41BB	Multiple including lung adeno-carcinoma IV, IP and intrapleural routes	Not known	Nil or Cy	1–3 x10^{7} to 1–3 x10^{8}/m^{2}	Not known	1 patient death due to respiratory failure (summarized in[24])	Not known
N/A	2017	Yu Fengiei	NCT03060343	PD-L1; CD80 CD86	Not known	NSCLC	Not known	Not known	1 x10^{5} to 1 x10^{7}/kg	Not known	Not known	Not known

(Continued)

Table 1 (Continued).

Ref	Year	Institution/Sponsor	Clinical Trial Register	Target	CAR Gen.	Disease	No. Treated	Conditioning	Cell Product and Dose	Clinical Outcome	Toxicity (>Grade 3)	Survival
[93]	2018	Chinese PLA General Hospital	NCT01935843	HER2	2G: 41BB	Multiple, including NSCLC (no NSCLC patients reported as yet)	11	Cy/ nab-paclitaxel	Median dose 2.1x10^{6}/Kg x 1–2 cycles	9% PR 45% SD	0% CRS 0% ICANS	Med PFS 4.8m
[94]	2018	Amgen	NCT03392064	DLL3	Not known	SCLC	5	1PR 2SD	3.3–10[5]/Kg x 1–2 cycles	Suspended	0% CRS 0% ICANS	Med PFS 3.7m
[95]	2018	The First Affiliated Hospital of Guangdong Pharmaceutical University	NCT03525782	MUC1	PD1 knockout	NSCLC	20	Not known	1–4 cycles of treatment	11 SD	0% CRS 0% ICANS	Not known
N/A	2018	Shenzen BinDeBio Ltd.	NCT03638206	Multiple	Not known	Multiple including lung cancer	Not known	Cy/Flu	Not known	Trial status unknown	Not known	Not known
N/A	2018	Baylor College	NCT03740256	HER2	Combined with intratumoral oncolytic adenovirus	Multiple including lung cancer	Not known	Not known	Not known	Not known	Not known	Not known
[96]	2019	UPenn	NCT02159716	MSLN	2G: 41BB	Multiple including malignant pleural mesothelioma	15	Nil or Cy	1–3x10^{7}or 1–3x10^{8}/m^{2} cells	73% SD	0% CRS 0% ICANS	Med PFS 2.1m
[97]	2019	Tmunity Therapeutics	NCT04025216	MUC1 Tn	2G: 41BB	Multiple including NSCLC	6	Nil or Cy/Flu	1–6 x 10^{7}/kg	3SD	0% CRS 0% ICANS	Not known
[98]	2019	Sun Yat-sen University	NCT04153799	EGFR	Armored with CXCR5	NSCLC	11	Not known	0.5–5x10^{6}/Kg	2 PR 5SD	Not known	Not known
N/A	2019	Shenzhen Geno-Immune Medical Institute	NCT03356808	Multiple	Not known	Lung cancer	Not known	Not known	1–10 x 10^{6}/kg	Not known	Not known	Not known
N/A	2020	Chongqing Precision Biotech Co. Ltd	NCT04348643	CEA	Not known	Lung cancer and other CEA+ cancers	Not known	Not known	1–3 IV doses	Not known	Not known	Not known
N/A	2020	Wuhan Union Hospital	NCT04489862	MSLN	Armored with anti-PD1 nanobodies	NSCLC and mesothelioma	Not known	Cy	1x10^{5}-3x10^{6}/Kg	Not known	Not known	Not known

[99]	2020	MSKCC	NCT04577326	MSLN	2G: CD28 (1XX zeta domain) armored with PD1 dominant negative receptor. Intrapleural delivery	Mesothelioma		Cy	Up to 3x10^{7}/Kg	Not known	1 fatal SAE causing temporary pause to recruitment in 2022	Not known
N/A	2021	Second Affiliated Hospital of Guangzhou Medical University	NCT04952272	Not known	Armored with OX40 scFv. Combined with intra-tumoral drug-eluting beads and CpG-ODN	Multiple including lung cancer	Not known	Not known	Not known	Not known	Not known	Not known
N/A	2021	Second Affiliated Hospital of Guangzhou Medical University	NCT05060796	EGFR	Armored with CXCR5	NSCLC	Not known	Not known	0.5–5x10^{6}/Kg	Not known	Not known	Not known
N/A	2021	Beijing Immunochina Medical Science and Technology Company	NCT05117138	Not known	Not known	Multiple including NSCLC	Not known	Not known	Not known	Not known	Not known	Not known
N/A	2021	PersonGen Bio-Therapeutics (Suzhou) Co. Ltd.	NCT04864821	B7-H3	Not known	Multiple including NSCLC	Not known	Not known	Not known		Not yet recruiting	
N/A	2021	SOTIO LLC	NCT05120271	Glypican-3	2G: 41BB Glutamic oxaloacetic transaminase 2 armoring[41]	Multiple including NSCLC	Not known	Cy/Flu	Three dose levels during dose escalation	Not known	Not known	Not known
N/A	2022	PersonGen Bio-Therapeutics (Suzhou) Co. Ltd.	NCT05190185	B7-H3	Not known	Multiple including NSCLC	Not known	Not known	1x10^{6}-1x10^{8}/Kg	Not known	Not known	Not known
[100]	2022	Poseida Therapeutics	NCT05239143	MUC1-C	Allogeneic CAR T-cells TCRB knock out by Cas-Clover Piggy-Bac gene delivery	Multiple including NSCLC	3	Cy/Flu ± rituximab	0.5–15x10^{6}/Kg	Not known	0% CRS 0% GvHD	Not known
N/A	2022	Second Affiliated Hospital of Guangzhou Medical University	NCT05341492	EGFR/B7-H3	Not known	Lung cancer and triple negative breast cancer	Not known	Not known	2x10^{6}/Kg	Not known	Not known	Not known

(*Continued*)

Table 1 (Continued).

Ref	Year	Institution/ Sponsor	Clinical Trial Register	Target	CAR Gen.	Disease	No. Treated	Conditioning	Cell Product and Dose	Clinical Outcome	Toxicity (>Grade 3)	Survival
N/A	2022	UNC Lineberger Comprehensive Cancer Center	NCT05620342	GD2	Armored with IL-15 and inducible caspase 9	Lung cancer	0	Not known	Not known	Not yet recruiting		
[101]	2022	Lyell Immunopharma, Inc.	NCT05274451	ROR1	Genetically and epigenetically reprogammed CAR T-cells	Multiple including NSCLC	Not known	Cy/Flu	Not known	Not known	Not known	Not known
N/A	2023	Legend Biotech USA	NCT05680922	DLL3	Not known	SCLC and large cell neuroendocrine lung cancer	0	Not known	Not known	Not yet recruiting		
[102]	2023	A2 Biotherapeutics Inc.	NCT05736731	CEA and HLA-A2 loss	Logic gated CAR-T employing LIR-1 based inhibitor CAR	Multiple including NSCLC	0	Not known	Not known	Not yet recruiting		

Abbreviations: 2G, 2nd generation; CEA, carcinoembryonic antigen; CR, complete response; CRS, cytokine release syndrome; Cy, cyclophosphamide; EGFR, epidermal growth factor receptor; FAP, fibroblast activation protein; Flu, fludarabine; ICANs, immune cell-associated neurotoxicity syndrome; IL, interleukin; IP, intraperitoneal; IV, intravenous; MSKCC, Memorial Sloan Kettering Cancer Center; MSLN, mesothelin; N/A, not applicable; NCI, National Cancer Institute; NSCLC, non-small cell lung cancer; PR, partial response; SCLC, small cell lung cancer; SD, stable disease; TGF-b, transforming growth factor b; UPenn, University of Pennsylvania.

undertaken by a group in Shanghai using PiggyBac transposon-engineered EGFR-re-targeted CAR T-cells, administered as two doses. Once again, infusions were generally well tolerated and one durable PR that lasted 13 months was reported in a series of 9 patients.[91]

Given the frequent expression of ROR1 in lung cancer, a clinical trial of 2G (4–1BB) ROR1-targeted CAR T-cells was initiated in subjects with ROR1^{+} NSCLC and TNBC (NCT02706392).[90] Following condition with cyclophosphamide and fludarabine, cells were infused intravenously but demonstrated poor trafficking to the site of disease. Two patients with NSCLC achieved a mixed response, with reduction in tumor burden observed at some metastatic sites of disease.[103] Subsequent pre-clinical mouse studies demonstrated that conditioning with oxaliplatin resulted in macrophage activation and chemokine release, favoring enhanced CAR T-cell recruitment to tumors and providing a rational direction for future clinical testing.[90]

Mesothelin was the target of a Phase 1 clinical trial employing a 2G (CD28) CAR. One patient with lung cancer and malignant pleural disease was treated with intrapleural CAR T-cells without prior lymphodepletion and survived for approximately 12 months.[104] Two mesothelioma patients treated with a similar CAR T-cell approach after cyclophosphamide conditioning and combined with pembrolizumab achieved complete metabolic responses, as detected using Positron emission tomography (PET) using[18] fluoro-deoxyglucose (FDG).

PD-L1 has also been the subject of a CAR T-cell clinical trial in patients with NSCLC (clinicaltrials.gov reference NCT03330834).[92] One patient was conditioned with cyclophosphamide and fludarabine and treated with a 2G (4–1BB) scFv-based CAR (scFv derived from atezolizumab) at doses of 1×10^5/kg on day 1, 3×10^5/kg on day 4 and 6×10^5/kg on day 8. CAR T-cell persistence at 3.3% of peripheral blood T-cells was detected on day 29. He presented on day 47 with pyrexia and adult respiratory distress syndrome, accompanied by elevated CRP and IL-6 (but not other cytokines). Investigators felt that this was atypical for cytokine release syndrome and instead most likely represented on-target off-tumor toxicity. The patient was treated with tocilizumab and methylprednisolone with rapid resolution and the study has been terminated.

Finally, MUC1 is a popular choice for clinical studies and was targeted in 20 NSCLC patients using CAR T-cells in which PD1 had been knocked out. However, stable disease was the best response observed, an outcome reported in eleven subjects.[95] Similarly, stable disease (achieved in 3 of 6 treated subjects) was the best response achieved using CAR T-cells specific for the Tn glycoform of MUC1.[105]

Perspectives and Conclusions

Despite considerable effort, CAR T-cell immunotherapy has achieved little meaningful clinical impact against lung cancer. The main limitation has been lack of efficacy rather than unacceptable safety. Recent studies of the biology of NSCLC from the TRACERx study highlight significant issues of intra-tumoral heterogeneity, varied pathways to metastasis and the remarkable propensity of these tumors to undergo clonal evolution under selective pressure applied by chemotherapy and perhaps immune selection.[106] The profound adaptability of lung cancers highlights the significant challenge in developing broadly applicable therapeutic approaches for this disease, including CAR T-cell solutions. Nonetheless, immune checkpoint blockade can achieve long-term survival in a subset of NSCLC patients, demonstrating the principle that sustained disease control can be achieved by immune effector cells even in advanced disease.[107]

The first key obstacle to consider is the need for efficient CAR T-cell homing towards and infiltration of solid tumors such as lung cancer. Co-expression of appropriate chemokine receptors in CAR T-cells offers one possible solution as described above, although exemplification thus far in lung cancer has involved enforced expression of cognate chemokines in tumor cells. One additional option entails the co-expression of CXCR2 in these cells[15] which binds to chemokines such as IL-8 that are naturally over-expressed in NSCLC and derived cell-line models.[108] Tumor infiltration by CAR T-cells may also be facilitated by the co-expression of matrix degrading enzymes such as heparanase.[109]

Given the heterogeneity of lung tumors described above, it is probable that single target antigens will not be uniformly expressed by all malignant cells. This highlights the need to consider combinatorial targeting strategies, as described above. Alternatively, CARs may be engineered to engage multiple targets using a single targeting moiety. One example involves the use of NKG2D-based CARs[110] which bind to eight discrete ligands that are highly expressed in NSCLC.[111] Alternatively, the panErbB-specific T1E28z CAR employs a promiscuous ligand, T1E, that can bind to eight

discrete ErbB homo- or heterodimers.[112] Although ErbB dimers are highly expressed in lung cancer,[113] this CAR may be unsuited to systemic administration owing to risks of on-target off-tumor toxicity due to low-level ErbB expression in normal tissues. To mitigate risk, a hypoxia-sensing derivative has been developed whereby CAR expression is restricted to the hypoxic TME.[114] These "hypoxiCAR" T-cells elicit therapeutic activity against ErbB-expressing solid tumors without toxicity in mice, despite the ability of T1E to bind mouse ErbB orthologues with high efficiency.

The tumor microenvironment (TME) is the battleground within which CAR T-cells must operate. The physical, chemical and biological hurdles which operate at that site together with generic strategies that may help to overcome these obstacles have recently been reviewed elsewhere.[115–117] Additionally, a number of specific approaches have been tested to modulate the TME in lung cancer in favor of CAR T-cell activation. For example, disruption of the NSCLC tumor microenvironment using a nanozyme potentiates anti-tumor activity of B7-H3-specific CAR T-cells.[118] Moreover, microwave ablation has been used to remodel the TME in order to facilitate efficacy of AXL targeted CAR T-cells against NSCLC.[33]

To potentiate impact, armoring strategies have also been employed to modulate inhibitory influences operative within the tumor microenvironment. High interstitial pressure within lung tumors can lead to the upregulation of the PD-L1 immune checkpoint, thereby mediating resistance to CAR T-cell immunotherapy.[119] Li et al investigated whether armoring of CAR T-cells to secrete PD1-specific scFvs could enhance anti-tumor activity.[120] They used a H292 lung cancer xenograft model in which expression of CD19 was enforced and observed that CD19-specific CAR T-cells that constitutively produced anti-PD1 scFvs outperformed CAR T-cells alone, or when combined with systemic PD1 blockade. Unfortunately, however, this principle was not exemplified using an endogenously expressed lung cancer target.

Cytokine armoring strategies represent another commonly used approach that has also been applied to CAR T-cells directed against lung cancer. Illustrating this, anti-tumor activity of CEA-specific 2G (CD28) CAR T-cells was potentiated by armoring with nuclear factor of activated T-cells (NFAT)-inducible IL-18, an intervention which increased M1-polarized macrophages and natural killer cells, while reducing regulatory T-cells within the tumor microenvironment.[37] Similarly, DLL3-specific CAR T-cells have been armored to express constitutively active IL-18, boosting anti-tumor activity due to stimulatory actions on the CAR T-cells themselves and on myeloid cells in the TME.[59] Interleukin 12 also represents an attractive armoring cytokine, albeit limited by high potential for toxicity. Immunotherapy using tumor-infiltrating lymphocytes in which IL-12 expression was directed by an NFAT (nuclear factor of activated T-cells) promoter potentiated efficacy in patients with melanoma but at the risk of severe toxicity.[121] Similar considerations apply to the use of IL-18, establishing the desirability of tightly regulated control systems that restrict biological activity of these potently pro-inflammatory cytokines to the TME.

A further intriguing option to target solid tumors such as lung cancer entails the use of macrophage-targeted CARs.[52,122] Macrophages have a natural tumor tropism and Sanchez-Paulete et al have shown that such cells can infiltrate orthotopic lung tumors and impede tumor progression.[52] Clinical efficacy data using this novel approach are awaited.

In conclusion, lung cancer presents a substantial challenge to the development of effective CAR-based immunotherapies. Nonetheless, advanced genetic engineering technologies that allow precise delivery of cells to the site of disease, recognition of a desired target portfolio, re-education of the immunosuppressive TME and also the stimulation of endogenous immune reactivity and epitope spreading[123–127] together offer hope for future success in this daunting quest.

Disclosure

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