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CAR T cells for solid tumors: armed and ready to go?

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Abstract

CAR T cells face a unique set of challenges in the context of solid tumors. To induce a favorable clinical outcome, CAR T cells have to surmount a series of increasingly arduous tasks. First they have to be made specific for an antigen whose expression clearly demarcates tumor from normal tissue. Then they must be able to home and penetrate the desmoplastic stroma that surrounds the tumor. Once within the tumor they must expand, persist and mediate cytotoxicity in a hostile milieu largely composed of immunosuppressive modulators. While a seemingly herculean task, all of the above requirements can potentially be met effectively through both intrinsic and/or extrinsic modifications of CAR T cells. In this review we delineate the barriers imposed by solid tumors on CARs and strategies that have and should be undertaken to improve therapeutic response.

Keywords

immunotherapy; T cells; gene therapy; chimeric antigen receptors; solid tumors

Introduction

T cells - the armed forces of our immune repertoire are constantly coursing through the bloodstream on the lookout for foreign antigens. On encountering a solid tumor - a complex and dynamic mass composed of rogue host cells, T cells often fail to mount an effective response. Even when tumor responsive T cells extravasate to tumor sites, they are faced with a barrage of immunosuppressive factors that render them non-responsive. Thus, even the most adroit natural eliminator is exhausted in the tumor environment. Fortunately, there exist genetic approaches that can reprogram ordinary circulating T cells to highly specific slayers of cancer cells.¹⁻³

Genetically modifying T cells with chimeric antigen receptors (CARs) is the most commonly used approach to generate tumor specific T cells.¹ As outlined in detail in other contributions to this themed journal issue, CARs consist of an ectodomain, commonly derived from a single chain variable fragment (scFv), a hinge, a transmembrane domain, and

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an endodomain with one (1st generation), two (2nd generation), or three (3rd generation) signaling domains derived from CD3Z and/or co-stimulatory molecules.⁴ While CAR T cells have been successful in early phase clinical studies treating CD19-positive hematological malignancies,⁵⁻⁸ the success of CARs in the purview of solid tumors has been greatly hampered by the lack of unique tumor associated antigens, inefficient homing of T cells to tumor sites and the immunosuppressive microenvironment of solid tumors. In this review we delineate the barriers imposed by solid tumors on CARs and strategies that have and should be undertaken to improve therapeutic response.

Solid tumor antigens

Tumor associated antigens can be divided into several groups including antigens that 1) contain novel peptide sequences due to gene mutation, 2) are expressed in a tissue/lineage specific fashion, 3) are normally expressed during fetal development or at immunoprivileged sites, or 4) are expressed at higher than normal levels on tumor cells compared to non-malignant host cells.

Mutated antigens

Ideally, the targeted antigen should contain a novel peptide sequence, limiting its expression to tumor cells. One example is a splice variant of the epidermal growth factor receptor (EGFRvIII).⁹ Other antigens that may provide some target exclusivity include those with altered post-translational modifications or those that present conformational epitopes unique to the tumor microenvironment. Examples for the former include abnormal, tumor-specific glycosylation patterns of MUC-1,¹⁰ and for the latter include i) conformational EGFR and erythropoietin-producing hepatocellular A2 receptor (EphA2) epitopes.^{11;12}

Tissue/Lineage antigens

Tissue/Lineage restricted antigens by definition show restrictive expression patterns and may seem particularly attractive. While tissue expendability is acceptable or correctable in certain conditions like B-cell aplasia with CD19 CART cells, the function of most solid organs cannot be readily replaced. However, tissue-specific antigens of organs that are dispensable such as prostate specific cancer antigen (PSCA),¹³ are actively being explored.

Developmental antigens

Antigens expressed during fetal development or at immunoprivileged sites such MAGE family members or NY-ESO-1 are actively being targeted with $\alpha\beta$ TCRs.^{14;15} However most of these antigens are in cytoplasm, making them inaccessible to scFv-based CARs that recognize antigens expressed on the cell surface. However, scFv have been developed that recognize peptide derived from cytoplasmic proteins in the context of HLA molecule, making them 'CAR targetable'.¹⁶

Overexpressed antigens

The majority of targeted antigens are only overexpressed in comparison to normal tissues, raising concerns about 'on target/off tumor' side effects, which in most cases cannot be adequately assessed in murine models. Nevertheless, a broad array of overexpressed solid

tumor antigens has been targeted in preclinical models with CAR T cells (Table 1) and several clinical studies have been conducted (Table 2).

Targeting multiple antigens

In contrast to conventional T cells that only recognize single antigens, CAR T cells can be genetically modified to recognize multiple antigens, which should allow the recognition of unique antigen expression patterns on tumor cells. One example include ‘split signal CARs’ that limit full T-cell activation to tumors expressing multiple antigens.¹⁷⁻¹⁹ Other strategies to target multiple antigens include tandem CARs (TanCARs), which contain ectodomains with two scFvs,²⁰ and so called ‘universal ectodomain CARs’ that incorporate avidin or a FITC-specific scFv to recognize tumor cells that have been incubated with tagged MAbs.^{21;22} Targeting multiple antigens should also limit the risk of immune escape.²³

In summary, numerous solid tumor antigens are being actively explored for CAR T-cell therapy, however only few are uniquely tumor specific. While genetic approaches can be used to increase specificity, there is a growing exigency to discover novel tumor antigens as CAR T cells become more potent.²⁴

Clinical trials with solid tumor-specific CAR T cells

Pre-clinical studies have targeted a broad array of solid tumor antigens with CAR T cells (Table 1). Antigens currently targeted in clinical studies include carbonic anhydrase IX (CAIX), CD171, folate receptor alpha (FR- α), GD2, human epidermal growth factor receptor 2 (HER2), mesothelin, EGFRvIII, fibroblast activation protein (FAP), carcinoembryonic antigen (CEA), and vascular endothelial growth factor receptor 2 (VEGF-R2) (Table 2). The majority of clinical studies so far have used 1st generation CAR T cells, and while the studies have demonstrated feasibility, the clinical results have been in general disappointing.²⁵⁻²⁷ Nevertheless, these studies gave important insights into CAR T-cell biology. Lamers *et al.* infused renal carcinoma patients with polyclonal T cells expressing a 1st generation CAIX-specific CAR, and observed ‘on target/off cancer’ side effects, and the development of anti-CAR immune responses resulting in limited T-cell persistence.²⁵ Subsequently, pretreatment with CAIX MAbs prior to CAR T-cell transfer prevented hepatitis and abrogated the induction of anti-CAR immune responses.²⁶

Limited CAR T-cell persistence was also observed in neuroblastoma patients, who received CD8-positive T-cell clones expressing 1st generation CD171-specific CARs,²⁷ and in ovarian cancer patients, who received folate receptor (FR- α)-specific CAR T cells.²⁸ The latter study also highlighted that T-cell homing to solid tumor sites is limited, which at least in preclinical studies can be overcome by genetically modifying T cells with chemokine receptors.^{29;30} The most promising results (including complete responses) were achieved by Pule *et al.* with 1st generation CARs directed to GD2, which were expressed in Epstein-Barr virus (EBV)-specific T cells.^{31;32}

HER2 has been targeted with 2nd and 3rd generation CAR T cells.^{33;34} One patient developed acute respiratory distress syndrome and died after receiving lymphodepleting chemotherapy, and 10¹⁰ T cells expressing a 3rd generation HER2-specific CAR in

combination with IL2.³³ In a 2nd study up to 10⁸/m² T cells expressing a 2nd generation HER2-CAR T cells have been infused. While no overt toxicities were observed, the antitumor activity was limited. Clinical studies with mesothelin-, EGFRvIII-, VEGF-R2-, GD2-, and FAP-specific 2nd or 3rd generation CAR T cells are in progress or will be soon initiated (Table 3).

Genetic modification to enhance CAR T-cell function

Strategies to improve the antitumor activity of CAR T cells include the provision of co-stimulation, the careful selection of T-cell subsets in which to express CARs, and additional genetic modification to enhance CAR T-cell function. We will focus our discussion on additional genetic modifications (Table 3) since the first two strategies are being discussed in detail in other contributions to this themed journal issue.

The solid tumor microenvironment is extremely inhospitable and capable of inducing anergy in CAR T cells. T cells must therefore come armed with countermeasures to thrive in an environment that is replete with immunosuppressive cytokines, regulatory modulators and co-inhibitory receptors.³⁵

While inclusion of co-stimulatory signaling domains in the endodomain of CARs can render CAR T cells resistant to inhibitory T cells and/or TGF β , additional genetic modification strategies are actively being explored to enhance their function. Transgenic expression of cytokines such as IL15^{36,37} improves CAR T-cell expansion and persistence *in vivo*, and renders T cells resistant to the inhibitory effects of regulatory T cells (Tregs).³⁸

Alternatively, transgenic expression of IL12 in CAR T cells reverses the immunosuppressive tumor environment.³⁹ While there are safety concerns in regards to constitutive IL12 expression, inducible expression systems are available to restrict IL12 production to activated T cells at the tumor site.⁴⁰

Conversely, CAR T cells can be engineered to resist the effects of immunosuppressive cytokines that can inhibit their effector functions. Transforming growth factor (TGF) β is widely used by tumors as an immune evasion strategy,⁴¹ since it limits effector T-cell function and activates regulatory T cells (Tregs). These detrimental effects of TGF β can be overcome by expressing a dominant negative TGF β receptor II (DNR)^{42,43}, and this approach is currently being tested in clinical trials.⁴⁴ CAR T cells can also be genetically engineered to actively benefit from the inhibitory signals generated by the tumor environment, by expressing chimeric receptors that convert inhibitory signals provided by TGF β , IL4, or programmed death 1 (PD-1) into stimulatory signals.⁴⁵⁻⁴⁸ Lastly, silencing genes that render T cells susceptible to inhibitory signals in the tumor microenvironment may also improve CAR T-cell function⁴⁹ or the transgenic expression of constitutively active signaling molecules.⁵⁰

Targeting the tumor stroma with CAR T cells

Most solid tumors have a stromal compartment that supports tumor growth directly through paracrine secretion of cytokines, growth factors, and provision of nutrients, and contributes to tumor-induced immunosuppression.⁵¹ For example, T cells expressing CARs specific for

FAP expressed on cancer associated fibroblasts (CAFs) has potent antitumor effects.⁵² To prevent on target/off tumor toxicity,^{53;54} transient expression of FAP CAR-T cells may be sufficient to weaken the desmoplastic stroma and allow infiltration of tumor specific CAR T cells. Targeting the tumor vasculature with CARs is another attractive strategy.^{55;56} While the initial CAR ectodomain was based on VEGF to target VEGF receptor 2 (VEGF-R2), more recent studies have used a VEGF-R2-specific scFv, and targeting the tumor vasculature in addition to tumor cells synergized in inducing tumor regression in preclinical models.⁵⁶

Combinatorial CAR T-cell therapy

Combining CAR T cells with other therapies offer the potential to improve antitumor effects. For example, the solid tumor microenvironment abounds in co-inhibitory receptors like cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and PD-1, that are known to limit the efficacy of any directly stimulatory strategy.⁵⁷ Systemic administration of antagonists (blocking antibodies) to these co-inhibitory receptors resulted in remarkable response rates even in refractory solid tumors,⁵⁸⁻⁶⁰ and combining blocking antibodies with CAR T cells should boost antitumor effects. Other strategies include epigenetic modifiers that upregulate the expression of tumor associated antigens⁶¹ or the use of targeted therapies that inhibit tumor cells growth, but are not detrimental to T cells.⁶²

Conclusions

The ultimate goal of CAR T-cell therapy for solid tumors is to be curative. This requires the development of a potent product that can withstand and thrive in the solid tumor microenvironment. Significant strides have been made towards this end in preclinical studies. While the options are varied, a judicious assessment of a given solid tumor and its microenvironment can help narrow them down to those that would render CAR T cells most successful in inducing a therapeutic response. Clinical trials comparing different genetic modification strategies⁶³ will be crucial in the future to optimize CAR T cells, transitioning CAR T cells from merely “promising” to being “effective” treatments for solid tumors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Solid tumor antigens for CAR T-cell therapy

Antigen	scFv	Ref [*]
B7-H3	8H9	S1
B7-H6	Nkp30	S2
CD70	CD27 ^{**}	S3
CEA	IgCEA, L45	S4;5
CSPG4	225.28S	S6
EGFRvIII	139, RAb ^{DMvIII} , MAb108	S7-9
EphA2	4H5	S10
EpCAM	M13-57	S11
EGFR family	T1E ^{***}	S12
ErbB2 (HER2)	FRP5, 4D5	S13-15
FAP	MO36, OS4, F19	S16-18
FR α	MOv19	S19
GD2	14g2a	S20;21
GD3	MB3.6	S22
HLA-A1+MAGE1	Fab-G8	S23;24
IL-11R α	IL11 ^{**}	S25
IL-13R α 2	IL13 mutein ^{**}	S26
Lewis-Y	Hu3S193	S27
Mesothelin	P4, SS1	S28;29
Muc1	HMFG2	S30
Muc16	4H11	S31
NKG2D Ligands	NKG2D ^{**}	S32;33
PSMA	J591, 3D8	S34;35
ROR1	R12	S36
TAG72	N13	S37
VEGFR2	VEGF ^{**} , DC101	S38;39

* Table references are listed in supplementary material

** ligand,

*** peptide

Table 2

Clinical studies with CAR T cells targeting solid antigens

Disease	CAR	2 nd gene	Cell type	Biological	Identifier
Advanced stage Cancers	HER2.CD28.ζ + DNR	DNR TGFβ	EBV-CTL	-	NCT00889954
Advanced stage Cancers	CEA.CD28.ζ	-	T cells	IL2	NCT01723306
Advanced stage Cancers	VEGF-R2.CD28.41BB.ζ	-	T cells	Flu, Ctx, IL2	NCT01218867
GBM	HER2.CD28.ζ	-	CMV-CTL	-	NCT01109095
GBM	EGFRvIII.CD28.41BB.ζ	-	T cells	Flu, Ctx, IL2	NCT01454596
HNSCC	T1.CD28.ζ	-	T cells	-	NCT01818323
Neuroblastoma	GD2.CD28.OX40.ζ	iC9	T cells	AP1903	NCT01822652
Neuroblastoma post-allo HSCT	GD2.ζ	-	Tri-virus specific CTLs	-	NCT01460901
Mesothelioma	FAP.CD28.ζ	-	T cells	-	NCT01722149
Sarcoma	HER2.CD28.ζ	-	T cells	None	NCT00902044
Sarcoma	GD2.CD28.OX40.ζ	iC9	VZV CTLs	VZV vaccine, AP1903	NCT01953900

AP1903: dimerizer drug; CMV: Cytomegalovirus, CTL: cytotoxic T cells; Ctx: cyclophosphamide, DNR: dominant negative receptor, EBV: Epstein Barr virus; Flu: fludarabine, HNSCC: head and neck squamous cell carcinoma, iC9: inducible caspase 9, T1: peptide that binds Erb family of receptors

Table 3

Genetic modification to improve CAR T cells for solid tumors

Obstacle	Genetic Solution
Specificity	multiple CARs, 'split signal CARs', TanCARs, universal ectodomain CARs
Trafficking to tumor sites	chemokine receptors
Inhibitory tumor microenvironment	cytokines (e.g. IL12, IL15) dominant negative receptors (e.g. TGF β RII), chimeric receptors ('signal converters'; e.g. IL4R/IL7R α) silencing inhibitory molecules (e.g. FAS)