RNA Therapeutics for Improving CAR T-cell Safety and Efficacy

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ABSTRACT

Autologous chimeric antigen receptor (CAR) T cells have recently emerged as potent tools in the fight against cancer, with promising therapeutic efficacy against hematological malignancies. However, several limitations hamper their widespread clinical use, including availability of target antigen, severe toxic effects, primary and secondary resistance, heterogeneous quality of autologous T cells, variable persistence, and low activity against solid tumors. Development of allogeneic off-the-shelf CAR T cells could help address some of these limitations but is impeded by alloimmunity with either rejection and limited expansion of allo-CAR T cells or CAR T cells versus host reactions. RNA therapeutics, such as small interfering RNAs, microRNAs, and antisense oligonucleotides, are able to silence transcripts in a sequence-specific and proliferation-sensitive way, which may offer a way to overcome some of the challenges facing CAR T-cell development and clinical utility. Here, we review how different RNA therapeutics or a combination of RNA therapeutics and genetic engineering could be harnessed to improve the safety and efficacy of autologous and allogeneic CAR T-cell therapy.

CAR T Cells: Room for Improvement

T cells can be genetically engineered to express chimeric antigen receptors (CAR) that target specific antigens. This may allow selective destruction of target antigen-expressing cells, e.g., tumor cells. Autologous CAR T cells are successfully deployed to treat B-cell malignancies, with four approved products targeting the B lymphocyte surface antigen CD19 and two targeting the B-cell maturation antigen (BMCA; ref. 1). Several other CAR T-cell products are under development with more than a hundred ongoing clinical trials for hematologic malignancies and solid tumors (2). CAR T cells have also been more recently used to treat autoimmune diseases and viral infections (3).

Despite the initial success, several challenges continue to hamper the widespread clinical application of CAR T cells. Primary resistance occurs in 10% to 20% (4), and relapse is also common with an incidence of 30% to 50% (4). Resistance mechanisms within the infused T cells include failure to expand *in vivo* due to early exhaustion, poor persistence, insufficient fitness (5), etc. The tumor itself may also contribute to resistance, for example, by escaping CAR T-cell control via antigen loss due to downregulation or mutation (5) or by exerting immune-suppressive effects hampering CAR T-cell function (e.g., particularly in the tumor microenvironment; ref. 6). In addition, CAR T cells may induce mild to severe toxic effects in the form of cytokine release syndrome (CRS) or immune effector cell–associated neurotoxicity syndrome (ICANS), which lead to fatal outcomes in around 1.5% of patients (7). Long-lasting and/or severe neutropenia as well as B-cell aplasia may furthermore lead to fatal infections (7, 8).

Several T-cell modulation strategies are being tested to reduce CAR T-cell resistance and toxicity and to expand the circle of targetable diseases. The currently approved CAR T cells are produced by lentiviral vector-based manufacturing. Precise genomic insertion of the CAR construct via CRISPR (9) or temporary CAR expression via mRNAs (10) may circumvent the potential side effects associated with incidental lentivirus integration. Furthermore, some CAR T-cell clones appear more efficacious than others. This heterogeneity is attributed to (i) lentiviral vector-based manufacturing leading to incidental genomic integration (11) and (ii) phenotypic heterogeneity of the apheresis product. Efficacious CAR T-cell clones proliferate (12, 13), eliminate targeted cells, do not get exhausted, and persist *in vivo* for several months up to 1 year (14). In contrast, hyperactive CAR T-cell clones—with too much proliferation and no exhaustion—cause excess toxicity (15).

Thus, there seems to be an ideal therapeutic window in which effective, nontoxic CAR T cells persist *in vivo* (**Fig. 1**). This therapeutic window is defined not only by the initial dose but also by CAR T-cell proliferation capacity. Therefore, the timing of a modulation strategy is crucial to maintain this therapeutic window. Proliferation and tissue penetration should be accelerated and exhaustion inhibited early on to reach the therapeutic threshold. Proliferation should, however, be slowed down at a later phase to flatten the curve and to prevent reaching the toxicity threshold (**Fig. 1A**). Thus, CAR T-cell modulation strategies should ideally take into account the proliferation rates.

RNA-Treated CAR T Cells

Regulatory RNAs modulate cellular phenotypes by fine-tuning transcription (16), splicing (17), translation (18), and mRNA lifespan (19, 20). RNAs, therefore, can be used for the rational phenotype design of, e.g., CAR T cells.

RNA therapeutics encompass various reversible and titrable inhibitors of gene expression, including RNA interference–based therapeutics (miRNAs, ref. 21; or siRNA, ref. 19) and antisense oligonucleotides (ASO; ref. 22).

RNA interference was first observed in petunia flowers in the 1980s (23), mechanistically described in *C. elegans* in 1998 (19), resulted in the first drug approval in 2018 (24), and displays five approved drug products today (25). RNA interference is an antiviral defense mechanism in plants (26) and a gene expression regulator in mammals (27). The key effectors of RNA interference, short interfering RNAs (siRNA; ref. 19) and microRNAs (miR; ref. 28), bind

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Cancer Res 2023;83:354–62

doi: 10.1158/0008-5472.CAN-22-2155

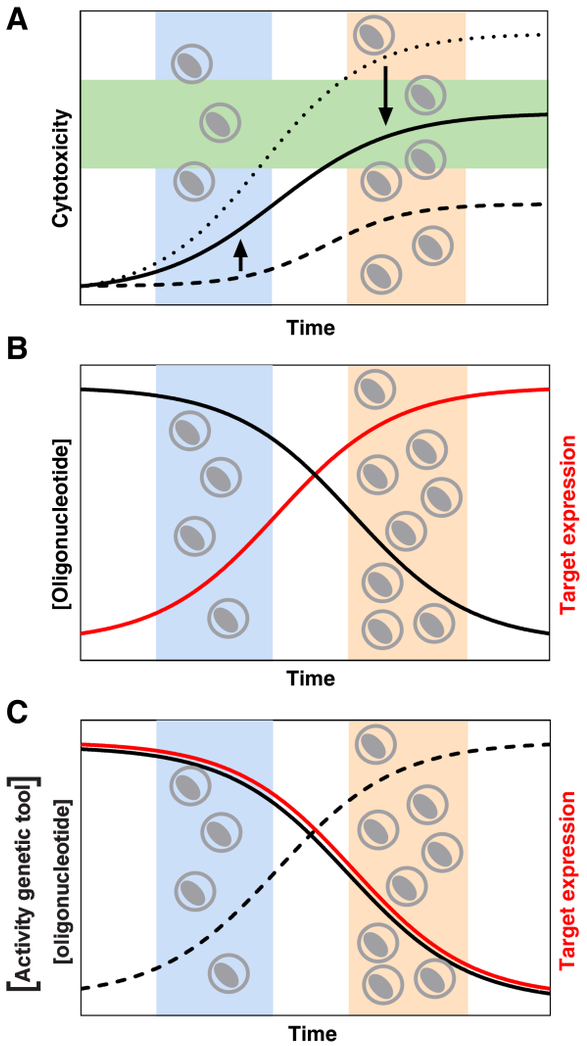

Figure 1.

CAR T-cell modulation in specific time windows. **A,** CAR T cells (depicted in gray) have to substantially proliferate (black curve) upon administration in order to reach the therapeutic threshold (lower limit of the green therapeutic window). Too much (dotted curve) proliferation would result in crossing the toxicity threshold (upper limit of the green therapeutic window). Too little (dashed curve) proliferation would, however, lead to lack of efficiency and failure to reach the therapeutic threshold. Early intervention (blue; only few cells present in the circulation/targeted tumor) should accelerate proliferation (left arrow) and tissue penetration as well as prevent exhaustion to enable reaching the therapeutic threshold. Late intervention (orange; many cells present in the circulation/targeted tumor) should rather slow down (right arrow) proliferation to prevent toxicity and promote persistence and effector functions to enable a deep and long-lasting remission. **B,** Intracellular concentration of an oligonucleotide RNA therapeutic (red) is highest directly upon delivery to cells (left) and maximal levels of mRNA silencing is reached shortly after (usually 3 to 7 days). During cell proliferation, the intracellular concentration of the oligonucleotide RNA therapeutic decreases with each cell division (red curve). Once the intracellular oligonucleotide "depot" filled at delivery gets depleted due to proliferation, target mRNA silencing cannot be maintained and target mRNA expression will rise (black curve). Therefore, target mRNA will be inhibited early within an expansion burst (blue) and expression will be restored later (orange). **C,** Intracellular concentration of the oligonucleotide (i.e., siRNA, miRNA, ASO, or anti-miR) is highest shortly upon delivery (left) and decreases during repeated cell divisions (red curve). The oligonucleotide inhibits the activity of a virally delivered genetic engineering tool (i.e., shRNA, miR sponge, mRNA; purple) so that the lowest activity coincides with the highest oligonucleotide concentration early during an expansion burst (blue) and activity will rise with cell proliferation reaching its maximum late during an expansion burst (orange). The genetic engineering tool is inhibiting a target (i.e., mRNA, miRNA; black). Therefore, low target expression coincides with high genetic tool activity late during an expansion burst (orange).

complementary mRNAs to induce mRNA degradation (21, 29, 30). Although siRNAs show a full complementarity to their target and catalyze cleavage (29), miRNAs are only partially complementary to their targets (31) and induce mRNA decay (21, 30). siRNAs are typically exogenously introduced for the sequence-specific silencing of mRNAs as a research tool or as a therapeutic (32). miRNAs, on the other hand, are typically endogenously produced in mammalian cells and are a major means of gene expression regulation (27). siRNAs can be introduced to cells as chemically synthesized short RNA duplexes (33) or in the form of short hairpin RNAs (shRNA) encoded in viral vectors (34). It is possible to exogenously introduce artificial miRNAs to a cell (either chemically synthesized artificial miRNA, ref. 35; or viral shRNA, refs. 36, 37) or to suppress miRNA effects via antisense sequences complementary to an miRNA (chemically synthesized anti-miRs, ref. 38; or virally encoded miR sponges, refs. 38, 39). RNA interference typically targets mRNAs; however, there is evidence that noncoding transcripts such as long noncoding RNAs (lncRNA) can also be targeted (40).

ASOs were first described in 1978 as translational inhibitors of the Rous sarcoma virus (22). Today, 10 ASO drugs are approved featuring diverse mechanisms of action including pre-mRNA cleavage and modulation of splicing or translation (41). All ASO drugs are single-stranded, short, chemically modified RNA or DNA sequences that bind complementary mRNA and either induce its cleavage or competitively inhibit the binding of certain proteins (41).

RNA therapeutics are being explored to potentiate CAR T cells. Several studies have demonstrated the feasibility and utility of RNA interference or ASO-mediated silencing in primary T and CAR T cells (**Table 1**). siRNAs (42–52) as well as ASOs (53–56) can be efficiently delivered to T cells *ex vivo* using either electroporation (57, 58), lipid-conjugated siRNAs (59), aptamer-conjugated siRNA (52, 60), lipid nanoparticles (61), or viral vectors (small hairpin RNA, shRNA; refs. 62–72). Three clinical trials (70, 73, 74) are currently testing the clinical utility of RNA-modified CAR T cells (**Table 1**). The combination of RNA therapeutics against multiple targets may be of advantage when modulating a complex system such as a CAR T cell (74).

Proliferation-Sensitive Control of Gene Expression

We propose a combination of RNA therapeutics and genetic engineering to achieve proliferation-sensitive gene expression control in CAR T cells.

RNA therapeutics (e.g., siRNAs, artificial miRNAs, ASOs, and anti-miRs) can be introduced to cells in the form of chemically synthesized oligonucleotides (33, 35) or via genetic engineering (viral delivery; ref. 34). The major difference is the timeline: The effect of oligonucleotides is temporary whereas the effect of genetic engineering is permanent.

The duration of the effect of an oligonucleotide RNA therapeutic depends on (i) its metabolic stability and (ii) the cell proliferation rate. Extensive chemical modifications (75) enhance the metabolic stability

Table 1: RNA therapeutics in (CAR) T cells.

Reference	Technology	Target	Cell type	Application
Peer D et al. (2007; ref. 42)	siRNA	LFA-1	T cells	Mice
Kumar P et al. (2008; ref. 43)	siRNA	CCR5, HIV	T cells	Humanized mice
Mantei A et al. (2008; ref. 44)	siRNA	GATA3	T cells	Mice
Kim S et al. (2010; ref. 45)	siRNA	CCR5	T cells	Mice
Iwamura K et al. (2012; ref. 46)	siRNA	PD-L1, PD-L2	T cells	*In vitro*
Hermann A et al. (2014; ref. 47)	siRNA	STAT3	TIL	Humanized mice
Berezhnoy A et al. (2014; ref. 48)	siRNA	mTORC1	T cells	Mice
Freeley M et al. (2015; ref. 49)	siRNA	Screening	T cells	*In vitro*
Rajagopalan A et al. (2017; ref. 50)	siRNA	CD25, Axin1	T cells	Mice
Simon B et al. (2018; ref. 58)	siRNA	PD-1, CTLA4	CAR T	*In vitro*
Ligtenberg MA et al. (2018; ref. 59)	siRNA	PD-1	TIL	*In vitro*
Zhou J et al. (2018; ref. 60)	siRNA	HIV	T cells	Humanized mice
Wheeler LE et al. (2011; ref. 52)	siRNA	CCR5, HIV	T cells	Humanized mice
Keil TWM et al. (2020; ref. 51)	siRNA	GATA3	Pulmonary T cells	Mice
Festag J et al. (2020; ref. 53)	ASO	CD39, CD73	T cells	*In vitro*
Ceccarello et al. (2021; ref. 54)	ASO	IFNβ, PRF1, GrB	T cells	*In vitro*
Kashyap et al. (2019; ref. 55)	ASO	CD39	T cells	Xenograft mouse model
Marasca F et al. (2022; ref. 56)	ASO	LINE-1	TIL	*In vitro*
Banerjea et al. (2003; ref. 63)	shRNA	HIV	T-cell precursors	Humanized mice
Liu G et al. (2021; ref. 64)	shRNA	PD-1	CAR T	*In vitro*
Jafarzadeh L et al. (2021; ref. 65)	shRNA	TIM3	CAR T	*In vitro*
Masouimi E et al. (2020; ref. 66)	shRNA	ADORA2A	CAR T	*In vitro*
Zhou JE et al. (2021; ref. 62)	shRNA	PD-1	CAR T	Xenograft mouse model
Kang L et al. (2020; ref. 67)	shRNA	IL6	CAR T	Xenograft mouse model
Liu G et al. (2021; ref. 68)	shRNA	ADORA2A	CAR T	Xenograft mouse model
Mane MM et al. (2020; ref. 69)	shRNA	LDHA	CAR T	Xenograft mouse model
Chen L-Y et al. (2020; ref. 70)	shRNA	IL6	CAR T	Clinical trial (NCT03064269)
CYAD-211 Celyad oncology (73)	shRNA	CD3ζ	CAR T	Clinical trial (NCT04613557)
Zhao L et al. (2020; ref. 71)	shRNA	ACAT1	CAR T	Xenograft mouse model
Lee Y-H et al. (2021; ref. 74)	shRNA	PD-1, TIM-3, LAG3, CTLA4, TIGIT	CAR T	Clinical trial (NCT04836507)
Zhang H et al. (2022; ref. 72)	shRNA	IL6, IFNγ	CAR T	*In vitro*

of RNA therapeutics and extend their duration of effect to 6 months in nondividing hepatocytes of patients (76). However, the intracellular concentration of an RNA therapeutic gets halved with every cell division, and the duration of effect in rapidly dividing cells remains largely unknown. In the case of siRNAs, 50- to 3,000-fold excess siRNA (forming a "depot"; ref. 77) can be delivered to cells than what is necessary for silencing. Therefore, a dilution effect is expected to appear after 5 to 12 cell divisions. Indeed, we observed a silencing duration of 5 to 20 divisions in primary immune cells and immune cell lines.

The single or combined use of an oligonucleotide RNA therapeutic and a genetic engineering strategy enables attaining different levels of sensitivity to cell proliferation:

(i) **Proliferation insensitive**: A genetic engineering tool (i.e., shRNA, miRNA sponge) may be expressed from a sequence integrated into the genome (78) or from an episome (79). (Episomes are extrachromosomal DNA replicating with the host genome but refrain from integration; ref. 80). Both chromosomes and episomes get duplicated during mitosis and copy numbers remain unchanged in daughter cells (81, 82). Therefore, the expression of the RNA-based engineering tool depends only on the promoter used and remains largely insensitive to cell proliferation.

(ii) **Proliferation-sensitive inhibition:** An oligonucleotide RNA therapeutic (i.e., siRNA, artificial miRNA, ASO, and anti-miR) enters the cell at a certain concentration and gets diluted with each cell division. The duration of the effect can be set by adjusting the initial concentrations used (**Fig. 1B**).

(iii) **Proliferation-sensitive expression:** The combination of an oligonucleotide RNA therapeutic and a genetic engineering tool with opposing effects (i.e., anti-miR–shRNA, miRNA–miRNA sponge, siRNA–mRNA) is delivered to a cell. The oligonucleotide blocks the effect of the genetic engineering tool. As the oligonucleotide dilutes out during cell proliferation, the genetic engineering tool will get released from this blockage and start exerting its effects—i.e., expressing a protein (mRNA) or downregulating its targets (shRNA, miRNA sponge; **Fig. 1C**).

Specific Interventions in Selected Time Windows

Toxicity control

The timing of a T-cell modulation strategy may differentiate between enhanced toxicity and antitumor activity—two otherwise overlapping mechanisms (83).

Peak CAR T-cell concentration (late during an expansion burst) coincides with peak levels of released cytokines (i.e., IL6 and IFNγ) and correlates with both CRS and neurotoxicity (84). Therefore, delayed inhibition of CAR T-cell proliferation and cytokine release may prevent overactivation, help keep CAR T-cell levels within the therapeutic window, and, thus, represent an optimal toxicity management strategy. CRS driver cytokines (IL6, ref. 67; IL1, ref. 85; IFNγ, ref. 72; and TNFα, ref. 86) may be silenced in CAR T cells in a delayed fashion.

Because IL6 and IL1 release mainly originates from CAR T-cell–activated macrophages, humoral (GM-CSF, ref. 87) and contact-dependent (CD40L, ref. 88; CD69, ref. 89; LAG3, ref. 90) macrophage activation mechanisms could also be subject of delayed silencing. Alternatively, CAR T cells may be engineered to express IL1/IL6 inhibitors (91) in a delayed fashion. In order to slow down proliferation, several genes involved in mitosis could be silenced.

Lower expression levels of the CAR construct (92) or reduced CAR T-cell number (4) may reduce toxicity while preserving or enhancing the therapeutic activity. The CAR expression level may be controlled via a precise genetic integration site (93) or perhaps by pretreatment or post-administration treatment with RNA therapeutics. Delayed silencing of the CAR construct may specifically inhibit overactivation.

On and off switches represent a genetic engineering strategy for CAR T-cell toxicity management. On and off switches are typically CAR construct modifications or fusion proteins that either get activated or inactivated via a small-molecule drug. For example, lenalidomide-gated (67), asunaprevir-gated (85), and rimiducid-gated (86) switches have been successfully applied in CAR T cells in preclinical settings. Proliferation-sensitive inhibition may be superior to genetically engineered on and off switches for toxicity control, because proliferation-sensitive inhibition enables prevention whereas on/off switches only curtail toxicities that already occurred.

Phenotype optimization

Most successful CAR T cells initially display a memory-like phenotype that guarantees robust antitumor activity and limited toxicity (94).

The scarcity of memory T cells makes it difficult to rely on apheresis for enrichment. Therefore, several strategies have been developed to induce memory phenotype (small-molecule inhibitors, ref. 95; and interleukins, ref. 96) and are now tested in clinical trials (97, 98). However, some of these treatments may limit the differentiation capacity of memory T cells into effector T cells, thus impairing antitumor effects (99). In contrast, the transient nature of RNA treatment may enable enrichment for the memory phenotype early on, while letting cells differentiate and exhaust in a delayed fashion in order to promote antitumor efficacy and a healthy lifespan.

Memory T-cell expression fingerprint includes the enrichment of *TCF7* (100), *BCL6* (101), *BMI1* (102), *FOXO1* (103), *KLF2* (104), *LEF1* (105), *CD27* (106), *CCR7* (107), and *SELL* (108). Initial exogenous expression (via for example viral delivery of additional gene copies) of these genes may be able to reprogram cells into a memory phenotype. On the other hand, inhibiting negative modulators of "memory" genes, such as *BRD4* (109), *EP300* (109), *TET2* (110), *MIR15/16* (111), or *MIR150* (112), leads to memory phenotype enrichment and superior antitumor activity. Overexpression of miR-143 (113) or downregulation of the miR-143 target GLUT1 (113) via siRNA also promotes memory T-cell formation. However, cell differentiation requires a decrease in the expression of "memory" genes. Therefore, the above interventions should be restricted to the *ex vivo* production phase and the beginning of the expansion burst upon administration.

The effector T-cell phenotype is characterized by the expression of *TOX* (114), *PRDM1* (115), *NR4A* (116), *BATF* (117), *IRF4* (118), *NFAT* (119), *KLRG1* (120), *EOMES* (121), *PRDM1* (122), *GZMA* (123), *PRF1* (123), *IFNG* (124), among others. Effector T cells may also express the exhaustion markers *PDCD1* (125), *HAVCR2* (126), *LAG3* (127), *TIGIT* (128), *CTLA4* (129), and miR-146a (130). DNMT3A is also associated with exhaustion (131). Early inhibition of the "effector" genes may successfully eliminate effector cells from the CAR T-cell product and block early exhaustion. However, reexpression of "effector genes" should be allowed for natural cell differentiation and successful antitumor effect. Persistence of effector T cells is promoted by miR-17-92 (132) and miR-155 (133). The "persistence genes" should ideally be expressed in a delayed fashion coinciding with T-cell differentiation genes.

It still remains to be elucidated whether expression of the above genes only correlates with, or by itself drives, memory/effector phenotypes. Phenotype driver genes would be ideal targets for RNA preprogramming of CAR T cells. Evidence-based rather than mechanism-based target identification may emerge from RNAi (49) or CRISPRi (134) screens of CAR T cells as well as from analyses of integration sites in approved CAR T-cell products (92).

CAR T-cell Enhancement in Solid Tumors

The precise timing of gene expression may potentiate the so-far insufficient activity of CAR T cells targeting solid tumors.

Infiltration

Infiltration could be enhanced by suppressing inhibitory receptors on CAR T cells, such as PDCD1 (135), TIM3 (135), LAG3 (135), or CX3CR1 (136). In fact, the combined silencing of these factors via shRNAs is a concept that has already entered the clinical trial phase (74). Overexpression of miRNAs targeting the above receptors has also proved to be beneficial (miR-27a-5p, ref. 137). Restricting the suppression of inhibitory receptors to early stages of CAR T-cell expansion may enhance safety.

CAR T cells engineered to coexpress IL7 and CCL19 (138) or IL7 and CCL21 (139)—chemokines important to establish T-cell zones in lymphoid organs—show better infiltration and higher activity against solid tumors when compared with conventional CAR T cells. However, IL7 (140), CCL19, and CCL21 (141) overexpression is potentially carcinogenic, and thus late inhibition of the above engineered factors may enhance safety.

Overexpression of receptors recognizing chemokines that are highly abundant in solid tumors (CCR2, ref. 142; CCR5, ref. 143) and silencing their inhibitors (let7; ref. 144) appear promising strategies to promote infiltration. However, CCR5 may mediate a nonclassic apoptosis pathway within the tumor, resulting in CAR T-cell death (145). Hence, CCR5 is an ideal candidate for delayed silencing.

Microenvironment

Several genetic engineering strategies have been proposed to overcome therapy resistance induced by the tumor microenvironment. The tumor vasculature may be targeted via CARs (VEGFR1, ref. 146; $EIIIB^{+}$ fibronectin splice variant, ref. 147). CAR T cells may express matrix-degrading enzymes (heparinase; ref. 148). Immunosuppressive solute receptors may be inhibited via either dominant-negative knock-in (TGFβ; ref. 149), shRNAs (adenosine; ref. 66), or CRISPR (TGFβ; ref. 150). Alternatively, immunosuppressive solutes can be targeted via CAR T cells (151). Immunosuppressive cell types, such as Tregs (152), tumor-associated macrophages (153), myeloid-derived suppressor cells (154), or cancer-associated fibroblasts (155), can also be targeted via CAR T cells.

Generally, degradation of the tumor microenvironment should be a local reaction. Thus, CAR T cells should ideally exert a tumor microenvironment inhibiting or degrading activity once docked on the

tumor. Therefore, tumor microenvironment targeting should rather happen in a delayed fashion.

Armed CAR T cells

CAR T cells may be "armed" to produce proinflammatory cytokines normally expressed by other cell types. CAR T cells expressing IL12, IL18, IL15, IL7, TGFβ, and IL23 achieve higher efficacy against solid tumors (156, 157). However, constitutive expression of these cytokines may associate with severe toxicities (158, 159). Therefore, cytokine production should occur only once CAR T cells are docked on the tumor and proliferate locally. Early inhibition/delayed expression may achieve this goal, enhancing the safety of armed CAR T cells.

miR-155 overexpression has also been shown to enhance T-cell activity against solid tumors (160). An artificial miRNA here may be able to prevent toxicities due to overactivation of T cells.

Antigen recognizing

Cancer stem cell targeting has been pursued as a strategy to prevent relapse and overcome tumor-associated antigen heterogeneity (161, 162). However, cancer stem cell markers are often not specific to tumors, leading to severe on-target off-tumor toxicities (163–166). For example, CD133—the most commonly used cancer stem cell marker (167)—is also expressed in hematopoietic stem cells, human embryonic stem cells, and epithelial cells of several tissues (168). Ideally, cancer stem cell targeting should occur only when CAR T cells infiltrate the tumor and proliferate locally. Early expression of a tumor-associated antigen targeting CAR combined with late expression of a cancer stem cell targeting CAR may increase the specificity, safety, and efficacy of CAR T cells targeting solid tumors.

Allogeneic CAR T Cells

"Off-the-shelf" allogeneic CAR T cells may be a game-changer in the field, shortening production length and improving initial T-cell fitness (169, 170). However, allogeneic T cells may be rejected by the host's immune system or, vice versa, by themselves attack the host inducing graft-versus-host disease (171).

MHC I knockout (β2-microglobulin locus; ref. 172) has been pursued to overcome allogeneic CAR T-cell rejection. However, MHC I–deficient CAR T cells are vulnerable to NK cell–mediated destruction. This NK-mediated "missing-self" reaction may be avoided by expressing NK-inhibiting receptors, such as HLA-E or HLA-G (173) or SIGLEC7/9 (174) on allogeneic CAR T cells. Class II MHC molecules may also be knocked out by targeting CIITA (175). Alternatively, allogeneic CAR T cells can be "armed" with an alloimmune defense receptor (targeting 4-1BB) and eliminate activated T cells of the host (176). However, complete blockage of alloimmune inhibition eliminates an important means of proliferation control and may lead to overactivation and/or CAR T-cell–derived malignancies (as shown in irradiated mice; ref. 177). Therefore, alloimmune inhibition may be restricted to early proliferation phase to enhance safety.

T-cell receptor (TCR)-deficient allogeneic CAR T cells do not attack the host. One strategy is to knock out the constant α chain of the TCR (178–179). Alternatively, shRNA-mediated silencing of the TCR has been successfully used in adoptive T-cell therapies (1). Another strategy is the exchange of the TCR for the CAR construct via CRISPR (92). However, the TCR is thought to be involved in low-level "tonic" signaling, important for T-cell maintenance and effector function (4, 23). Therefore, the TCR is a promising target for delayed silencing.

Conclusion

Small RNA therapeutics enable the proliferation-sensitive control of endogenous and exogenous gene expression in CAR T cells. RNA treatment would enable CAR T cells to more often reach the therapeutics threshold, do not surpass the toxicity threshold, and switch memory phenotype into effector phenotype at the right time. RNA therapeutics would further enable sequential expression of different CARs in T cells for combinatorial antigen targeting. RNA treatment may also optimize allogeneic CAR T-cell production.

Precise and target-specific fine-tuning of silencing duration is, however, necessary to realize the full potential of RNA-treated CAR T cells. Initial RNA therapeutic concentrations, metabolic stability of the RNA therapeutic, delivery strategies, and genetic engineering tools need to be optimized.

RNA treatment promises to increase the safety and efficacy of CAR T-cell therapies, expands the circle of targetable diseases, and pushes these cell therapies to the next level.

Authors' Disclosures

P. Schaible reports a patent for RNA-treated CAR-T cells pending. W. Bethge reports grants and personal fees from Miltenyi GmbH, Gilead GmbH, Novartis GmbH, and BMS GmbH outside the submitted work. R.A. Haraszti reports grants from German Cancer Aid (70113948) and Faculty of Medicine, University of Tübingen (473-0-0) during the conduct of the study and from Else Kröner Fresenius Stiftung outside the submitted work; in addition, R.A. Haraszti has a patent for nucleic acid modified biological cell with expansion-dependent gene expression pending. No disclosures were reported by the other author.

Acknowledgments

Tha authors thank J. Brandstetter, L. Lo Presti, and R. Sapkota for comments and assistance. R.A. Haraszti is funded by the German Cancer Aid (70113948) and the Faculty of Medicine, University of Tübingen (473–0-0). C. Lengerke is funded by the ERC Consolidator Grant HemStem.

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Received July 4, 2022; revised November 2, 2022; accepted December 9, 2022; published first December 13, 2022.

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