Adoptive Cell Therapy don't over engineer it: Alternatives to gene editing to improve cell-based therapeutics for the treatment of cancer

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Introduction

Treatment of solid tumors with adoptive cell therapy (ACT) has proven to be difficult, resulting in the development of alternative approaches including TCR and NK cell-based therapies. In order to overcome resistance to ACT for the treatment of solid tumors, most developers look to further improve the cells during manufacture. To date, these approaches predominantly include further genetic engineering of the cells.

An alternative approach to certain forms of gene editing is gene silencing by RNAi, especially in cases where permanent gene modification is not required or undesirable. RNAi compounds, have shown great promise for the improvement of ACT therapies without the need for gene editing. Here we present the case for utilizing self-delivering RNAi technology as an alternative and/or complement to certain genetic engineering approaches.

Delivery & Efficiency

CRISPR / Cas9

Need to deliver both cas9 enzyme and guide RNA of targeted gene through physical delivery or viral vector delivery[1].

Physical delivery

- Microinjection
- Electroporation
- Hydrodynamic force

Physical delivery allows for delivery of cas9 gene and the sgRNA; or delivery of cas9 protein with the sgRNA in a ribonucleoprotein complex.

Viral vector delivery

- Adeno-associated virus (AAV) most common viral delivery vector
- Deliver cas9 gene and guide RNA in separate vectors or single vector (single vector more challenging due to overall vector size).

RNAi

Exogenous siRNA delivery options include similar delivery approaches to CRISPR / Cas9 but also include direct chemical engineering, obviating the need for delivery tools / platforms.

Physical delivery

- Electroporation
- Lipid-based nanoparticles (LNPs)

Viral vector delivery

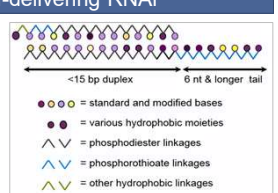
- shRNA delivery by lentiviruses (LVs), adenoviruses (AVs), or adeno-associated viruses (AAVs)

Chemical engineering

- Chemical modifications directly incorporated into siRNA to enhance cellular uptake of compounds, such as INTASYL™ (chemically modified self-delivering RNAi).

INTASYL – chemically modified self-delivering RNAi

INTASYL compounds are RNAi compounds with an asymmetric duplex structure, a small duplex region (≤ 15 base pairs) and a single-stranded phosphorothioate tail.



Furthermore these compounds are chemically modified with stabilizing and hydrophobic modifications which confer stability, efficient cellular uptake and reduced inflammatory response.

These compounds can be efficiently delivered to immune cells through addition to cell culture media.

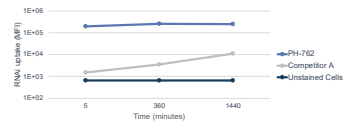
[1] Lino et al. Drug Delivery 25: 1234–1257 (2018)

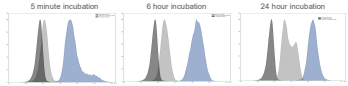
INTASYL – results in more rapid and more efficient transfection of T cells compared to traditional siRNA and gene editing

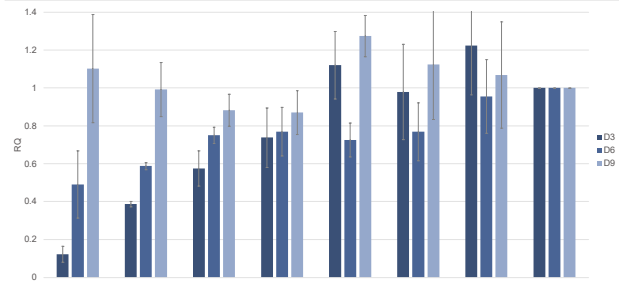
Activated T cells were incubated with fluorescently labeled PH-762, an INTASYL compound against *PD1*, or a chemically modified siRNA (comp. A) against the same target sequence.

siRNA uptake was monitored by flow cytometry.

PD1 mRNA was assessed by RT-qPCR.







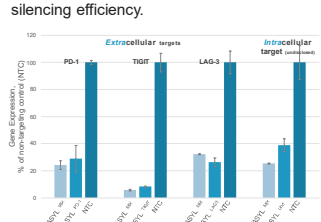
PH-762 allows for **≥ 90% gene silencing** of *PD1* in activated human T cells, but effect is transient.

In comparison, CRISPR / Cas9 gene editing efficiency of precise homology-directed repair (HDR) is substantially lower, but permanent.

- **20% gene disruption of *PD1*** in human TCR T cells [2]
- **50% protein silencing of PD-1** in CD19 CAR-T [3]

Targeting multiple genes – RNAi allows for easier and more efficient cell modification

INTASYL technology can silence multiple genes at once without negative impact on silencing efficiency.



Whereas multiple targets can be silenced with CRISPR, efficiency is much lower (low frequency of silencing of all targeted genes in one cell). For example: Silencing of *TRAC*, *TRBC* and *PD1* in TCR[2]. Frequency of silencing of all three genes in one cell is low:

- Cells having one mutation: 40%
- Cells having two mutations: 20%
- Cells having three mutations: 10%

[2] Stadtmauer et al. Science 367: 1001 (2020)
[3] Rupp et al. Scientific Reports 7: 737 (2017)

Safety considerations

RNAi / INTASYL	CRISPR / Cas9
DURATION	
INTASYL gene silencing is transient. • Duration of silencing is sufficient to enhance efficacy of transferred cells without long term risks of permanent gene silencing	CRISPR/Cas9 gene editing confers permanent genetic alterations [1] Off target gene editing can lead to permanent off target toxicity such as: • activation/inactivation of off-target genes giving toxic phenotypes • activation of oncogenes • unwanted chromosomal translocations Cas9 nuclease activity can cause a p53-dependent G1-phase arrest and potential decrease in precision of gene editing [4]
IMMUNE RESPONSE	
Undesired immune responses seen with large, unmodified siRNAs. No immune responses to INTASYL in NHP and human studies.	Undesired immune responses related to gene editing include • Inherent immune response to bacterial cas9 [5] • Immune response to viral vectors • Generation of immunity to cas9 delivered in RNPs [6]

Logistics and manufacturing considerations

	INTASYL	Genetic engineering	
CoGs	👍	👎	10-fold lower CoGs of INTASYL vs AAV (~$4M difference for a typical Phase 1 study).
Manufacturing	👍	👎	CDMO contracting wait time for viral vector manufacturing is 16 months on average [7]. Complex and difficult scale-up of viral vectors.
Ease of Use	👍	👎	INTASYL can be applied directly to immune cells without requirement for further manipulation (e.g. electroporation).
Longevity of effect	👍 / 👎	👍 / 👎	Depending on gene target: INTASYL optimal for transient modification & gene editing optimal for permanent genetic modification.

Regulatory considerations

RNAi / INTASYL	CRISPR / Cas9
INTASYL is reviewed as a Drug for direct therapeutic applications and as Ancillary Material for cell product manufacturing	CRISPR is reviewed as an Investigational Cellular and Gene Therapy Product
Regulatory path is well defined with several RNAi drugs approved by FDA and EMA and INTASYL technology has a proven regulatory track for clinical development	Regulatory path is an ongoing evolutionary process

Conclusions

In order to overcome resistance to ACT for the treatment of solid tumors, most developers look to further improve the cells during manufacture. Two alternative approaches to enhancing ACT for solid tumors are genetic modification with CRISPR/Cas9 and use of RNAi. Advances in the RNAi field, specifically in the discovery and development of self-delivering RNAi compounds such as the INTASYL platform, have great promise for the improvement of ACT therapies without the need for gene editing.

[4] Haapaniemi et al. Nature Med 24: 927–930 (2018)
[5] Charlesworth et al. Nature Med 25:249-254 (2019)
[6] Crudele et al. Nature Commun 9:3497 (2018)
[7] J.P. Morgan: Cell & Gene Therapy Deep Dive (2019)