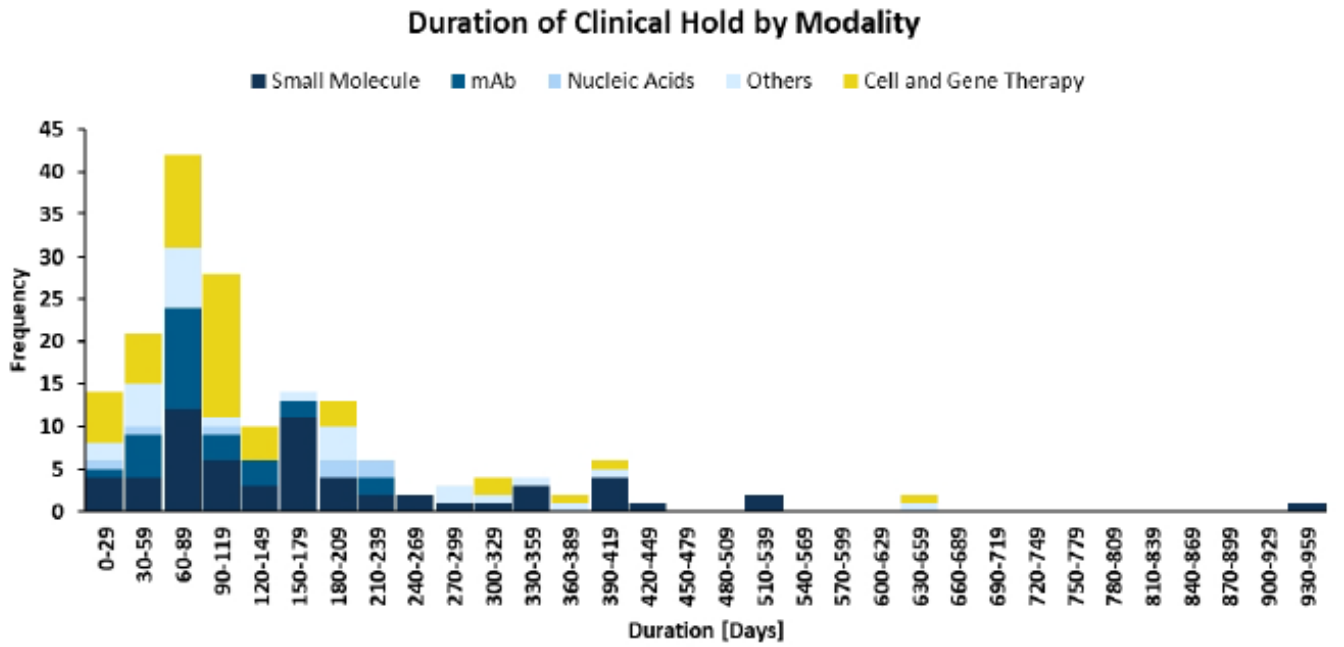
Emerging Technologies and Innovation in Manufacturing Regenerative Medicine Therapies

SESSION IV:
Quality Control and Regulatory Considerations

Sadik H. Kassim, Ph.D.
CTO, Danaher Genomic Medicines
17 October 2023

Clinical Holds in the Genomic Medicines Space

Exhibit 4 - Clinical hold duration is evenly distributed across modalities.



Source: Biomedtracker, Jefferies Research.

"NIH-listed CGT clinical trials total less than 2% of all listed clinical trials, yet they are responsible for approximately 40% of all clinical holds*."

Yee MJ, Tsai A, Ding D, Wen J, Song YC. FDA Clinical Holds Now Double the 445 Historical Average. *Jefferies Equity Research: Biotechnology.* Jefferies; 2022:1-6

*Wills CA, Drago D, Pietrusko RG, Clinical Holds for Cell and Gene Therapy Trials: Risks, Impact, and Lessons Learned, *Molecular Therapy: Methods & Clinical Development* (2023),

Complexity of Genomic Medicines
.....and Their Raw Materials

Small molecules	Biologics	AAV or lentivirus	Cell therapy

Aspirin
21 atoms

Humira
20 067 atoms

Viral vector
10^5 to 10^7 atoms

CAR T
10^{22} atoms

Guide RNA

CRISPR Nuclease

Viral Vector

iPSC Line

Cytokines

RNP

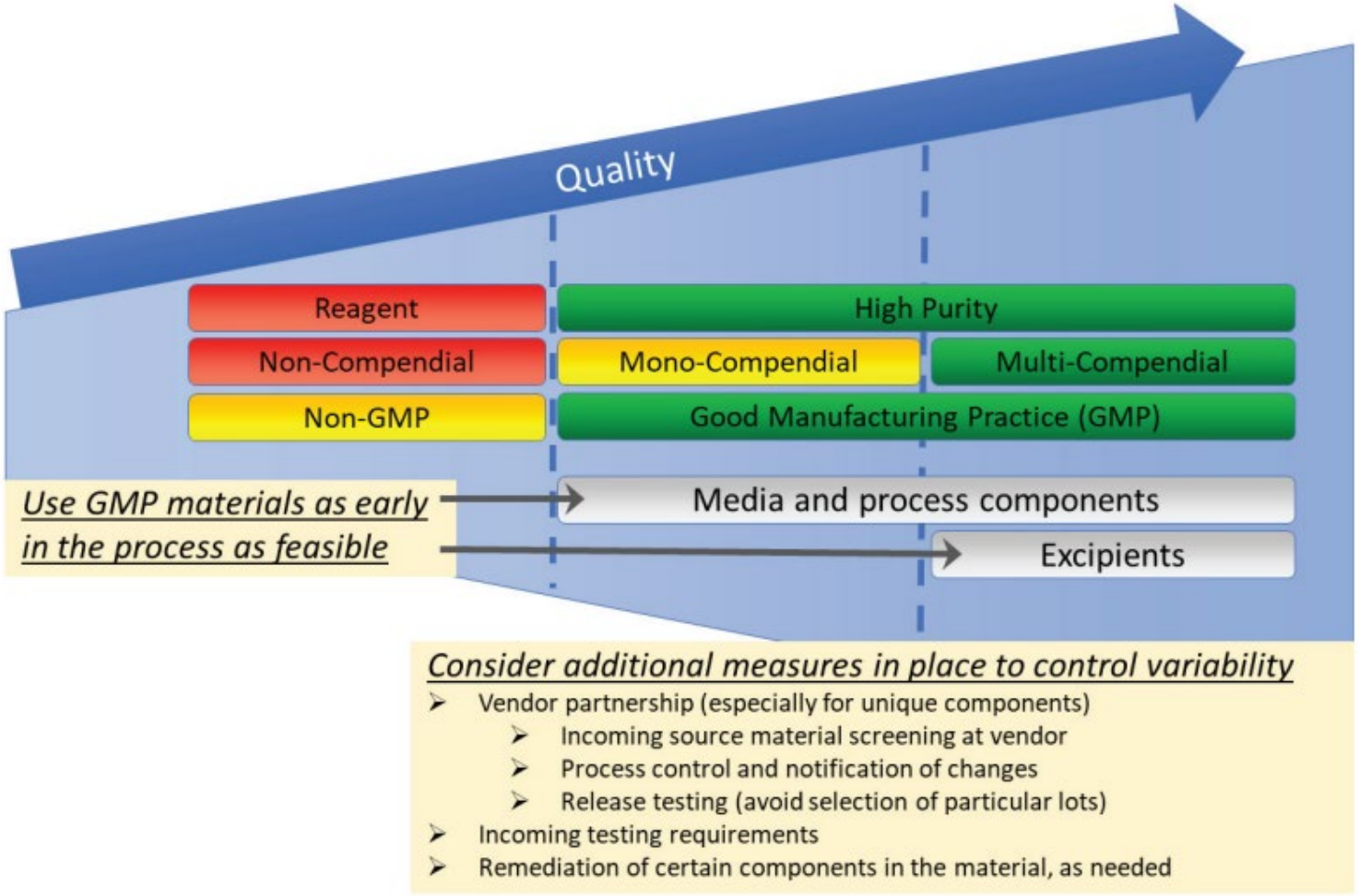
mRNA/LNP

Cell Bank

Growth Factors

Plasmid DNA

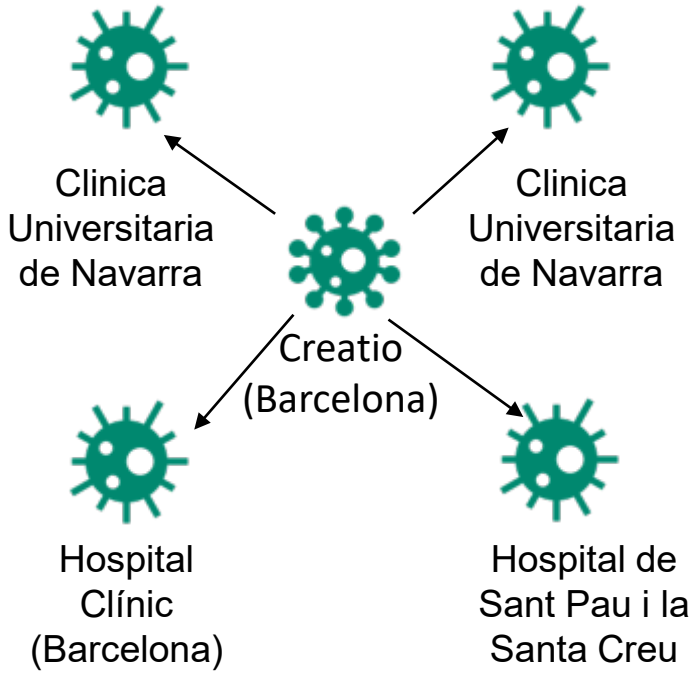
Raw Material Quality is a Major Determinant of Reproducible Manufacturing



"Consistency and reliability of raw materials are critical to ensuring consistent manufacturability and comparability of product through development."

Tsokas, K., R. McFarland, C. Burke, J. L. Lynch, T. Bollenbach, D. A. Callaway II, and J. Siegel. 2019. Reducing risks and delays in the translation of cell and gene therapy innovations into regulated products. *NAM Perspectives*. Discussion Paper, National Academy of Medicine.

CAR-T ARI-0001 Case Study: Centralized Raw Material Manufacturing, Decentralized Drug Product Manufacturing



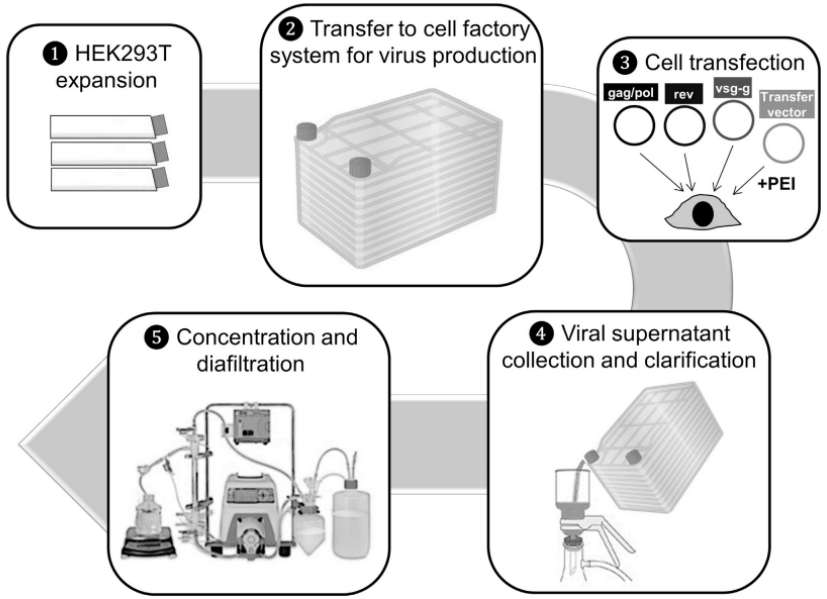


Table 1. Results and Quality Controls of GMP-Grade Viral Productions of 3 Supernatant Lots

Parameter	Method	Acceptance Criteria	Lot 1	Lot 2	Lot 3
Appearance	visual inspection	yellowish liquid solution	cloudy liquid solution	cloudy liquid solution	cloudy liquid solution
Viral titer	limiting dilution	$>3.75 \times 10^7$ TU/mL	2.29×10^8 TU/mL	1.68×10^8 TU/mL	1.10×10^8 TU/mL
Sterility	microbial growth	sterile	sterile	sterile	sterile
Mycoplasma	PCR	absent	absent	absent	absent
Identity	PCR	positive	positive	positive	positive
RCL (replication-competent lentivirus)	real-time PCR	absent	absent	absent	absent

Molecular Therapy: Methods & Clinical Development Vol. 12 March 2019

Lentivirus Vectors Have Proven Safe Across Clinical Trials and Indications

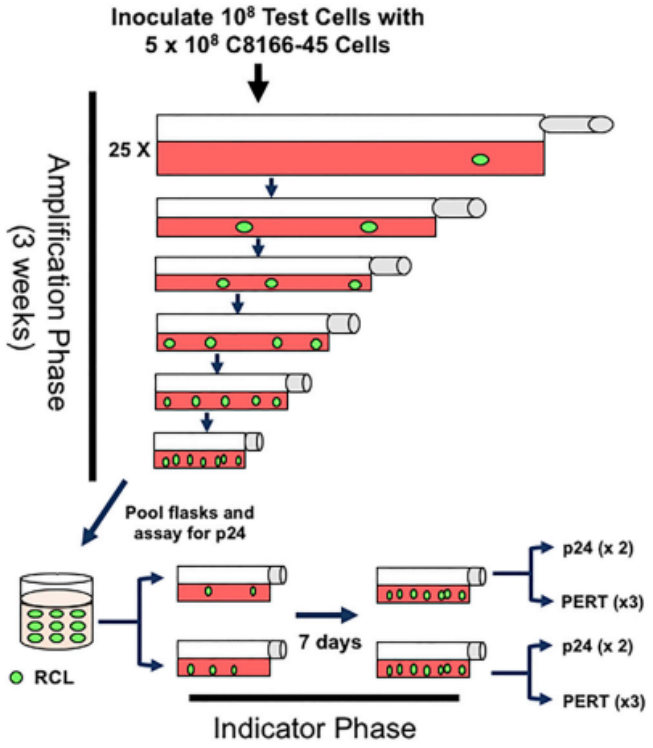


Table 3. Follow-Up Testing for RCLs in Subjects Infused with Gene-Modified T Cell Products

Study No.	Principal Investigator	No. of Products Infused	No. of Subjects Infused	No. of Subjects with RCL Follow-Up[a]	Method of RCL Detection	Level of Sensitivity per DNA
11-2	C.H.J.	2	2	1	VSV-G DNA PCR	25 copies per 1 μg
11-3	C.H.J.	19	17	16	VSV-G DNA PCR	25 copies per 1 μg
11-4	C.H.J.	2	2	2	VSV-G DNA PCR	25 copies per 1 μg
11-11	C.H.J.	24	24	21	VSV-G DNA PCR	25 copies per 1 μg
11-12	C.H.J.	14	13	13	VSV-G DNA PCR	25 copies per 1 μg
11-13	C.H.J.	1	1	0	VSV-G DNA PCR	25 copies per 1 μg
12-4	C.H.J.	36	36	34	VSV-G DNA PCR	25 copies per 1 μg
12-16	M.J.	3	3	2	VSV-G DNA PCR	10 copies per 50 ng
13-4	C.H.J.	32	32	23	VSV-G DNA PCR	25 copies per 1 μg
13-12	G.B.-S.	31	31	24	VSV-G DNA PCR	5 copies per 100 ng
13-15	S.F.	5	5	5	VSV-G DNA PCR	2.5 copies per 50 ng
14-9	C.H.J.	25	25	14	VSV-G DNA PCR	25 copies per 1 μg
14-10	C.H.J.	34	34	30	VSV-G DNA PCR	25 copies per 1 μg
14-12	C.J.T.	76	76	49	VSV-G DNA PCR	10 copies per 1 μg
14-18	T.F.	14	14	11	VSV-G DNA PCR	10 copies per 200 ng
14-27	S.F.	7	7	6	VSV-G DNA PCR	2.5 copies per 50 ng
15-9	S.F.	6	4	3[b]	VSV-G DNA PCR	2.5 copies per 50 ng
15-10	S.F.	6	6	5	VSV-G DNA PCR	2.5 copies per 50 ng
15-11	S.F.	11	11	11	VSV-G DNA PCR	2.5 copies per 50 ng
15-26	M.J.	45	23	19	VSV-G DNA PCR	10 copies per 50 ng
15-36	S.F.	2	2	2	VSV-G DNA PCR	2.5 copies per 50 ng
16-1	M.J.	14	7	5	VSV-G DNA PCR	10 copies per 50 ng
Total		409	375	296		

VSV-G, vesicular stomatitis virus G protein.
[a] ≥ 30 Days post-infusion.
[b] 2 subjects were tested by PCR, and 1 subject was tested by serology.

"In 460 products tested, using a vigorous biologic assay for RCL, there was no evidence of replication competent lentivirus (RCL)."

"Therefore, screening T cell products for RCL does not add additional assurance of safety and should no longer be required when the lentiviral vector product has been successfully screened for RCL."

Molecular Therapy Vol. 26 No 1 January 2018

CRISPR-Based Gene Editing: Potential Factors that Impact Specificity and Efficacy

Figure 1. RNP Configuration

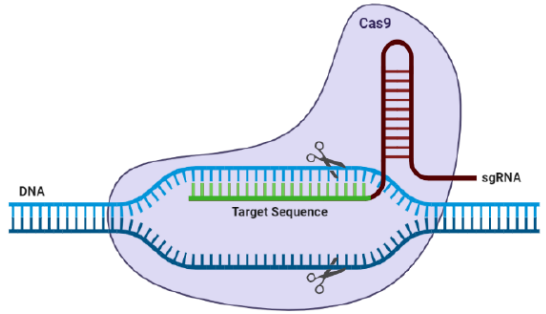
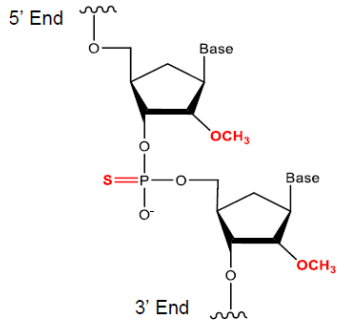
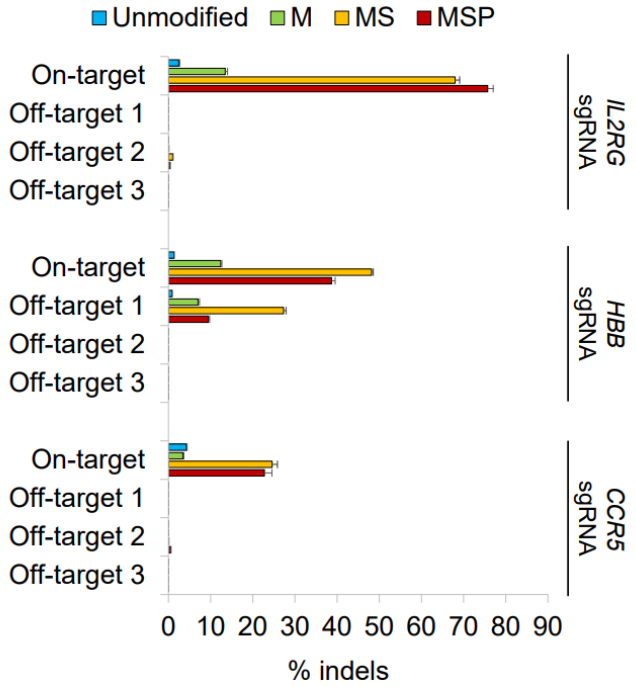


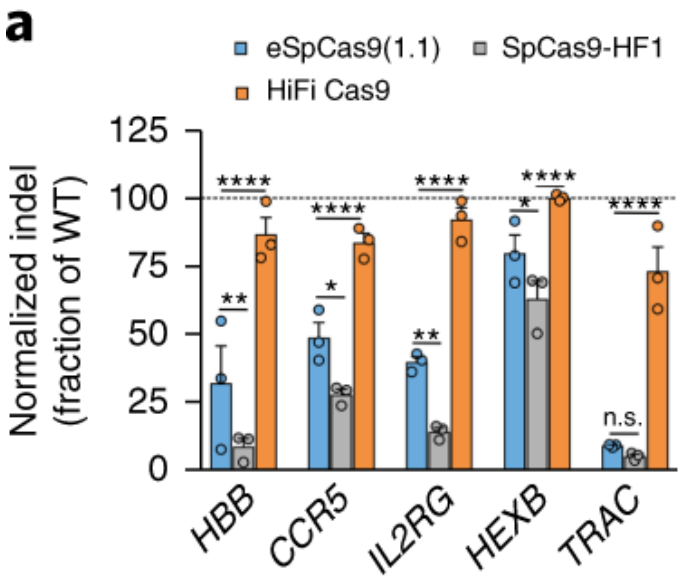
Figure 2. Modifications

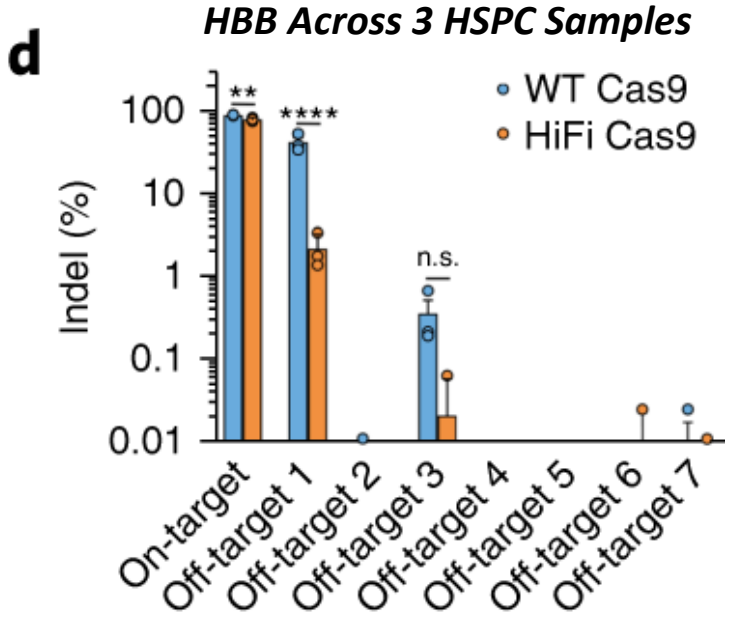




2′-*O*-methyl (M),
2′-*O*-methyl 3′phosphorothioate (MS),
2′-*O*-methyl 3′thioPACE (MSP)

Nat Biotechnol. 2015 Sep; 33(9): 985–989.

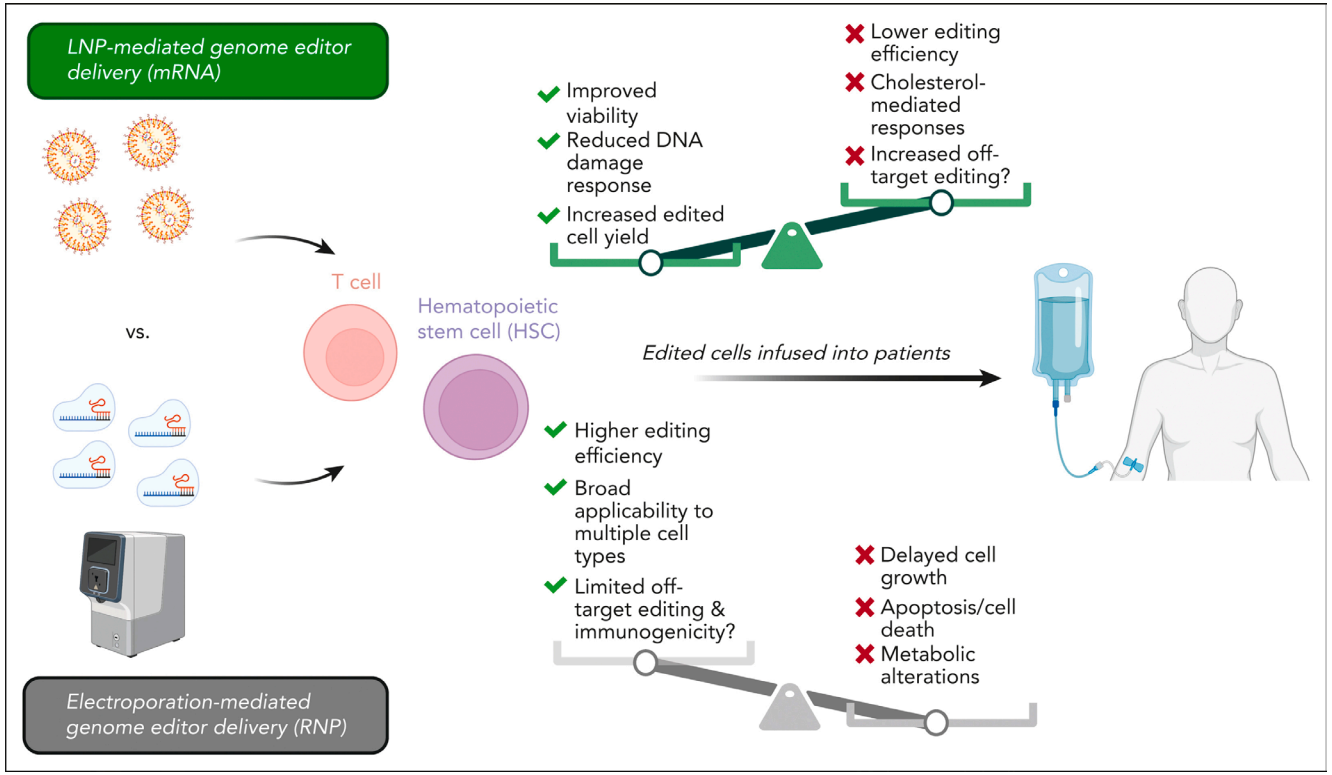




Nature Medicine volume 24, pages1216–1224 (2018)

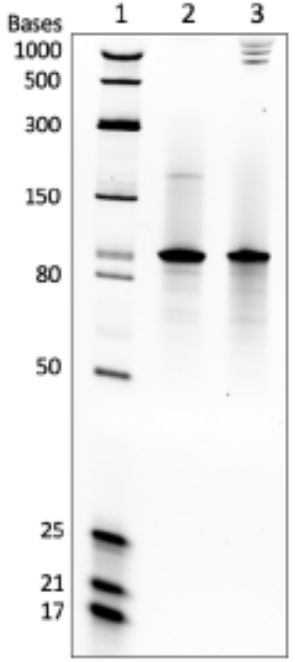
How Do Other Factors Impact CRISPR Gene Editing Specificity and Efficacy??

How does delivery method and format (mRNA vs Protein) impact specificity and efficacy?

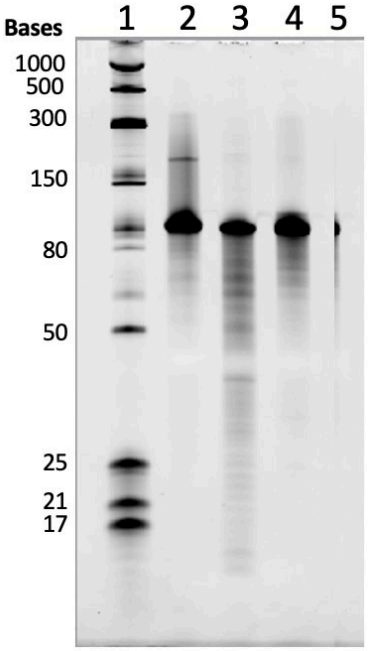


Blood (2023) 142 (9): 755–756.
Blood (2023) 142 (9): 812–826.

How does guide RNA purity impact specificity and efficacy?



1 – Ladder
2 – Vendor X, sequence A
3 – Vendor Y, sequence A



1 – Ladder
2 – Control Guide
3 – Vendor Y, HPLC-purified
4 – Vendor Y, new purification method

Aldevron, Internal Data

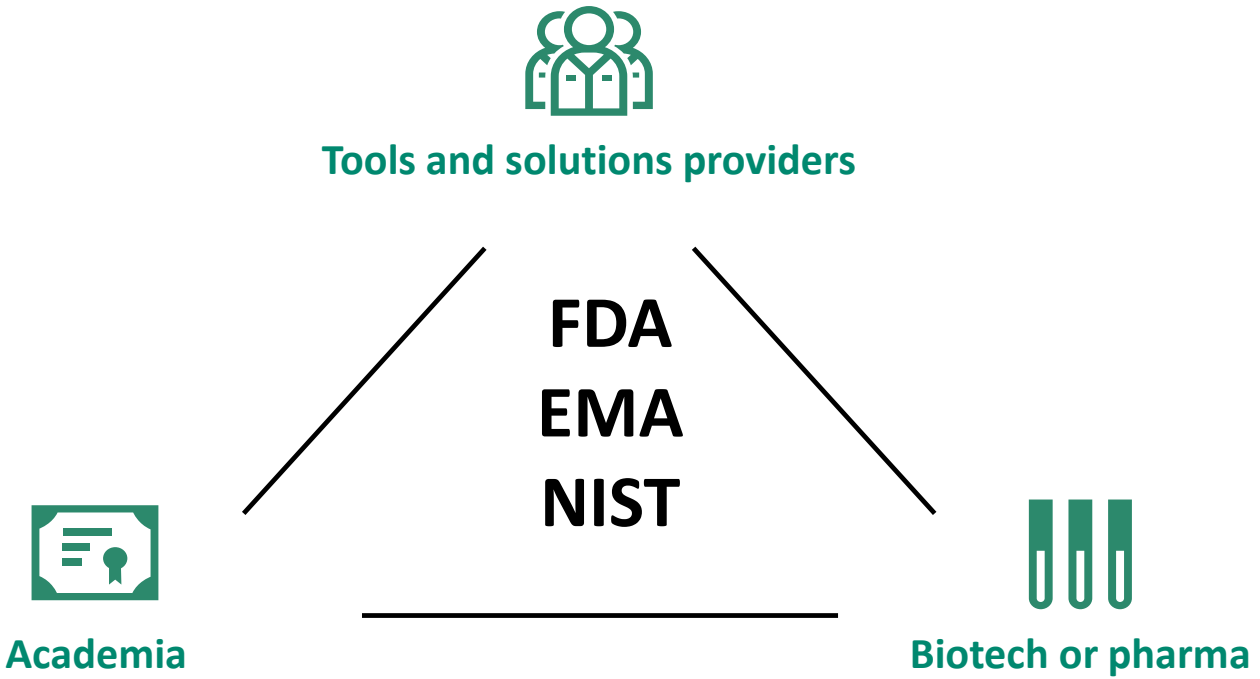
Proposed Future Directions: Raw Material Characterization and the Acceleration of Regenerative Medicine Manufacturing

- Robust clinical safety record and decades of manufacturing know-how and characterization of raw materials, may enable decentralized manufacturing of certain therapeutic modalities (e.g. lentivirus vector and CAR-T).

- In other instances, early technology, unknown design space, and minimal manufacturing know-how may limit the field's practical ability to accelerate translation and/or decentralize manufacturing (e.g. CRISPR nucleases, guide RNAs, and CRISPR gene-edited cell therapies).

- Federated-learning model may be a potential solution to accelerate adoption and distribution of novel technologies.

- Example: MELLODDY, a federated-learning project between 10 pharma companies focused on small-molecule drug development (www.melloddy.eu).



Innovation at the speed of life™