Lovo-cel's Journey:
Lessons Learned in the Development of LVV-based Cell & Gene Therapies

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Disclosure

I’m an employee of bluebird bio, Inc. and own stock in the company

agenda

1. Introduction to bluebird bio:
   - Products, clinical impact, experience, process overview
2. SCD & lovotibeglogene autotemcel (lovo-cel)
   - Sickle Cell Disease and the development of lovo-cel
   - CMC Challenges on the path to a successful product
3. Key learnings during development of lovo-cel:
   - Major process improvements are possible in late-stage development (but …)
   - Demonstration of CMC comparability across vector & drug product
   - Helpful advice for fellow travellers

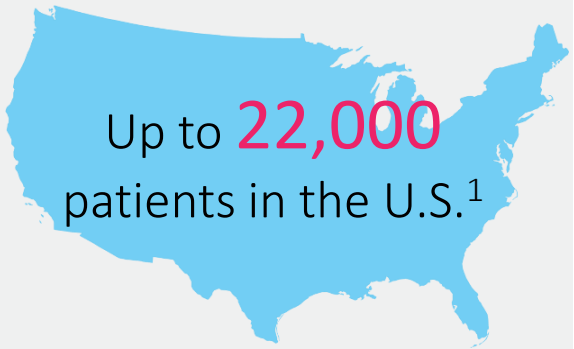
bluebird bio: a primer

10+ years
since inception

200+ patients
studied across
8 clinical trials

250+
drug product lots
manufactured across
3 programs

> 800 patient-years
of experience with gene therapies

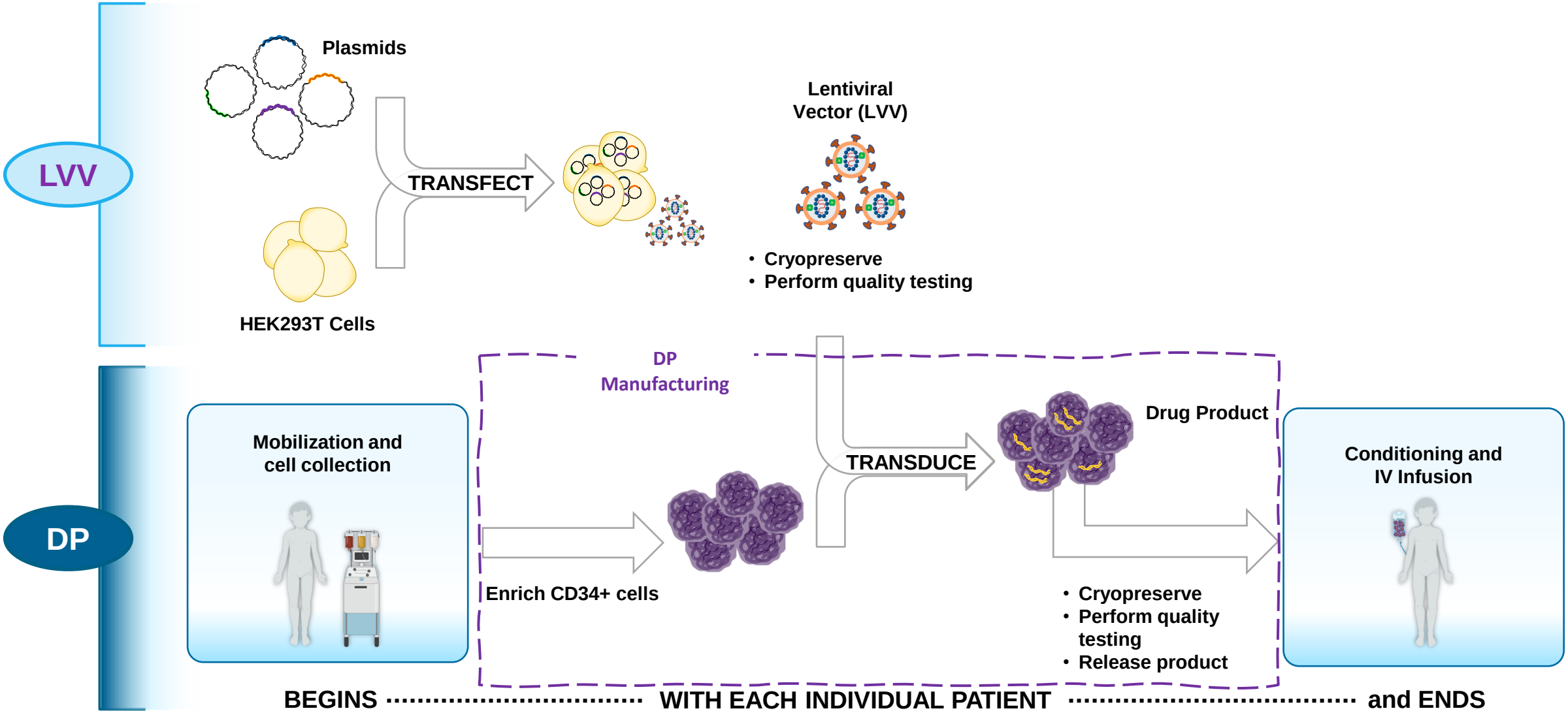


Focus on LVV-based autologous HSC gene therapies

3 FDA approved therapies for
3 rare genetic diseases

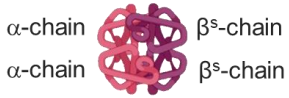
Hassell KL. Population estimates of sickle cell disease in the U.S. Am J Prev Med. 2010;38(4 Suppl):S512 521; Jul '21 bbb analysis of Komodo patient-level claims data (Apr '20 – Mar '21), IQVIA patient-level claims data (Aug '18 – Jul '19); Hulihan, Mary M., et al. State-based surveillance for selected hemoglobinopathies. Genetics in Medicine 17.2 (2015): 125-130.; Bezman L, et al. Adrenoleukodystrophy: Incidence, new mutation rate, and results of extended family screening. Ann Neurol. 2001;49:512–517; Moser HW, Mahmood A, Raymond GV. X-linked adrenoleukodystrophy. Nature Clin Pract Neurol. 2007;3(3):140-51

Manufacturing process for autologous, LVV-based cellular gene therapy

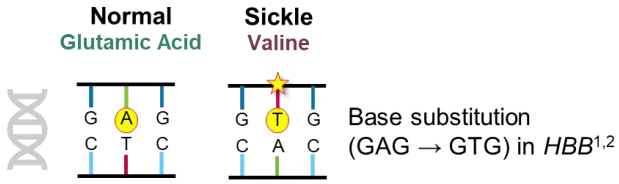


SCD is a Genetic Disease Caused by a Point Mutation in the β-globin Gene

HbS Molecule



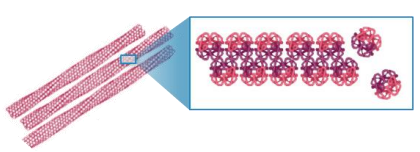
DNA

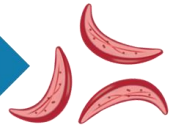


Base substitution (GAG → GTG) in *HBB*[1,2]

HbS Polymerization

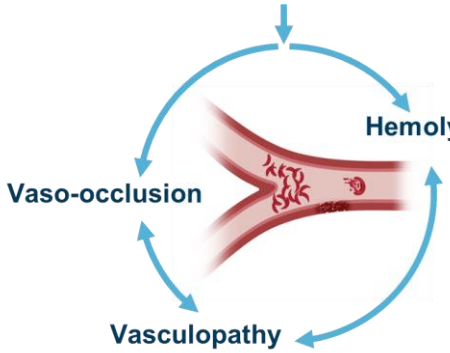
Long polymers of HbS align into fibers, which then form parallel rods.[1]

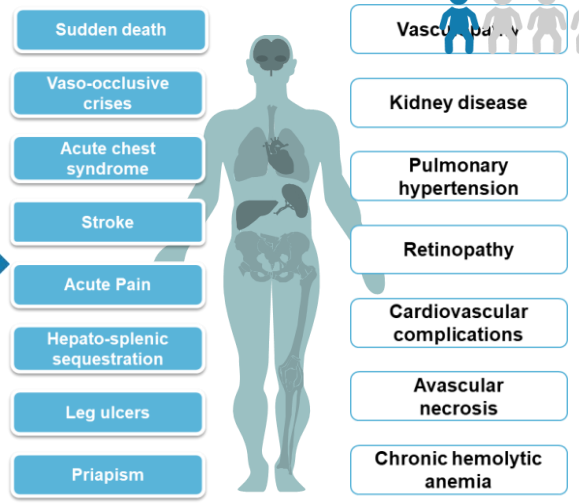




Sickled RBCs

HbS polymerization alters the shape and physical properties of RBCs. **Sickled, rigid RBCs** are prone to **hemolysis.**[1,2]





Acute

Chronic

US Incidence of SCD[3]

~1:365 African-American Births

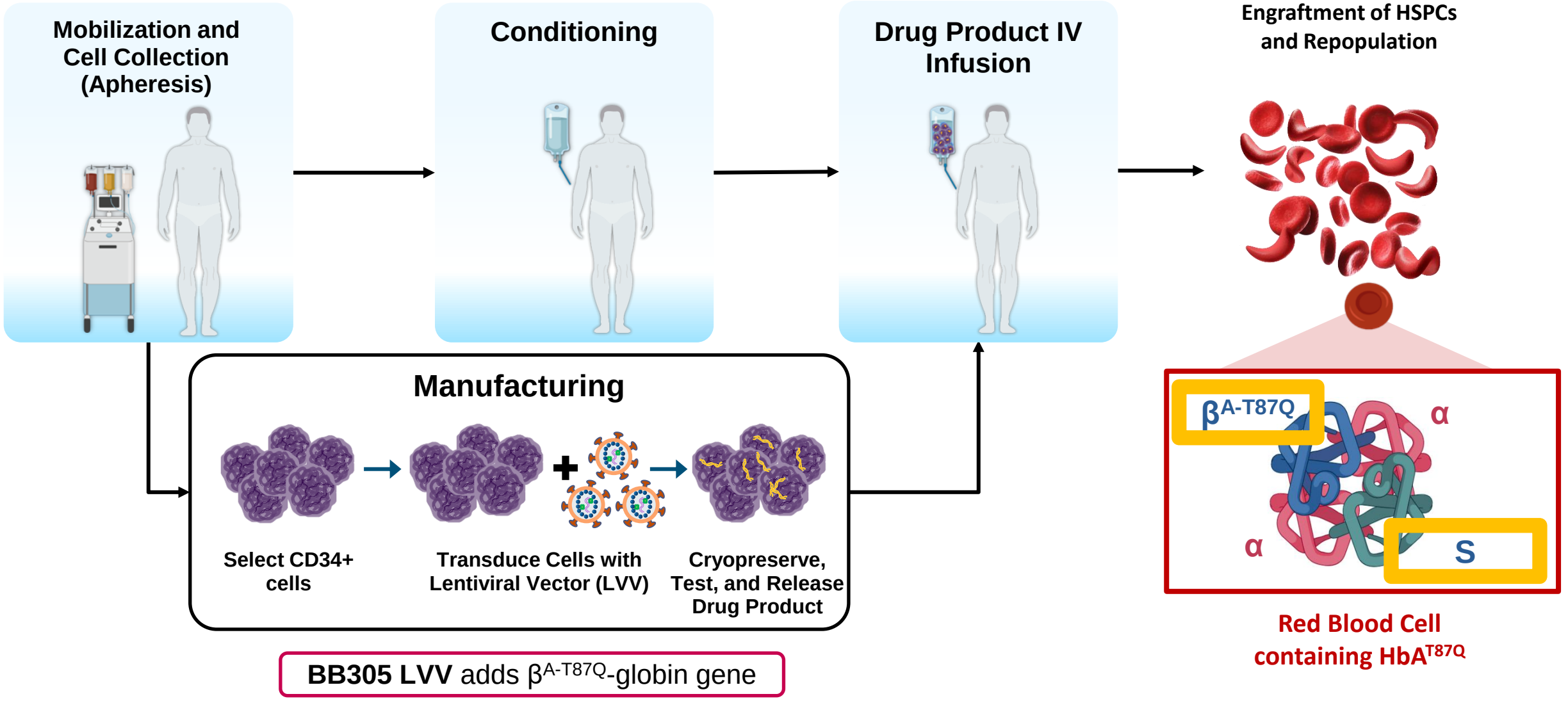
~1:16,300 Hispanic-American Births

Incidence of Sickle Cell Trait[3]

~1:13 African-American babies born with sickle cell trait

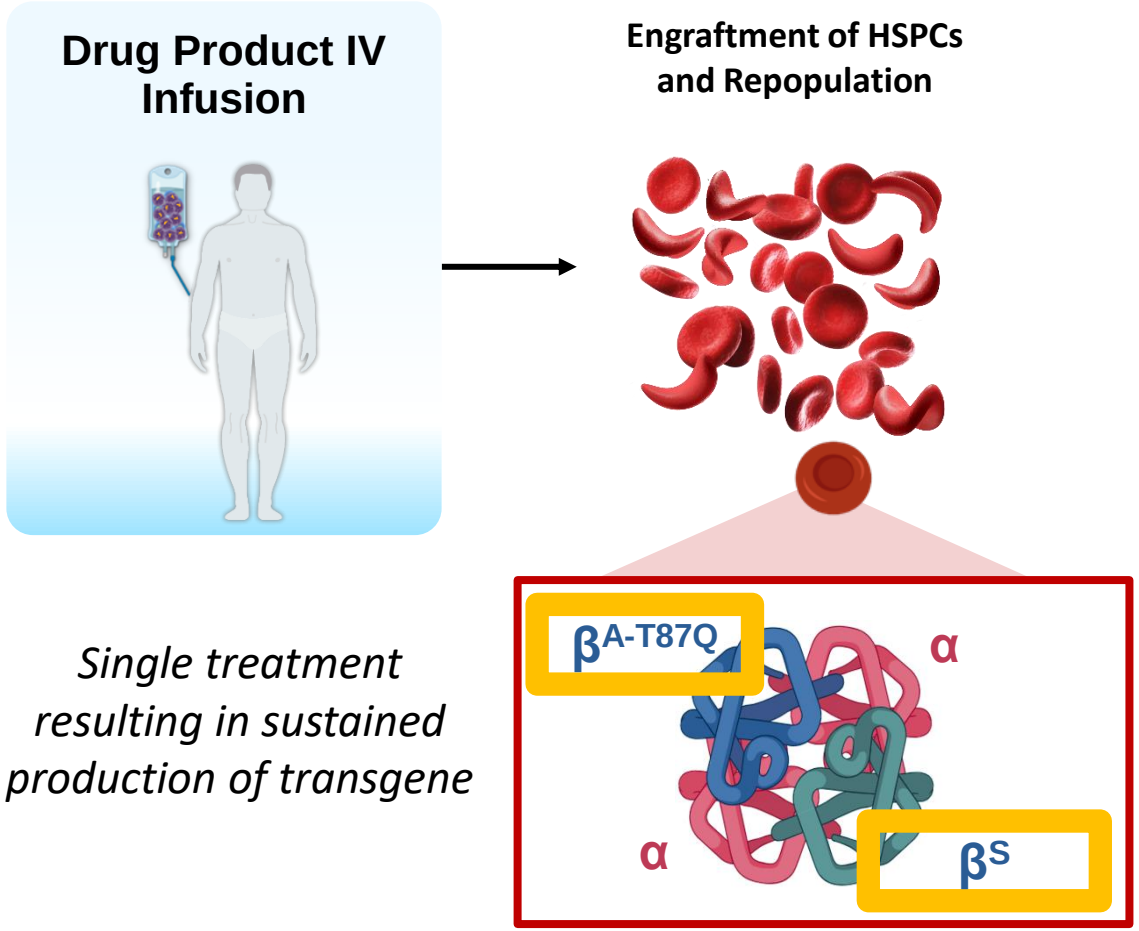
1. Kato, et al. *Nat Rev Dis Primers*. 2018;4:(18010):1-22; 2. Sundd P, et al. *Annu Rev Pathol*. 2019;14:263–292; 3. Hassell KL. Population estimates of sickle cell disease in the U.S. Am J Prev Med. 2010;38(4 Suppl):S512 521

lovo-cel Produces Functional Adult Hemoglobin Referred to as HbA^{T87Q}



HbA^{T87Q}: hemoglobin containing $\beta^{A\text{-}T87Q}$-globin; IV: intravenous

Development of lovo-cel for SCD



Single treatment resulting in sustained production of transgene

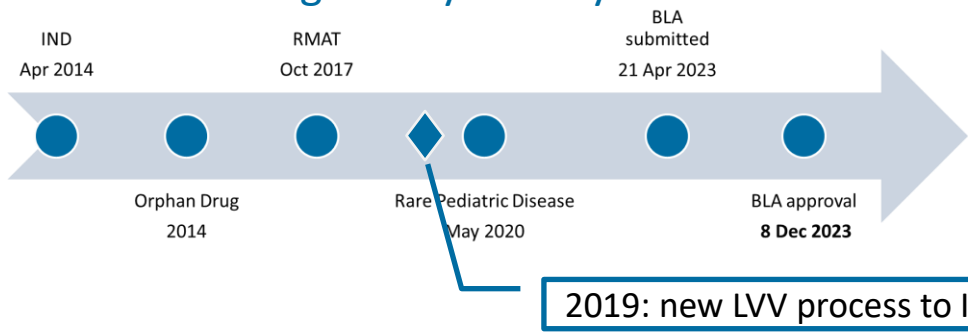
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 FEBRUARY 17, 2022 VOL. 386 NO. 7

Biologic and Clinical Efficacy of LentiGlobin for Sickle Cell Disease

J. Kanter, M.C. Walters, L. Krishnamurti, M.Y. Mapara, J.L. Kwiatkowski, S. Rifkin-Zenenberg, B. Aygun, K.A. Kasow, F.J. Pierciey, Jr., M. Bonner, A. Miller, X. Zhang, J. Lynch, D. Kim, J.-A. Ribeil, M. Asmal, S. Goyal, A.A. Thompson, and J.F. Tisdale

Regulatory History



HbA^{T87Q}: hemoglobin containing β$^{A\text{-}T87Q}$-globin; IV: intravenous; Lentiglobin for SCD: alternate name for lovotibeglogene autotemcel during development

1. Kanter, Walters et al. N Engl J Med 2022; 386:617-628

The CMC Challenge in Autologous CGTs:
Delivering on the Promise of Early Clinical Success for a Successful Product

Where we were (2019):

- Clinical manufacturing facilities
- Limited network of QTCs centered on clinical investigation sites
- Development-phase analytical strategy with multiple exploratory / characterization testing approaches
- Constrained supply for lentiviral vector with a “scale-challenged” manufacturing process

Where we needed to be:

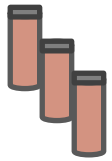
- Commercial manufacturing facilities with scale-out potential
- Broad network of Qualified Treatment Centers aligned with market geography
- Robust and efficient analytical control strategy for all components of manufacture
- Robust and efficient vector supply

Lentiviral Vector Supply Scenarios during Development of lovo-cel

Early LVV Manufacturing Process (adherent cell culture – aLVV)

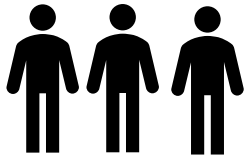
Cell culture flasks

Volumetric limits on culture scale limits fill volume



Lower titer process consumes more LVV vials per DP manufacture

Fewer patients treated per aLVV lot



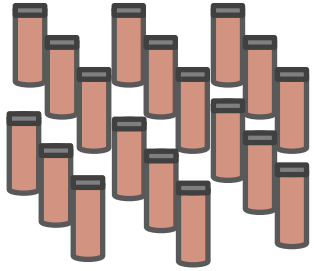
LVV Development Goals:

1. **Synergistic titer and production scale improvements vs. aLVV**
2. **Maintain potency (ability to transduce CD34+ cells) vs. aLVV**

Commercial LVV Manufacturing Process (suspension cell culture – sLVV)

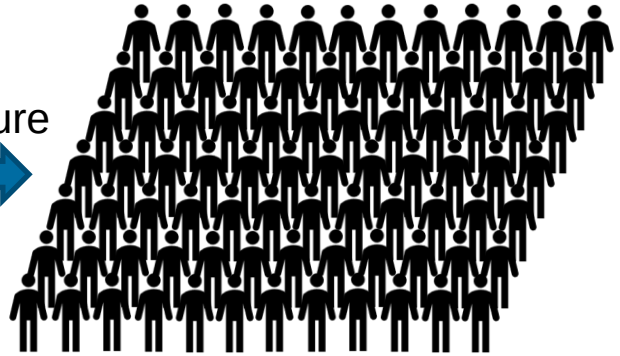
Single-Use Bioreactor

Larger culture volume yields more vials at fill

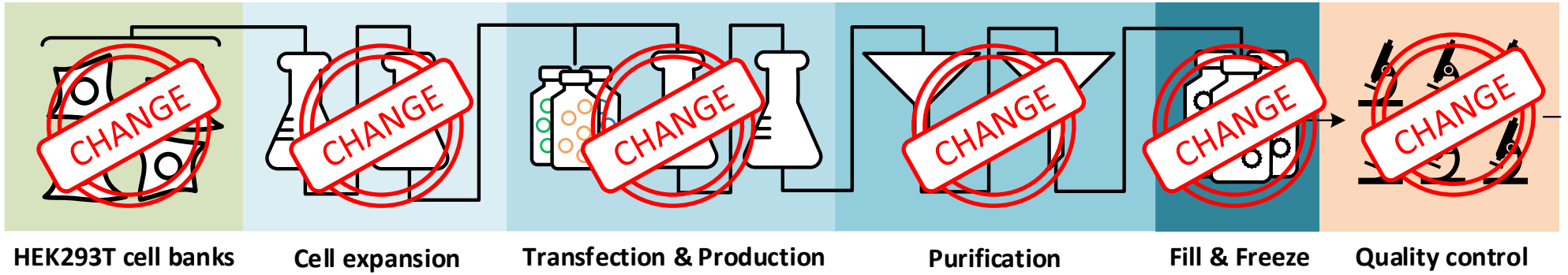


Higher titer process consumes fewer LVV vials per DP manufacture

Potentially **10 – 40X** more patients treated per sLVV lot



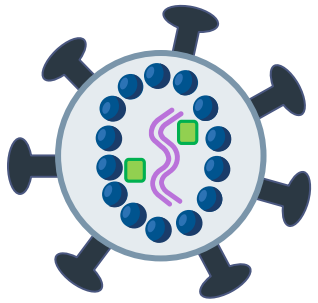
Summary of Process Changes for sLVV



- Suspension-adapted cell banks for production cell line
- New plasmid design to eliminate use of antibiotics in their manufacture
- Higher intensity, larger scale cell expansion and production
- Improved transient transfection process step
- Larger scale and more robust downstream purification steps
- Improved formulation and decoupling bulk and filling steps for ease of manufacturing
- Test method and site changes

Impact of Process Changes on Quality Attributes of LVV

Intended? Expected? Impactful?



- Pol (reverse transcriptase/integrase/protease)
- Gag (Capsid/Matrix/Nucleocapsid)
- Envelope

- Increased viral titer per unit volume
- Changes to product-related and process-related impurity profiles
- Extensive characterization panel showed LVV to have similar structural and biophysical properties
- Functional potency of LVV not adversely impacted as assessed by:
  - in vitro measures of transduction efficiency in CD34+ cells derived from health donors
  - in vitro measure of functional biological activity in differentiated CD34+ cells derived from SCD patients relative to untransduced control
  - in vitro measure of functional protein expression in a sustainable transducible cell line, reported relative to well characterized reference standard

… is comparability in vector component alone sufficient?

Figure adapted from https://en.wikipedia.org/wiki/Viral_vector accessed 25Apr2022

Overall Comparability Strategy Overview

1. CMC strategy to introduce late-stage process changes required the successful demonstration of ***multi-component***, multi-stage analytical comparability
2. Methodology for establishing "comparability acceptance criteria" was ***prospectively set***, scientifically and ***statistically justified***, and ***derived from*** supportive CMC data from lovo-cel lots dosed during clinical studies used to assess safety and efficacy in BLA

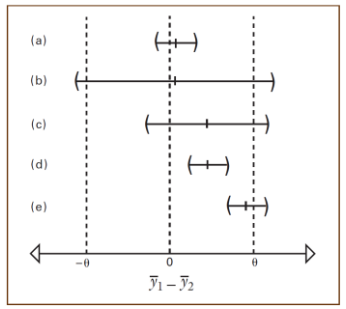
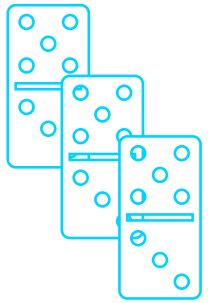


FIGURE 1. Comparison of two-sample *t*-test and TOST in terms of confidence intervals.

The conclusions for each scenario with a *t*-test and TOST, respectively, would be (a) equal and equivalent, (b, c) equal but not equivalent, (d) not equal but equivalent, (e) not equal and not equivalent.



3. For any Quality Attributes that is not determined to be analytically comparable, justification for no adverse impact on downstream element(s) is needed (LVV → drug product → clinical data)

Lessons Learned Along the Way …

1. Major process changes (even in late-stage development!) are possible but present challenges with respect to regulatory expectations and technical execution
2. Avoid making multiple simultaneous process changes to be able to resolve impact
3. Testing method and site changes during development compound the comparability challenge
   - Robust method bridging or equivalency studies and/or historical lot testing for stable attributes
4. Maintain sufficient retains for testing of pre-change lots with new and revised methods
5. Release Test Specification Acceptance Criteria setting strategy:
   - Understand sources of variability in process data and methods
   - Ideally, establish process consistency ranges from representative post-change process data
   - Leverage clinical experience to establish impurity limits
6. Maintain methods for product characterization through late development and early commercialization and for evaluation of major process changes
7. Establish continuity strategy in reference standard-based methods

thank you

Analytical Development

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