A Gene Therapy's Journey:

Lessons Learned in Commercializing an LVV-Based Autologous Gene Therapy

bluebird bio: a primer

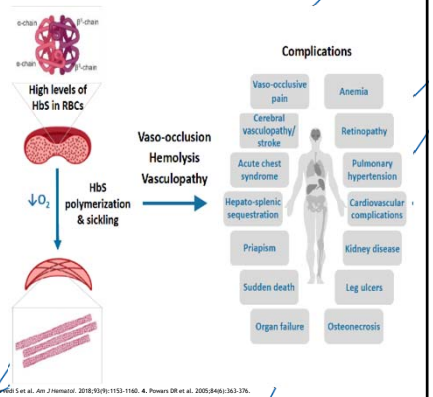
- 10+ years since inception
- 200+ patients studied across 8 clinical trials
- 250+ drug product lots manufactured across 3 programs
- > 800 patient-years of experience with gene therapies
- Up to 22,000 patients in the U.S.[1]
- Focus on LVV-based autologous HSC gene therapies
- 3 FDA approved therapies 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

SCD: Disease Overview

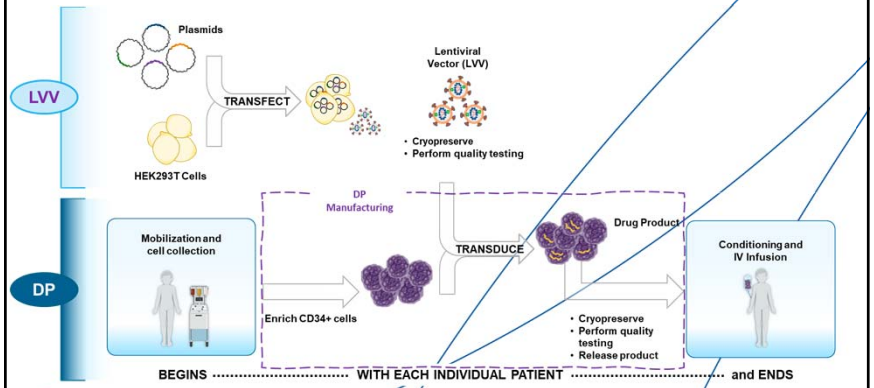
Disease State

- Genetic disease caused by a point mutation in the B-globin gene (*HBB*) that results in the production of an abnormal B-globin (β_S)[1]
- Hemoglobin S (HbS) polymerization, due to low oxygen tension, cellular dehydration, and acidosis, deforms RBCs into the sickle shape characteristic of the disease. Increased fragility and breakdown of RBCs leads to anemia and vascular bed damage.[1,2]
- The disease is associated with acute, unpredictable vaso-occlusion that leads to excruciating pain crisis and even death, as well as progressive damage to blood vessels, which can lead to end-organ damage. SCD results in significant medical burden, profound impact on quality of life, and premature mortality[3,4]

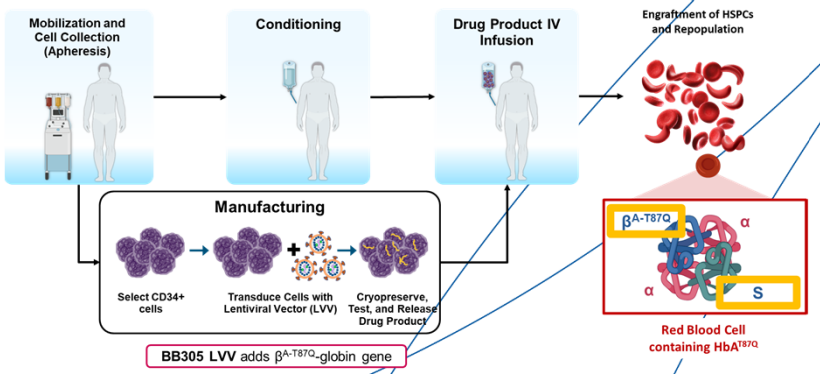


RBCs, red blood cells.
Reference: 1. Kato GJ et al. *Nat Rev Dis Primers.* 2018;4:18010. 387: 2554-64. **2.** Khemani K et al. *Ochsner Journal.* 2019; 19:131-137. **3.** Chakravorti S et al. *Am J Hematol.* 2018;93(9):1153-1160. **4.** Powars DR et al. 2005;84(6):363-376.
https://www.bluebirdbio.com/~/media/bluebirdbio/Corporate%20COM/Files/Lyfgenia/LYFGENIA_Prescribing_Information.pdf

Manufacturing process for autologous, LVV-based cellular gene therapy

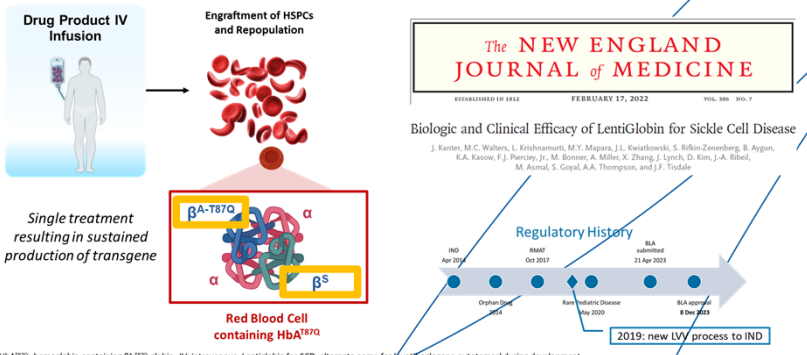


Iovo-cel Produces Functional Adult Hemoglobin Referred to as HbA^{T87Q}



HbA^{T87Q}: hemoglobin containing β^{A-T87Q}-globin; IV: intravenous

Development of Iovo-cel for SCD



HbA^{T87Q}: hemoglobin containing β^{A-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

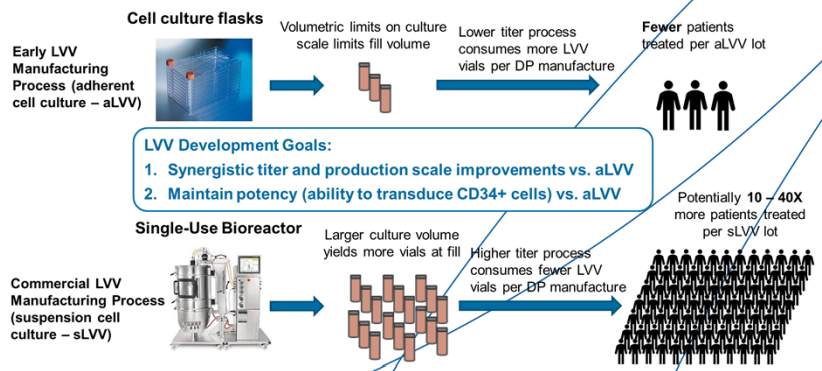
Where we were (2019):

- Clinical manufacturing facilities
- Constrained supply for lentiviral vector with a "scale-challenged" manufacturing process
- Development-phase processes with multiple exploratory & characterization testing approaches
- Transactional relationship with suppliers
- Limited network of clinical investigation sites

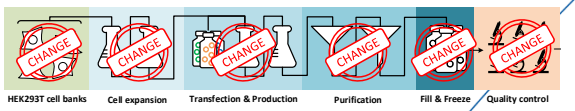
Where we needed to be:

- Commercial manufacturing facilities with scale-out potential
- Robust and cost-efficient vector supply
- Robust control strategy with validated methods for all components of manufacture
- Strategic and transparent collaborations with key suppliers
- Broad network of Qualified Treatment Centers aligned with market geography

Lentiviral Vector Supply Scenarios During Development



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?

- 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 three orthogonal transduction-based assays

… is comparability in vector component alone sufficient?

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

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

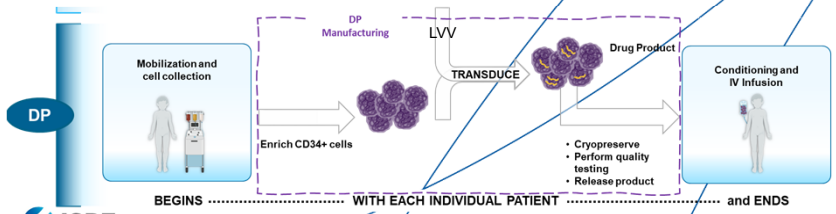
Lessons Learned Along the Way …

1. Major process changes (even in late-stage development!) are possible but require careful alignment with regulators on expectations for 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

Lessons Learned in the Drug Product Process

Challenges overcome during scale-up, process validation, and successful commercial readiness include:

- Starting Material Variability (Patient Cells)
- Process Performance Qualification Complexity
- Establishing an exceptional relationship with contract manufacturer



Starting Material Variability

From a manufacturing perspective, attributes of the patient cells are highly variable.

- Volume – 4-fold change
- Total nucleated Cells – 6-fold change
- CD34+ Cell Purity – 17-fold change

Sickle Cell Disease characteristics:

- Chronic anemia
- Increased cellular adhesion

These disease consequences, among other factors, contribute to the significant variability in the type and quantity of CD34+ hematopoietic and progenitor cells present in the bone marrow[1].

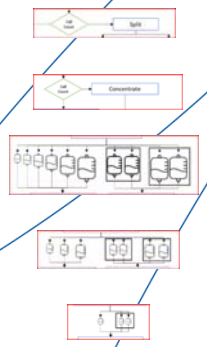
1. Cavazzana et al. 2017; Schroeder et al. 2017; Lagresle-Peyrou et al. 2018; Sundd et al. 2019a; Leonard et al. 2019

Starting Material Variability

The wide range of variability in starting material from Sickle Cell patients necessitates an autologous drug product manufacturing paradigm that can manage the variability at specified process steps using well defined decision trees and clearly defined options.

There are several decision points in the process where technicians select an option based on volume or cell counts. These options are designed to optimize conditions or eliminate/minimize the discard of patient cells to accommodate process limitations.

From a Quality point of view, options in the manufacturing process require careful consideration. Each option was thoroughly characterized and implemented in the clinical phase of the program resulting in a robust commercial process.



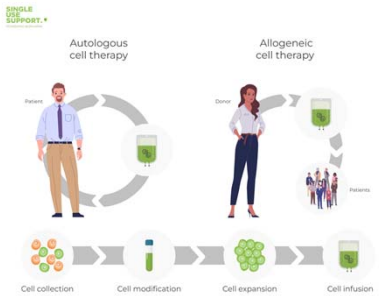
Process Performance Qualification Complexity

Process performance qualification (PPQ) is the stage where the process design is evaluated to determine if the process is capable of reproducible commercial manufacturing[1].

In biologics, vaccines, or pharmaceuticals, the product is held until a commercial license is granted.

1. FDA Guidance for Industry, Process Validation: General Principles and Practices

Process Performance Qualification Complexity



https://www.susupport.com/knowledge/cell-gene-therapy/autologous-allogeneic-cell-therapy

Autologous cell and gene therapies are one batch per patient who is both the donor and the recipient.

Process consistency can be demonstrated using healthy donor, or surrogate, material in place of patient cells. However, patient cells are required to demonstrate potency and the full circle of product traceability.

Process Performance Qualification Complexity

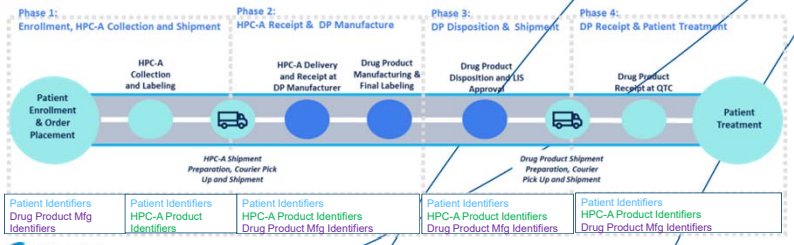
In autologous cell and gene therapies, holding PPQ batches until health authority approval is problematic.

From PPQ batch manufacture to approval could be 18 months or more which could be less than the initial expiry granted.

In our case, we had an ongoing clinical trial which allowed us to include patients in our PPQ campaign, obtain potency data, demonstrate the full circle of product traceability and perform clinical release on those batches.

Process Performance Qualification Complexity Traceability Overview

Drug product chain of identity (COI) or traceability involves the collective tracking and control of subject cells through procurement, drug product manufacture, and infusion.



Supplier Relationships

If you are using contract manufacturers, collaboration is essential to success.

- Communication channels at every level and across levels are critical.
  - Weekly, sometimes daily, joint working team meetings
  - Monthly or preferably bi-weekly joint leadership team meetings
  - Monthly or quarterly joint steering committee meetings
- Person in plant (PIP) is invaluable for gaining an inside perspective and troubleshooting.
- The tone of all this collaboration needs to be “We’re in this together, your success is our success.”
  - This approach unlocks another level of transparency and enables even more collaboration.
  - During Commercial Readiness phase, we had unprecedented access to the site.
    - Expanded PIP opportunities at multiple levels of the organization substantially enhanced site readiness as well as our own preparedness.
- This approach can apply to contract testing labs as well.
- Contracts are useful and essential, but the relationship is key to achieving success.

thank you

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