Optimizing Analytical Release Testing Through Reduced Volume and Turn-around Times

MICHAEL GIFFIN
ANALYTICAL DEVELOPMENT

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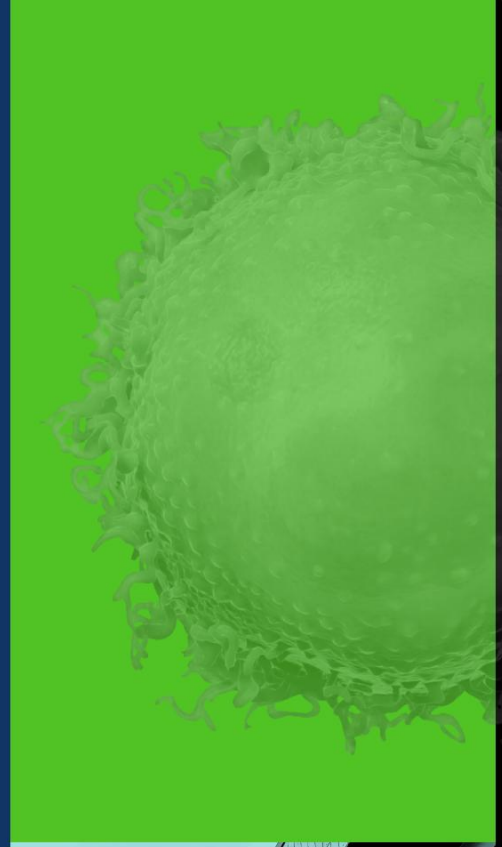
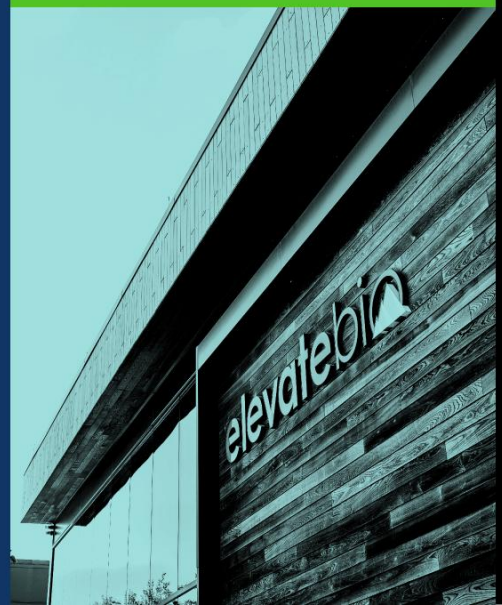


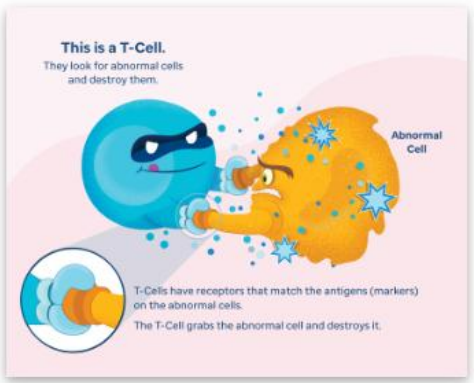


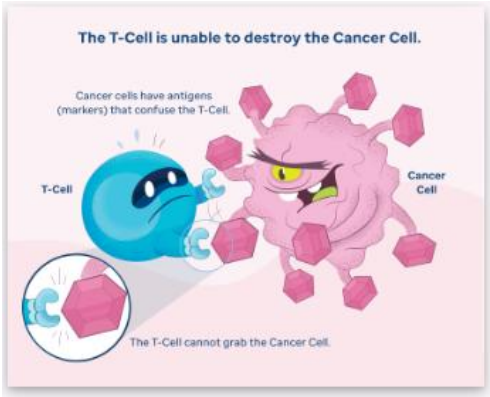
JUNE 12TH, 2025

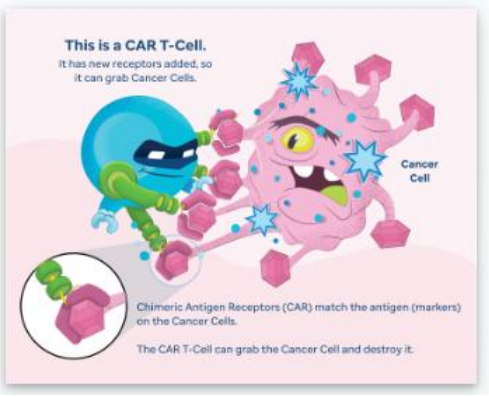
THE DAWN OF A NEW ERA

FDA Approval of Kymriah and Yescarta (2017)

- The first approved Cell and Gene therapies in the US by FDA were the CAR-T cell therapies for cancer treatment: Kymriah and Yescarta
- Since their approval in 2017, **thousands of patients** have been treated with Kymriah and Yestcarta worldwide.
- Kymriah is a chimeric antigen receptor T cell (CAR-T) therapy used to treat certain types of **aggressive B-cell lymphomas and acute lymphoblastic leukemia (ALL)** in children and young adults
- Yescarta is a chimeric antigen receptor T cell (CAR-T) therapy used to treat certain types of **aggressive non-Hodgkin lymphoma.**







How immunotherapy fights cancer cells
https://boostershotmedia.com/car-t-cell-therapy/

Photographs of Emily Whitehead.

Emily Whitehead (6 years old) & Dr Grupp, CHOP. Emily 1-, 5- and 10-years cancer free.

Emily Whitehead, diagnosed with acute lymphoblastic leukemia (ALL) became the first pediatric patient to be treated with a CAR-T therapy in 2012.

The CAR-T therapy she received (Kymriah®, antiCD19 CAR-T) became the first approved gene therapy by FDA in 2017, transforming cancer immunotherapy.

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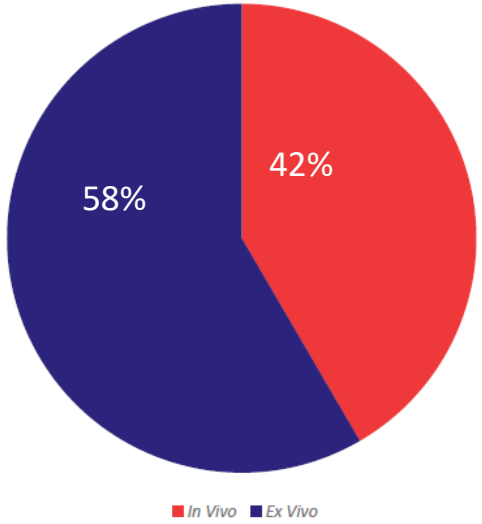
* See Novartis and Kite Pharma, a subsidiary of Gilead Sciences, for an accurate number of treated patients

CELL AND GENE THERAPIES Q1 2024 LANDSCAPE

Worldwide Gene Therapy Pipeline

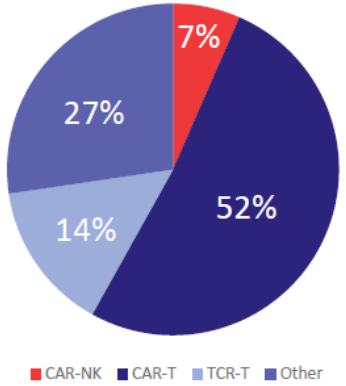
American Society of Gene + Cell Therapy

In vivo vs. *Ex vivo* genetic modification



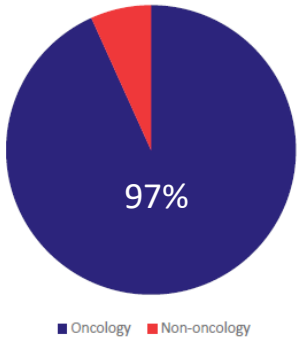
- *Ex vivo* genetic modification is more widely used for gene therapies in pipeline development
- In Q1 2024, *in vivo* delivery techniques were used in 42% of gene therapies

Genetically modified cell therapy breakdown



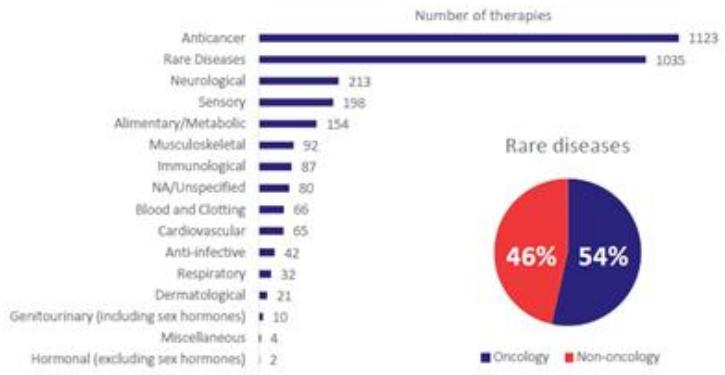
Other: TCR-NK, CAR-M, and TAC-T

CAR-T breakdown

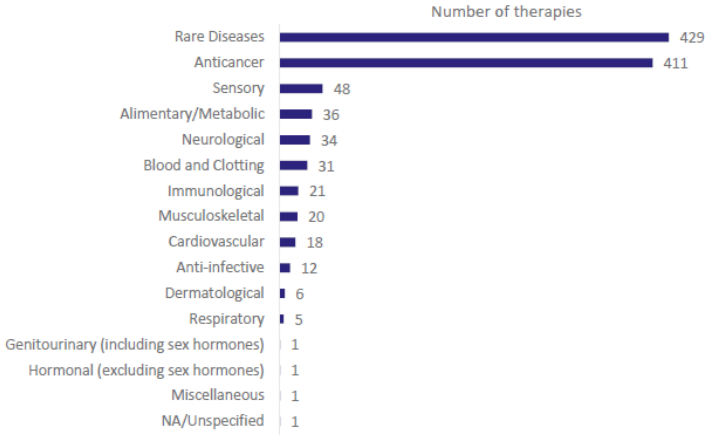


Non-oncology indications included scleroderma, HIV/AIDS, and autoimmune disease (unspecified)

Number of therapies from preclinical through pre-registration



Therapies in the clinic (excludes preclinical development)



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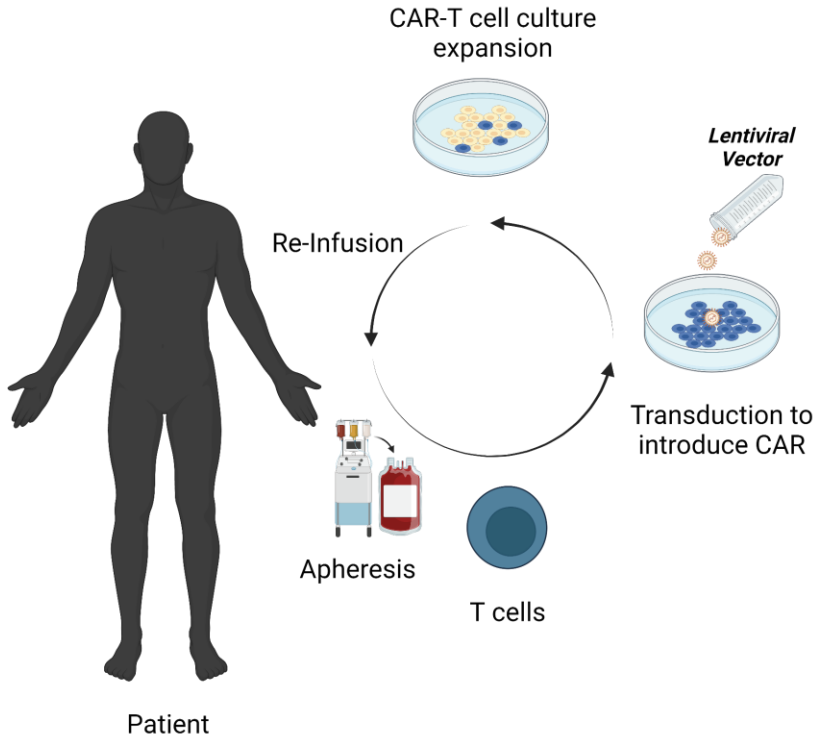
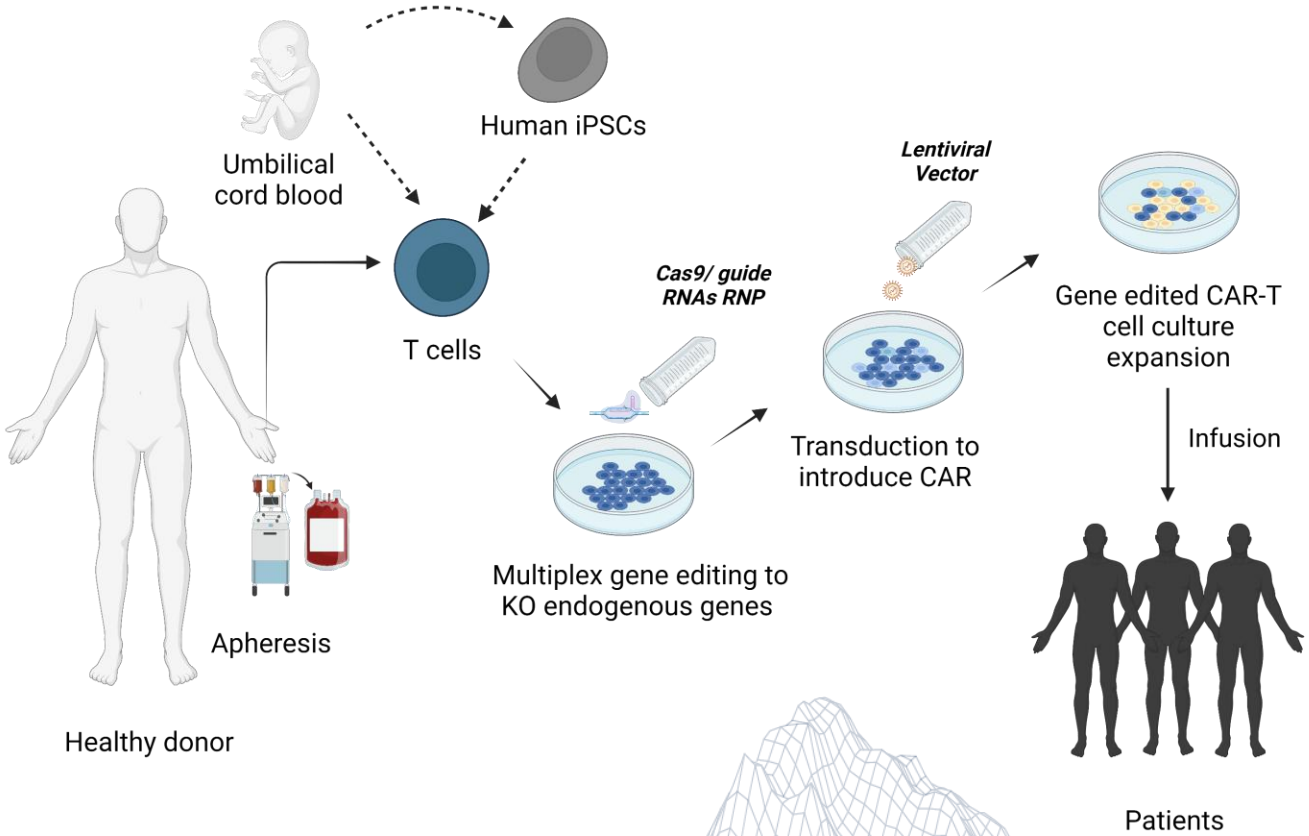
https://www.asgct.org/publications/landscape-report

Autologous CAR-T Is Still the Dominant Paradigm vs. Allogeneic



Autologous CAR-T cells



Allogeneic CAR-T cells

Autologous Cell Therapies: Transforming Patient Care

FDA/EMA Approved Autologous Cell Therapies

CAR-T Therapies:

- Kymriah® (2017) - 83% CR in pediatric ALL
- Yescarta® (2017) - 58% CR in DLBCL
- Tecartus® (2020) - 67% CR in MCL
- Breyanzi® (2021) - 73% ORR in LBCL
- Abecma® (2021) - 72% ORR in multiple myeloma
- Carvykti® (2022) - 98% ORR in multiple myeloma

HEMATOLOGIC MALIGNANCIES:

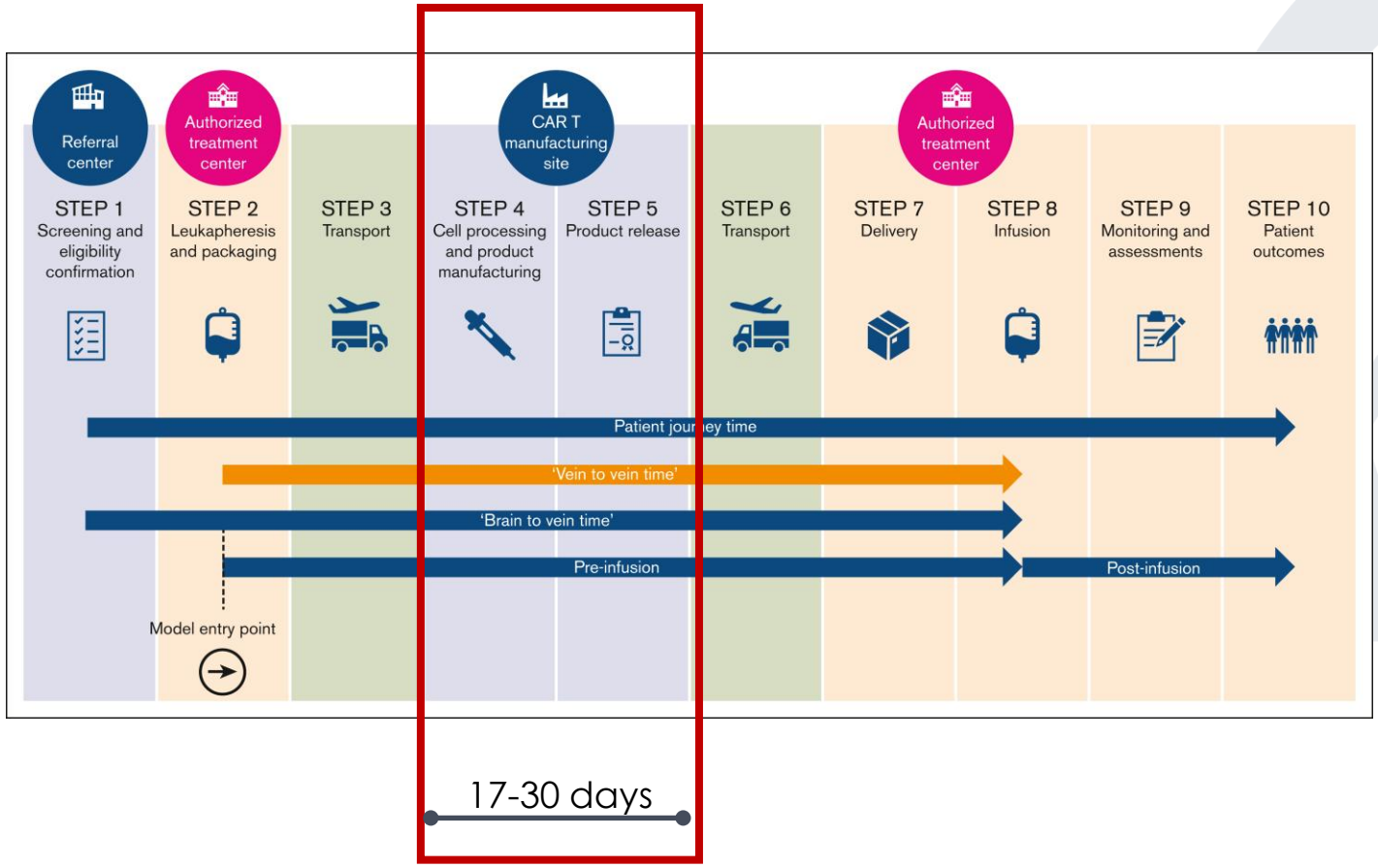
- DURABLE RESPONSES IN REFRACTORY/RELAPSED PATIENTS
- 40-50% LONG-TERM REMISSION IN LYMPHOMAS
- MEANINGFUL SURVIVAL BENEFIT IN MULTIPLE MYELOMA

REAL-WORLD EVIDENCE:

- MEDIAN SURVIVAL EXTENDED BY 2+ YEARS IN DLBCL
- QUALITY OF LIFE IMPROVEMENTS ACROSS INDICATIONS
- ONE-TIME TREATMENT VS. CONTINUOUS THERAPY

The Clock is Ticking: Time-Sensitive Manufacturing in Autologous Cell Therapy

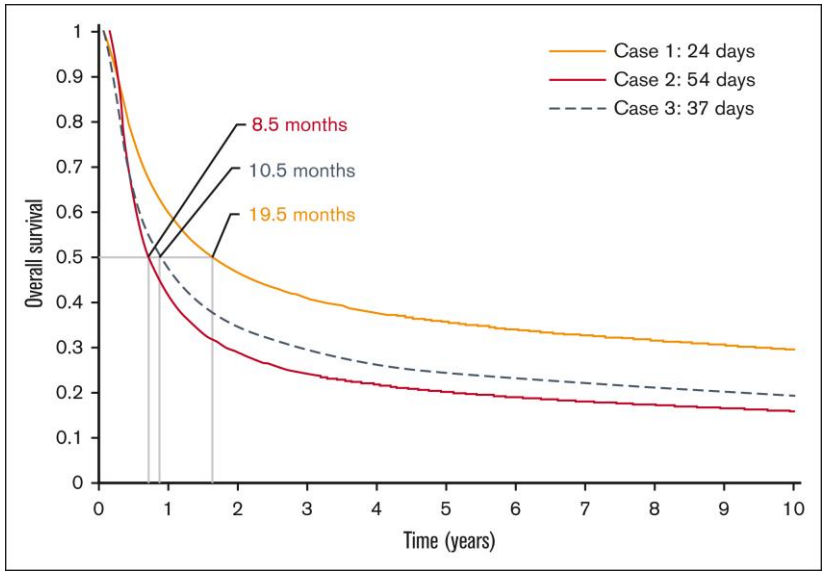
Product manufacturing and Release Testing Represent the majority of vein-to-vein time



The Vein-to-Vein Time May Influence Clinical Outcomes

DECREASED V2VT CORRELATES WITH INCREASED OVERALL SURVIVAL

Base-case survival extrapolations for all patients based on cohort average V2VT and median survival



Sachin Vadgama, et al, Blood Adv, 2024.

DECREASING TIME TO TREATMENT MAY IMPROVE PATIENT OUTCOMES

- Average vein-to-vein time (V2VT) timelines for commercial products (17-30 days)
- Correlations in time-to-treatment and patient outcomes
  - 10-15% of patients experience clinically significant disease progression during the manufacturing period
  - Higher complete response rates in patients with shorter time from enrollment to infusion[1]
  - Patients who progress during manufacturing window show 30-50% lower response rates overall[2]
  - Each additional week in manufacturing time is associated with a 5-11% reduction in complete response probability[3]

[1] Schuster et al., 2019; [2] Nastoupil et al., 2020; [3] Awasthi et al., 2020.

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Reducing V2VT Via Reduced Processing Time and TAT

REDUCED TESTING TURN-AROUND TIMES

- Use of alternative methods with reduced time-to-result
  - Rapid microbial methods (RMM)
  - Methods not described in a USP chapter should be validated according to USP <1223>
    - Acceptability of validation strategy should be confirmed with regulators

SHORTER MANUFACTURING PROCESS TIME

- May alter CQAs
- Typically results in reduced product yield, necessitating lower sample usage for release testing
  - Miniaturization of Assays
  - Optimization of Sampling Plans
  - Development of High-Sensitivity Assays
  - Multiplexing

Autologous Cell Therapy: Release Testing Turn-Around Times

Test Category	Typical Duration	% of Release Testing Time	% of Total Vein-to-Vein Time
Sterility	7-14 days	50-70%	10-20%
Mycoplasma	1-28 days*	10-50%*	2-15%*
Identity	4-24 hours	5-10%	1-3%
Potency	1-3 days	10-20%	2-5%
Safety (other)	1-3 days	10-20%	2-5%

- Validation of alternative methods for sterility and mycoplasma testing may allow significant reductions in overall product release turn-around time

USP Chapters on Alternative Sterility Testing Methods Offer a Simplified Path to Validated Rapid Microbial Methods

Activity	1	2	3	4	5	6	7	8	9	10	11	12	13	14	USP Chapter
Growth-based Methods															
USP <71>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
BacT/ALERT	■	■	■	■	■										72: Aug 1, 2025
Nongrowth-based Methods															
Solid Phase Cytometry	■	■													74: 2026?
ATP Bioluminescence	■	■	■	■	■										73: Aug 1, 2025
Flow Cytometry	■	■													
Molecular Methods															
PCR-based methods	■														75: TBD
NGS	■	■	■												

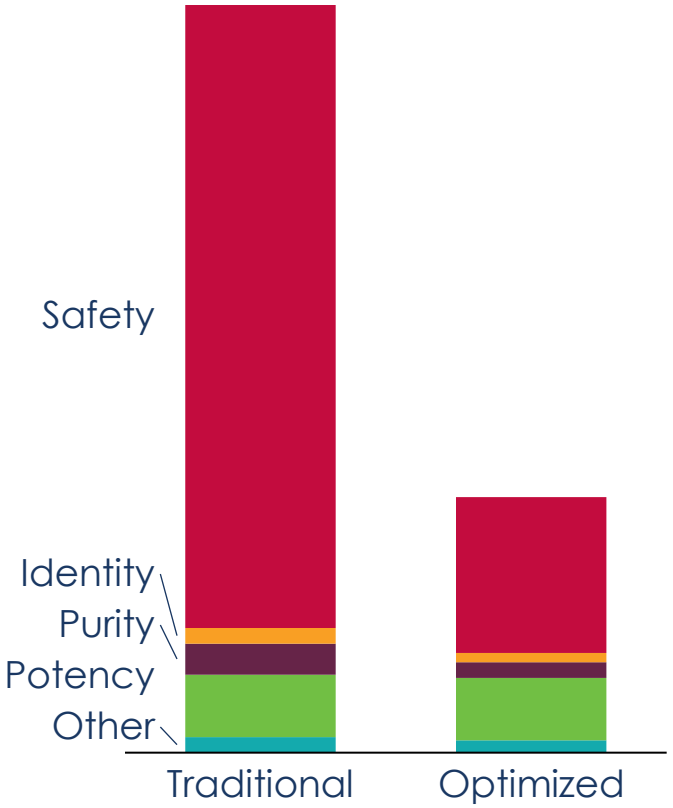
- Methods described in a USP <1000 chapter, eg 72 or 73, will need to complete primary validation and suitability.
- Methods not described in a USP chapter will need to complete full equivalence according to USP <1223>.

Reduce Time to Treatment with Shorter Duration Manufacturing

Manufacturing Protocol	Duration	Starting Cell Count	Final Cell Yield	Product Volume
Standard Protocol	10-14 days	1-2×10^9 cells	2-6×10^{10} cells	50-100 mL
Accelerated Protocol	5-7 days	1-2×10^9 cells	0.5-2×10^{10} cells	20-50 mL
Ultra-rapid Protocol	1-3 days	1-2×10^9 cells	0.2-0.8×10^{10} cells	10-30 mL

SHORTER MANUFACTURING TIMES PRODUCE LOWER TOTAL YIELDS IMPACTING TRADITIONAL SAMPLING VOLUMES

Test Methods Requiring Lower Sample Volume Will Reduce Impact on Release Testing Material Needs



Test Category	Traditional (mL)	Optimized (mL)	Volume Reduction (%)
Safety	15-25	2-5	~80%
Identity	0.2-0.5	0.2-0.5	~0%
Purity	0.3-1	0.3-1	~0%
Potency	1-3	1-3	~0%
Additional	0.2-0.5	0.2-0.5	~0%
TOTAL	**17-30**	**4-10**	**~70%**

Alternative Mycoplasma Test Methods May Allow Testing Smaller Sample Volumes

Parameter	Traditional Culture Method	PCR-Based Method	Reduction/ Improvement
Time to Result	28 days	1-2 days	93-96% reduction
Sample Volume Required	10 mL	0.2-1 mL	90-98% reduction
Minimum Cell Number	1×10^{7} cells	1×10^{5}-1×10^{6} cells	90-99% reduction
Sensitivity (LOD)	10-100 CFU/mL	1-10 CFU/mL	1-10× improvement
Specificity	Limited to cultivable species	All species (with proper primers)	Broader detection range
Suitability for Automation	Limited	Highly suitable	Improved throughput
Regulatory Status	Compendial method	Alternative method (requires validation)	Additional validation needed

- PCR-based methods offer significantly increased sensitivity compared to culture-based methods
- Validation is crucial to demonstrate that alternative method is equivalent to USP <63>/EP 2.6.7 in sensitivity and specificity
- USP <77> "Mycoplasma Nucleic Acid Amplification Tests" anticipated to publish in PF for review in mid-2026

Minimizing Sample Volume: Smart Sampling Plan Design

KEY STRATEGIES

- Method Miniaturization
  - Validate reduced-volume procedures
  - Leverage high-sensitivity instruments/methods
- Sequential Sample Utilization
  - Design workflows allowing sample reuse across tests
  - Implement hold-time stability studies as necessary to justify sequential workflows in QC labs
- Alternative Testing Strategies
  - Substitute in-process controls where appropriate
  - Implement surrogate markers consuming less material
  - Explore multiplexed analytical methods

IMPLEMENTATION CONSIDERATIONS

- Engage Regulatory Affairs in sampling plan design
- Document scientific justification/rationale
- Validate methods against standard procedures
- Implement progressive sampling plan improvements throughout product lifecycle

Transforming Cell Therapy Testing And Manufacturing

TESTING OPTIMIZATION	MANUFACTURING EVOLUTION	PATH FORWARD
• Dramatic volume reduction • TATs reduced from weeks to days • Preserves product available for dosing or retains • No compromise in product control strategy	• Reduced V2VT • Potential for improvements in clinical outcomes • Optimize process and yield considerations based on target product profile	• Regulatory: engage early on innovative testing strategies • Implement rapid methods across testing portfolio as justified by equivalency in method performance • Continuous improvement: iterative optimization throughout product lifecycle

Thank you

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