

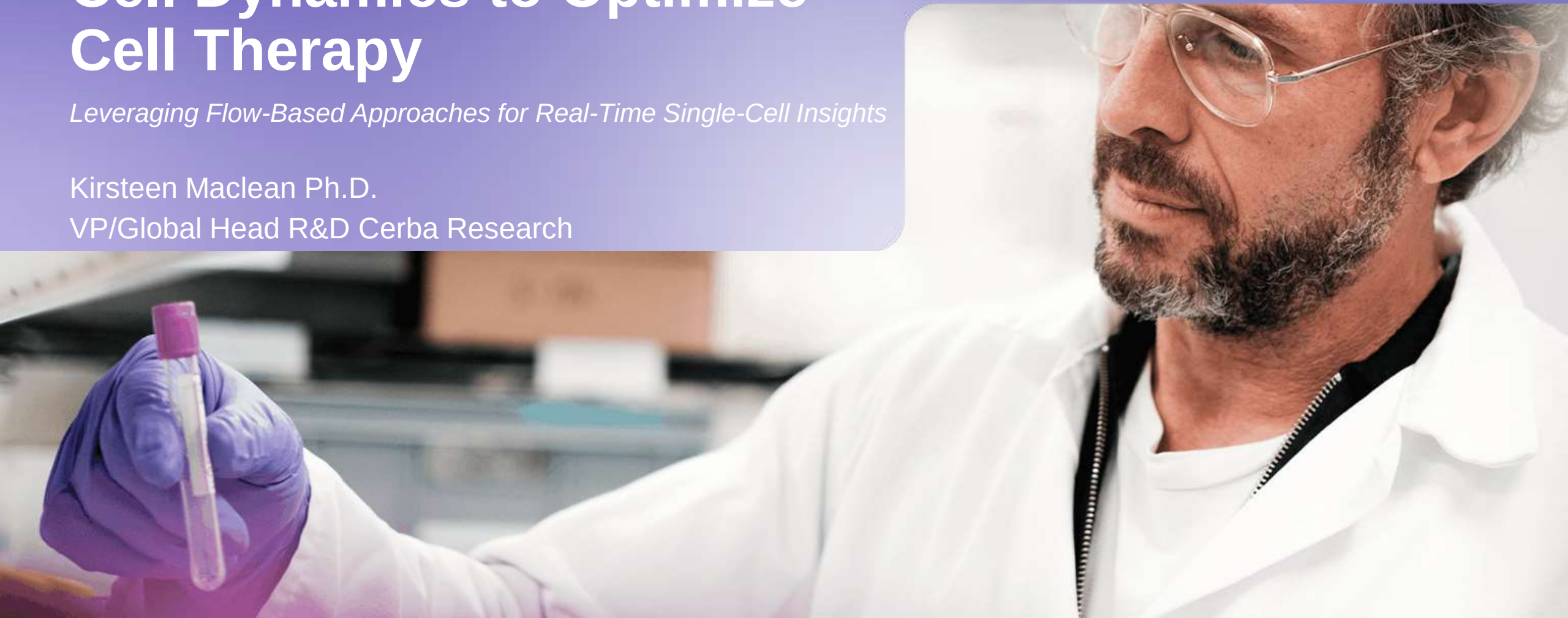
Presentation Deck



Mapping Killer and Bystander Cell Dynamics to Optimize Cell Therapy

Leveraging Flow-Based Approaches for Real-Time Single-Cell Insights

Kirsteen Maclean Ph.D.
VP/Global Head R&D Cerba Research



The opportunity: Redefining modern medicine

Cell therapy, often described as a "**living drug**," is advancing rapidly across a wide range of disease areas. However, its inherent biological complexity creates analytical, manufacturing, and regulatory challenges that differ significantly from those associated with traditional small-molecule and biologic therapeutics.



Oncology



Rare diseases



Hematology



Regenerative
medicine

Testing Challenges

Short Shelf-Life: Autologous cell therapies must be tested and infused quickly before the cells expire.

Complex Potency Assays: MoA is often complex.

Sample Volume: Limits the number of tests you can run.

Product Variability: Changes from patient to patient, making standard baseline testing difficult.

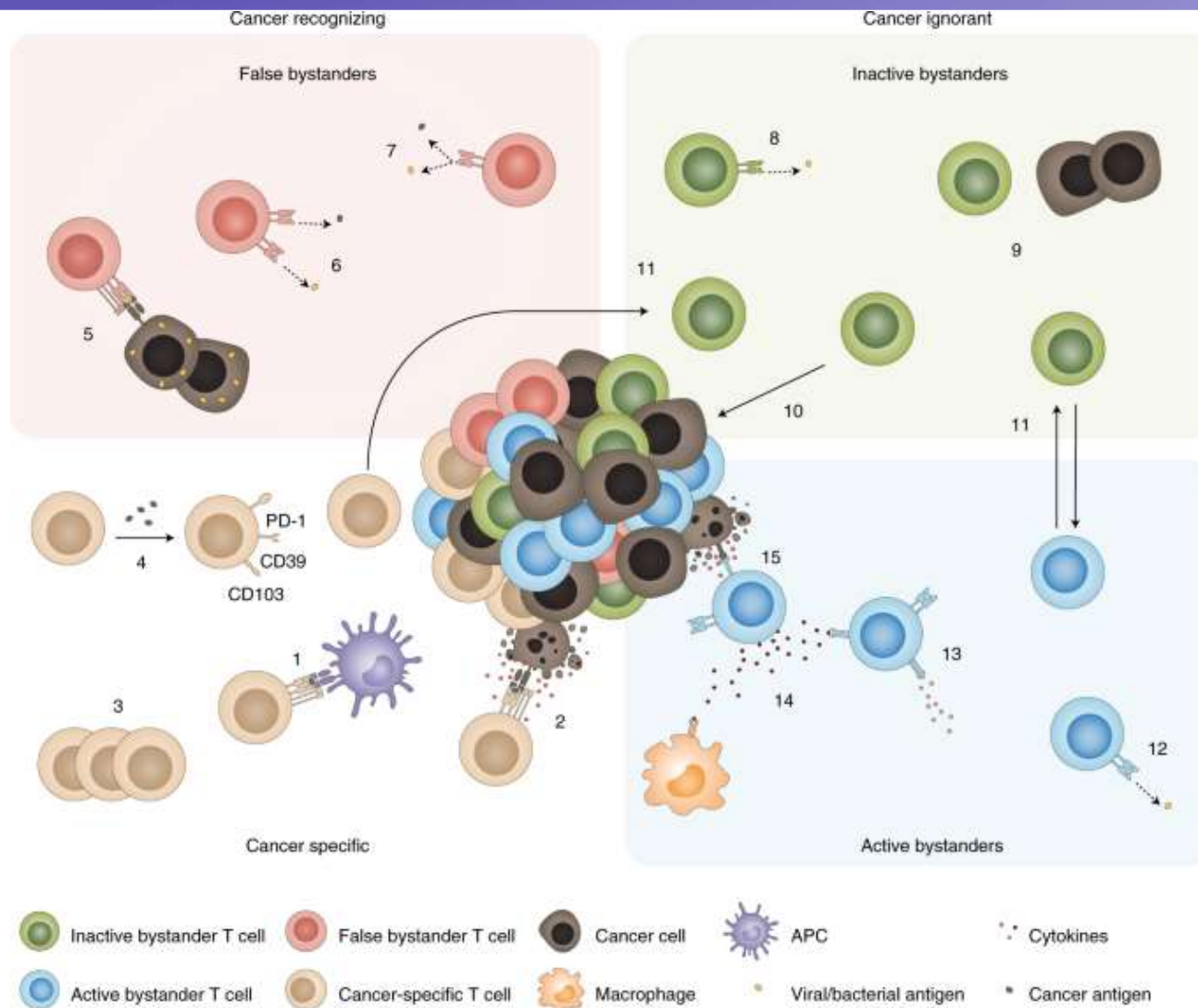
Monitoring Challenges

Long-Term Follow-Up: Regulatory require patient tracking for years to check for late side effects or vector persistence.

Immune Reactions: Detecting unexpected immune responses or toxicity,

Vector Shedding: Monitoring viral vector shedding in bodily fluids requires specialized assays to ensure safety for the environment

Mapping CAR T cell dynamics to support the next-generation cell therapy design



- Mapping killer and bystander cell dynamics allows researchers to improve cell therapy by harnessing un-targeted neighboring cells to clear tumors that evade direct detection
- **Killer Cells:** Cytotoxic T cells or natural killer cells seek out and destroy cells with specific target antigens
- **Bystander Cells:** Neighboring non-targeted cells, such as endogenous CD8+ T cells or macrophages, can be recruited to help attack the tumor

Why Cerba Research: End-to-end Laboratory Solutions

Scientific expertise, agility and scale for optimized solutions



Specialty Laboratory Services

Biomarker Services

- “Off- the-Shelf” panels
- Custom assay development
- Immunoassays
- **Flow Cytometry**
- Molecular testing
- Next Generation Sequencing
- DNA/RNA Extraction and qPCR
- Histopathology
- Cytogenetics
- BSL3 laboratory network

Bioanalytical Services

- Pre-clinical (GLP & non-GLP)
- Clinical (Primary, Secondary & Exploratory endpoints)
- Vaccines, Biologics and Biosimilars
- Full PK & Immunogenicity programs



Central Laboratory Services

- Safety and routine testing
- Global network capabilities
- PBMC Processing

Logistic Solutions for Kit & Sample Management

Standard and Premium Services for complex trials

High quality data harmonized across laboratories - Valid laboratory licenses and accreditations

- Cerba Research operates in Europe through several legal entities that qualify as Health Institutions under IVDR,
- Within these entities, LDTs are designed, manufactured, and used in compliance with the conditions set forth under Article 5(5), including their use under appropriate quality and governance frameworks, in accordance with applicable national requirements.

CGT Highlights Across Therapy Areas



~60

CGT trials
since 2018



85%+

Trials include
specialty testing



45%

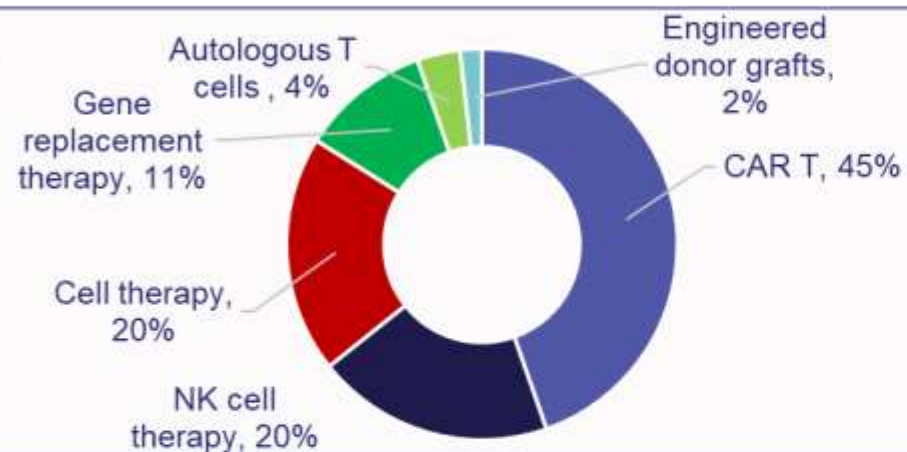
CAR T Driven



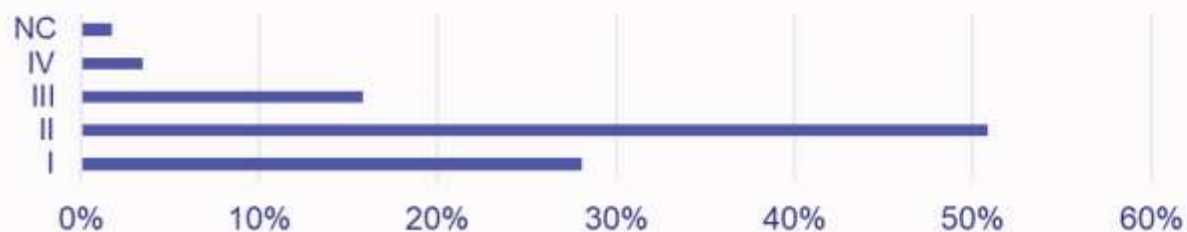
~60%

In hematological
malignancies

Asset Class



Clinical Trial Phases Overview



Market Authorization / Expansion



7

Market
authorizations /
expansions



**Approved
Indications**

- Multiple myeloma (MM)
- Diffuse large B cell lymphoma (DLBCL)
- Epidermolysis bullosa
- Spinal muscular atrophy (SMA)



3

CAR T

Approved Cell & Gene Therapies



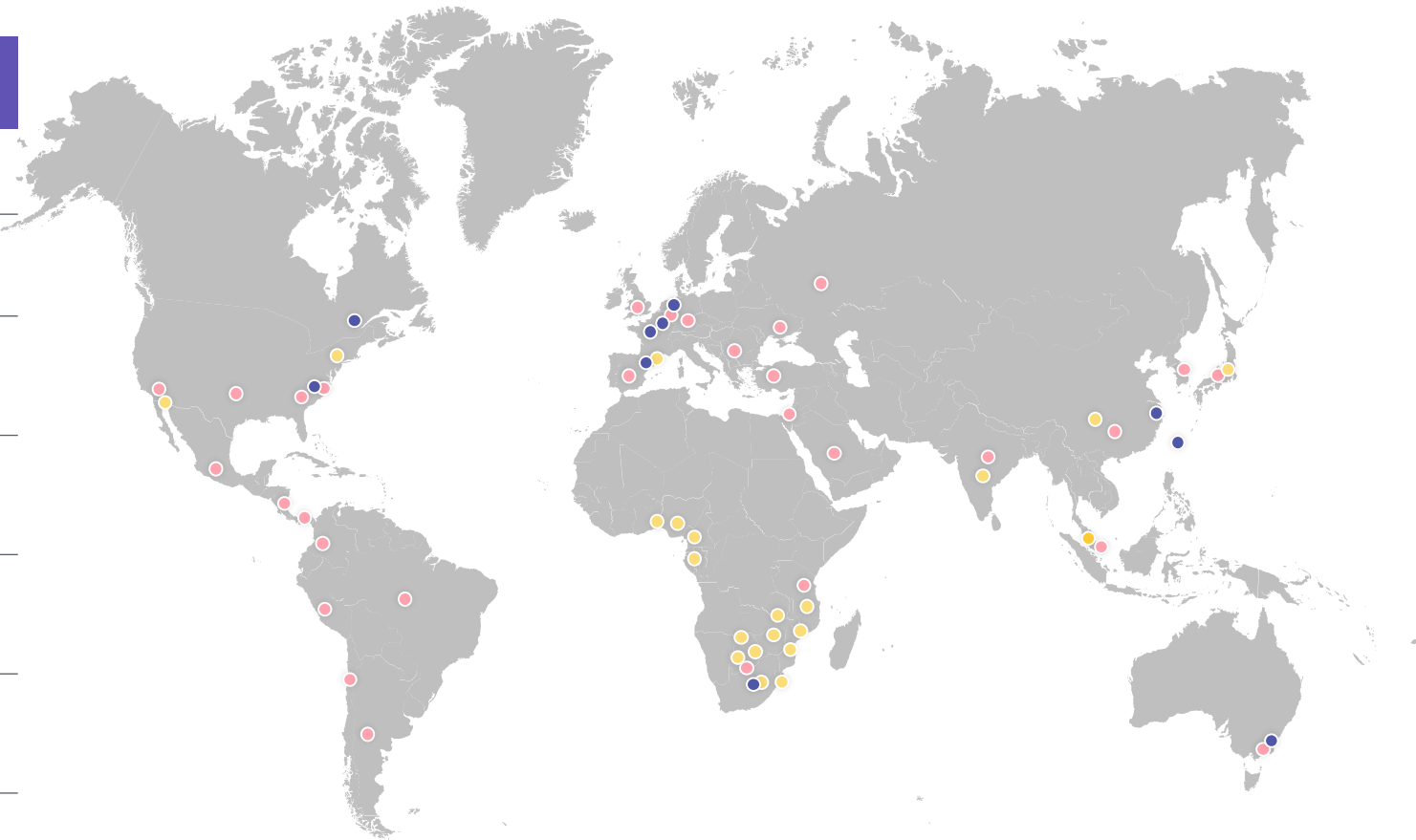
Trade name	Active ingredient	Genetic modification method	Vector type	EMA authorized indication / EMA authorization type	FDA authorized indication/ FDA authorization type
Strimvelis®	Ex vivo gene modified cells	Gene addition	Retroviral	Severe combined immunodeficiency due to adenosine deaminase deficiency (ADA-SCID)/ conditional approval	No FDA approved indication
Kymriah®	Tisagenlecleucel	CAR-T	Lentiviral	Relapsed or refractory B-cell precursor acute lymphoblastic leukemia (ALL), diffuse large B-cell lymphoma (DLBCL)/ standard approval	Relapsed or refractory B-cell pre-cursor acute lymphoblastic leukemia (ALL), large B-cell lymphoma (LBCL), follicular lymphoma (FL) / standard Approval
Yescarta®	Axicabtagene ciloleucel	CAR-T	Retroviral	Relapsed or refractory dif-fuse large B-cell lymphoma (DLBCL) and primary medi-astinal large B-cell lymphoma (PMBCL)/standard approval	Relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy, follicular lymphoma (FL)/standard approval
Zynteglo®	Betibeglogene autotemcel	Gene addition	Lentiviral	Transfusion-dependent beta-thalassemia (TDT)/conditional approval	No longer marketed in the United States
Libmeldy® Lenmeldy®	Atidarsagene autotemcel	Gene addition	Lentiviral	Metachromatic leukodystrophy (MLD)/conditional approval	Metachromatic leukodystrophy (MLD)/standard approval
Tecartus®	Brexucabtagene autoleucel	CAR-T	Retroviral	Relapsed or refractory mantle cell lymphoma (MCL), Relapsed or refractory B-cell precursor acute lymphoblastic leukemia (ALL)/standard approval	Relapsed or refractory mantle cell lymphoma (MCL), Relapsed or refractory B-cell precursor acute lymphoblastic leukemia (ALL)/ standard approval
Breyanzi®	Lisocabtagene maraleucel	CAR-T	Lentiviral	Relapsed or refractory dif-fuse large B-cell lymphoma (DLBCL), high-grade B-cell lymphoma (HGBCL), primary mediastinal large B-cell lymphoma (PMBCL), and follicular lymphoma grade 3B/ standard approval	Relapsed or refractory large B-cell lymphoma (LBCL) after two or more lines of systemic therapy, follicular lymphoma (FL)/standard approval
Abecma®	Idecabtagene vicleucel	CAR-T	Lentiviral	Relapsed and refractory multiple myeloma/standard approval	Relapsed and refractory multiple myeloma/standard approval
Skysona®	Elivaldogene autotemcel	Gene addition	Lentiviral	Cerebral adrenoleukodystrophy (CALD)/conditional approval	Cerebral adrenoleukodystrophy (CALD)/standard approval
Carvykti®	Ciltacabtagene autoleucel	CAR-T	Lentiviral	Relapsed and refractory multiple myeloma/standard approval	Relapsed and refractory multiple myeloma/standard approval
Casgevy®	Exagamglogene autotemcel	Genome editing (CRISPR-Cas9)	N/A	Severe sickle cell disease and transfusion-dependent beta-thalassemia (TDT)/conditional approval	Severe sickle cell disease/standard approval
Lyfgenia®	Lovotibeglogene autotemcel	Gene addition	Lentiviral	No EMA approved indication	Sickle cell disease/standard approval

Delivering Global Solutions with Extended Laboratory Network

Local operations for efficiency and risk mitigation



Region	Expertise
Belgium	Routine, Molecular Biology, Bioanalytical Lab, Flow Cytometry, Microbiology
The Netherlands	BSL3, Pre-clinical, Molecular Biology, Genetics/NGS, Pathology, Virology
France	Genetics/NGS, Molecular Biology, IVD, Flow Cytometry, Histopathology/IHC, Virology, Biorepository, BSL3
North America (Montreal and NY)	Routine, Genetics/NGS, Molecular Biology, IVD, Flow Cytometry, Bioanalytical Lab/PK, Histopathology/IHC, Virology
China (Shanghai)	Routine, Genetics/NGS, Molecular Biology, Flow Cytometry, Bioanalytical Lab/PK, Histopathology/IHC
South Africa (Johannesburg)	Routine, Genetics/NGS, Molecular Biology, Virology, Microbiology, BSL3, Biorepository
Australia (Sydney)	Routine, Molecular Biology, Flow Cytometry, Virology



- Specialty Laboratories
- Pre-Processing Laboratories
- Research & Partner Laboratories

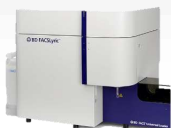
Flow Cytometry Laboratory Hubs

Delivering a Global Solution with Cerba Research's Extended Network



US (New York)

CAP/CLIA/NYS
BD FACS Lyric
Cytek Aurora*



*BD FACSLyric™: 3-laser
conventional Flow cytometry*



**Cytek® Aurora: 5-laser Spectral Flow cytometry*

Belgium (Ghent)

ISO15189 (BELAC) & CAP/CLIA
BD FACS Lyric
Cytek Aurora*

China (Shanghai)**

ISO15189 (CNAS) & CAP
BD FACS Lyric
Cytek Aurora*

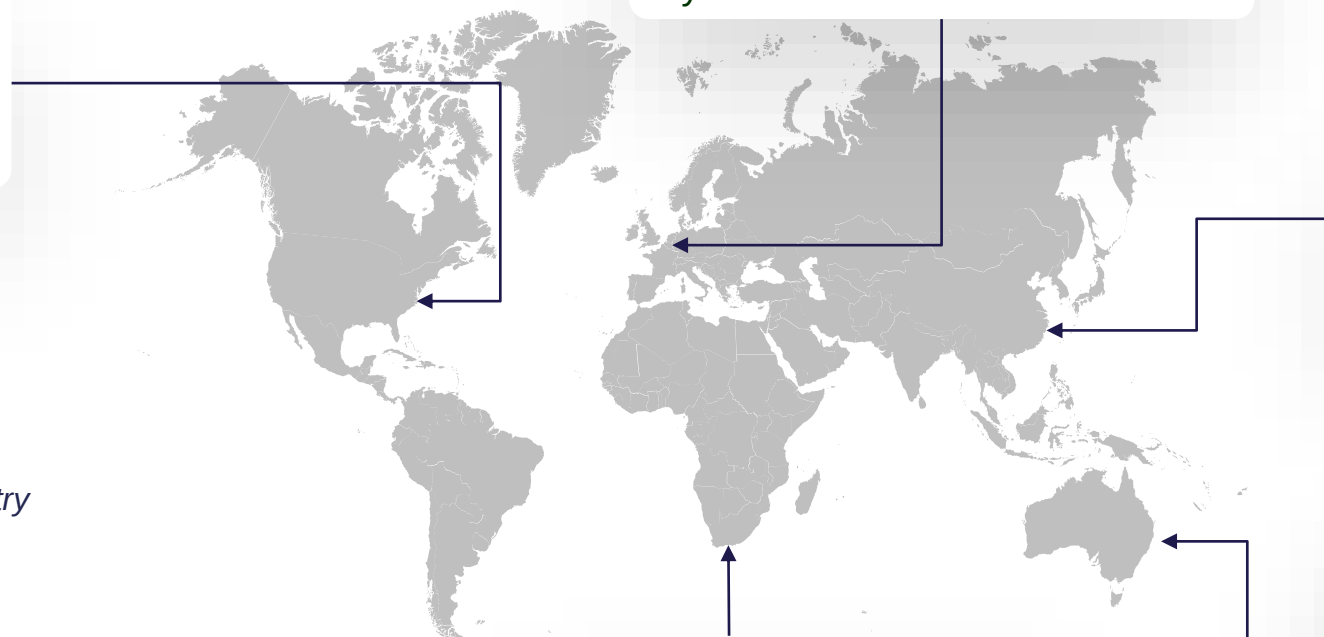
Australia (Sydney)

ISO15189 (SANAS)
BD FACS Lyric
Cytek Aurora*



South Africa

ISO15189
BD FACSCanto II



Customization: Choose from an array of assays as appropriate to your study

Areas of Expertise

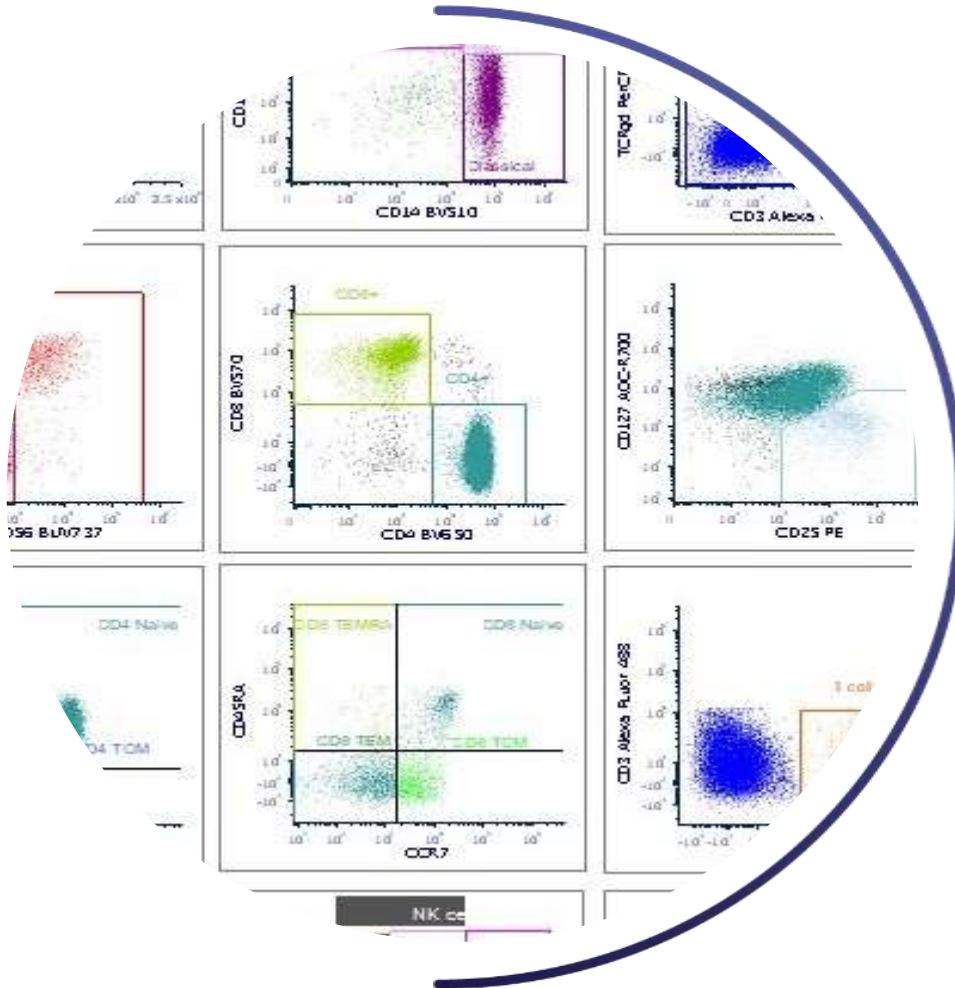
Monitor immunophenotyping and cell activation

Evaluate receptor occupancy of a drug

Monitor and characterize CAR-T cells

Detect intracellular cytokine production via intracellular staining (ICS) production.

Different matrices are used to validate the assays, including fresh and cryopreserved PBMC, blood and bone marrow aspirate.



Flow Cytometry Panels for CAR-T Therapy

CART Enumeration panel: Basic T cell markers and CAR detection antibody. Includes quantitation of CAR expression and absolute counts

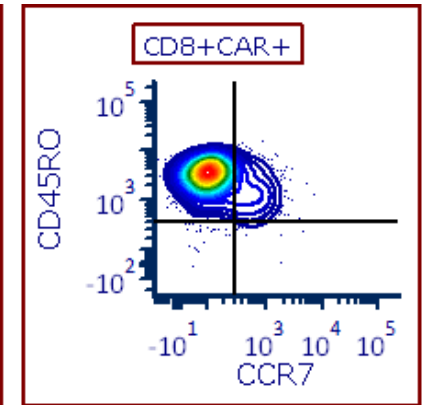
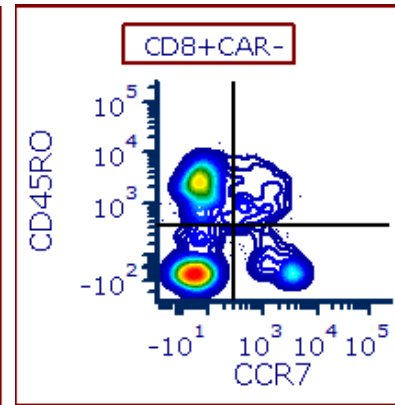
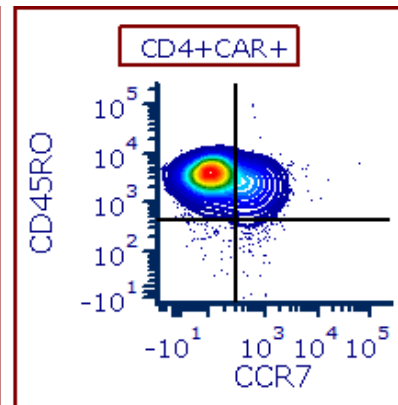
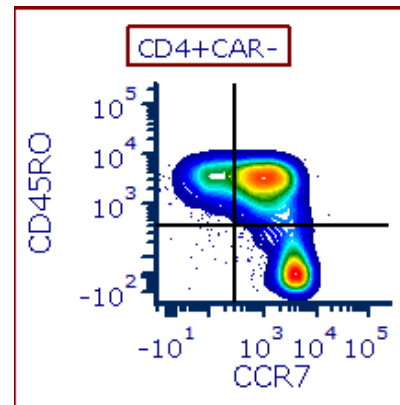
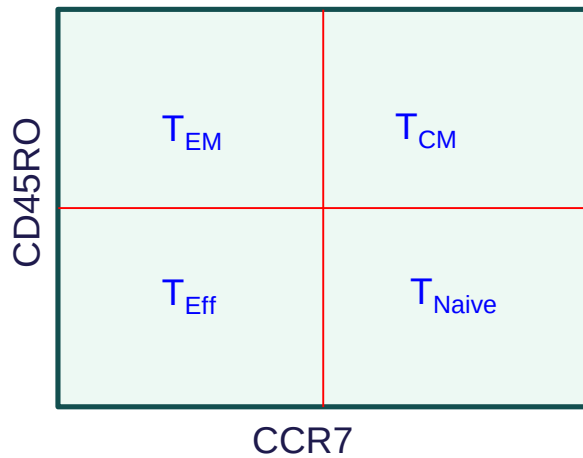
Fluorochrome	V500	PE	PerCP-Cy5.5	PE-Cy7	AF700	APC-H7
(Filter)	(528/45)	(586/42)	(700/54)	(783/56)	(720/30)	(783/56)
Tube 1 (FMO)	CD45	buffer	CD3	CD4	Viability	CD8
Tube 2 (FS)	CD45	CAR	CD3	CD4	Viability	CD8
Tube 3 (CAR quantitation)		Quantibrite				
Tube 4 (Trucount)	CD45					



*BD FACSLyric™: 3-laser
conventional Flow cytometry*

CAR-T Immunophenotyping Panel

CART Characterization/immunophenotyping panel: T cell activation/exhaustion markers and memory subsets, and Treg cells for endogenous T cells and CART cells



- ✓ It is critical to understand the differentiation of CAR+ T cells into distinct memory subsets, as variation in these subsets influences the effectiveness of CAR+ T-cell therapy across individual patients.

Validated Flow Cytometry Panel Examples

BD FACSLyric™



Panel Name*	Antigen Markers	Matrix	Sites
TBNK T cells, B cell, NK cells (relative % and absolute count)	Tube 1: CD3, CD4, CD8, CD16/CD56, CD19, CD45	Blood, BMA	US, EU, AUS, CHN
T cell NK panel T cell subsets (memory), gamma/delta T cells, NK cells (includes Ki67 marker)	Tube 1: CD3, CD4, CD8, (CD14 and Viability), CD16, CD45, CD56, Granzyme B, ICOS, Ki-67, PD-1, TCRγδ Tube 2: CD3, CD4, CD8, (CD14 and Viability), CD25, CD45, CD45RA, CCR7, FoxP3, Granzyme B, Ki-67, PD-1 (includes Trucount tube for absolute count calculation)	Blood	US, EU, AUS
PBMC IC panel (intracellular cytokine assay)	Tube 1: CD3, CD4, CD8, CD45, CD183, CD194, IL-2, IL-4, IL-6, IFNγ, TNFα, viability.	PBMC	US
T cell NK panel T cell subsets (memory), gamma/delta T cells, NK cells (includes Ki67 marker)	Tube 1: CD3, CD4, CD8, (CD14 and Viability), CD16, CD45, CD56, Granzyme B, ICOS, Ki-67, PD-1, TCRγδ Tube 2: CD3, CD4, CD8, (CD14 and Viability), CD25, CD45, CD45RA, CCR7, FoxP3, Granzyme B, Ki-67, PD-1 (includes Trucount tube for absolute count calculation)	Blood	US, EU, AUS

*These are off the shelf-panels. The majority of panels that Cerba Research implements are proprietary to client programs.

Tracking Killer vs. Bystander Cell Dynamics

Utility of a validated TBNK panel



Features/Population	What It Identifies	Insights on "Killer" vs. "Bystander" Effects
Effector/Killer Population	CD3+ CD8+ (T-cell therapy) or CD3- CD16+ CD56+ (NK therapy)	Expansion and Persistence: Quantifies the precise absolute count and percentage of the therapeutic "killer" cells over time to monitor their expansion post-infusion.
Endogenous Bystander T Cells	CD3+ CD4+ (Helpers) or native CD3+ CD8+	Host Immune Reconstitution & Recruitment: Shows if bystander T cells are expanding in response to cytokines (like IL-2 or IL-6) released during target killing, indicating an indirect cascade.
Bystander B Cells	CD3- CD19+ or CD20+	Off-Target/Collateral Depletion: In therapies like anti-CD19 CAR-T, B cells serve as a marker for the "killer" cell efficacy and off-target bystander aplasia (bystander B-cell elimination).
Bystander NK Cells	CD3- CD16+ CD56+ (when tracking T-cell therapy)	Innate Immune Activation: Measures if the patient's native NK cells are being recruited or enhanced by the local microenvironment to assist in tumor clearing.

Validated Spectral Flow Cytometry Panel

Examples: Cytex Aurora

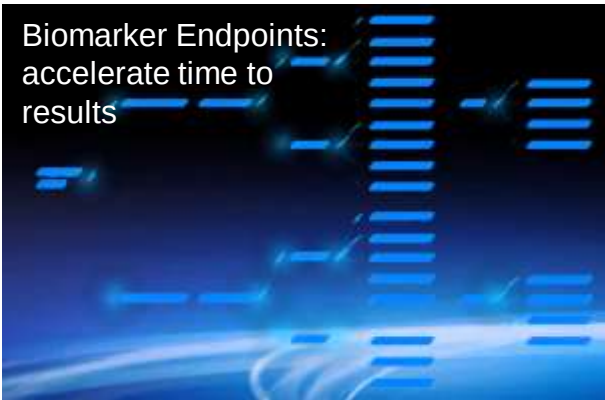


Panel Name*	Antigen Markers	Matrix	Sites
TBNKM (14-colors, T cells, B cells, NK cells and monocytes, and subsets)	Tube 1: CD3, CD4, CD8, CD14, CD16, CD19, CD25, CD27, CD45, CD56, CD127, CD45RA, CCR7, IgD, Viability	PBMC, Blood	US, EU
TBNKM DC (25-colors, T cells, B cells, NK cells, monocytes and DC, and subsets)	Tube 1: CD1c, CD3, CD4, CD8, CD11c, CD14, CD16, CD19, CD20, CD25, CD27, CD28, CD38, CD45, CD45RA, CD56, CD123, CD127, CD141, CCR7, HLA-DR, IgD, IgM, PD-1, TCRγδ	Blood	US, EU
Pan immune profiling spectral flow cytometry assay (PIP-01)	Tube 1: (48-marker panel) (Markers lists on subsequent slide)	PBMC	US (Q3 2026)

*These are off the shelf-panels. The majority of panels that Cerba Research implements are proprietary to client programs.

Spectral Flow global footprint and Ozette partnership

High-Parameter Panel Data Analysis | Automated platform



Spectral Flow High-Parameter Power: Measure 40+ markers in a single panel for deep immune profiling and rare cell detection across oncology, immunology, and cell therapy.

Improved Sensitivity: Advanced optics and spectral unmixing improve detection of low-abundance markers and rare populations (e.g., MRD, Tregs).

EndPoints Advanced Analytics: Rich spectral data supports machine learning, biomarker discovery, and multi-omic integration.



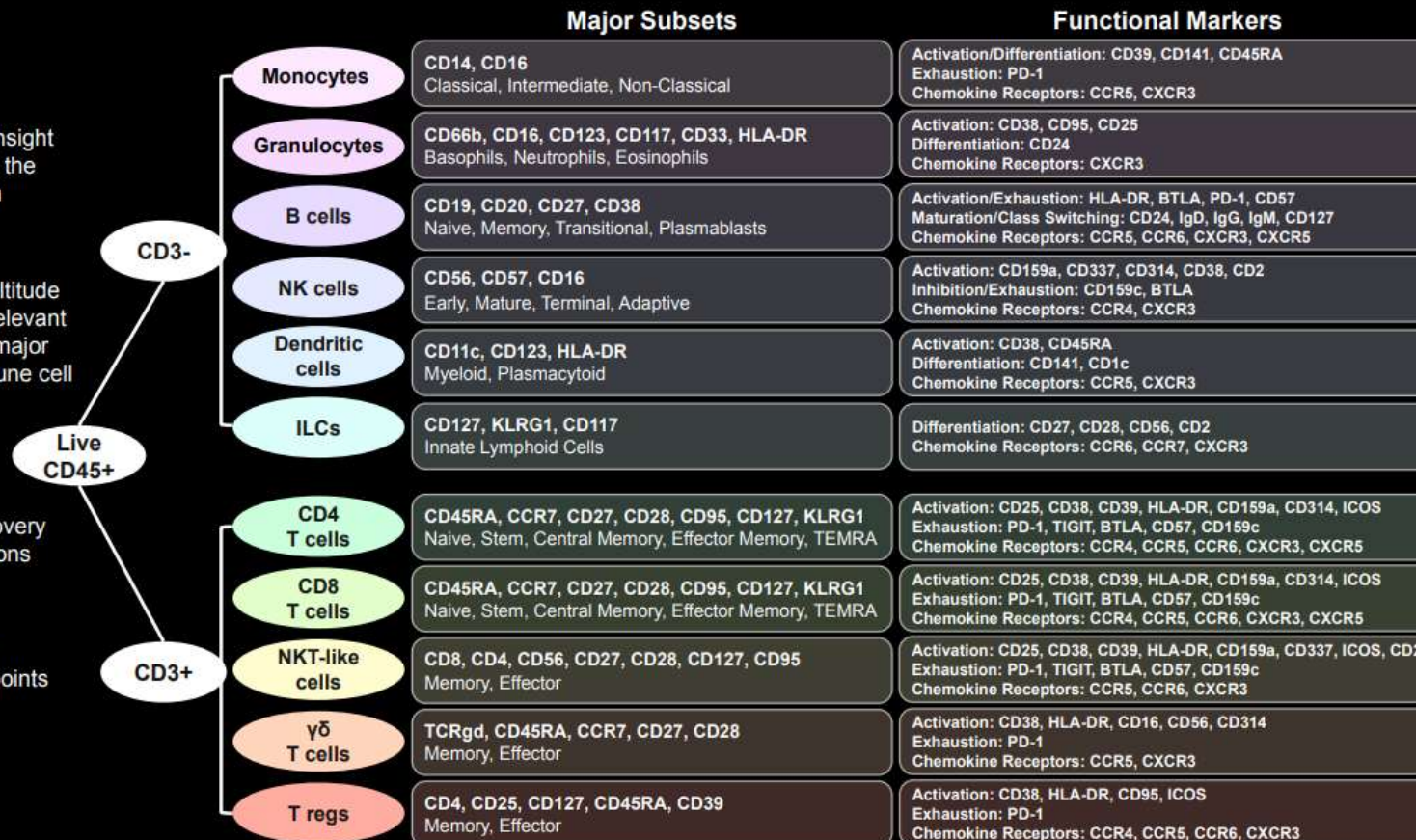
Ozette 48-Marker Spectral Reference Panel (PIP-01)

- Optimized 48-marker immune phenotyping panel for spectral flow
- Standardized backbone for discovery, translational, and early clinical studies
- Broad coverage of innate and adaptive immune lineages, including rare subsets
- Designed to maximize resolution while minimizing spectral spillover
- Enables consistent population definitions across multi-site and multi-sponsor studies

Ozette's 48-color pan immune profiling spectral flow cytometry assay (PIP-01)

Ozette's 48-color pan immune profiling spectral flow cytometry assay (PIP-01)

- Delivers rapid insight into the state of the immune system
- Measures a multitude of biologically relevant markers on all major circulating immune cell subsets
- Ideal for broad biomarker discovery based applications
- 171 predefined biomarker endpoints



Case Study: Immune cell phenotypes associated with product performance

Favorable

- ❑ Less differentiated cells
- ❑ Naive or stem-like memory phenotypes (CD45RA, CCR7)
- ❑ Co-stimulatory expression (CD27, CD28)

Unfavorable

- ❑ More differentiated cells
- ❑ Terminal effectors with reduced potential
- ❑ Checkpoint co-expression (PD-1, TIGIT)

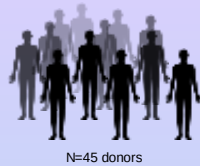
Most published biology is in the autologous CAR setting with patient material

Very little is known about healthy donor allo starting material

End-to-end sample processing and analysis of single-cell data yields interpretable insights into donor selection for allo cell therapy development

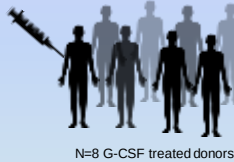
Multi-phased design links 3 types of healthy donor allogeneic starting material:

Phase 1: PBMC



Key donor metadata
(e.g. CMV status)

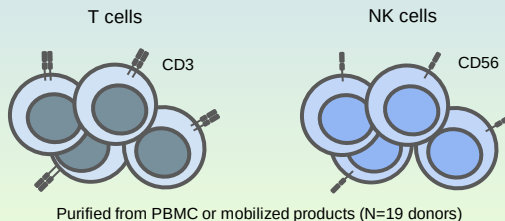
Phase 2: Mobilized apheresis products



Three samples per donor:
1. Pre
2. Post
3. Product

Mobilization scoring based on CD34+ stem cell counts

Phase 3: T and NK cell products

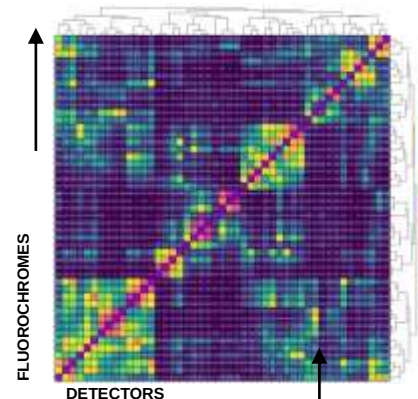


Process, stain and acquire using Ozette

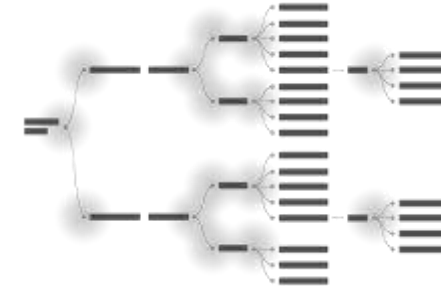


Validated 48-color pan immune profiling spectral flow assay

Spectral unmixing



Single-cell analysis with ozette endpoints™



Predefined biomarker tracking

+

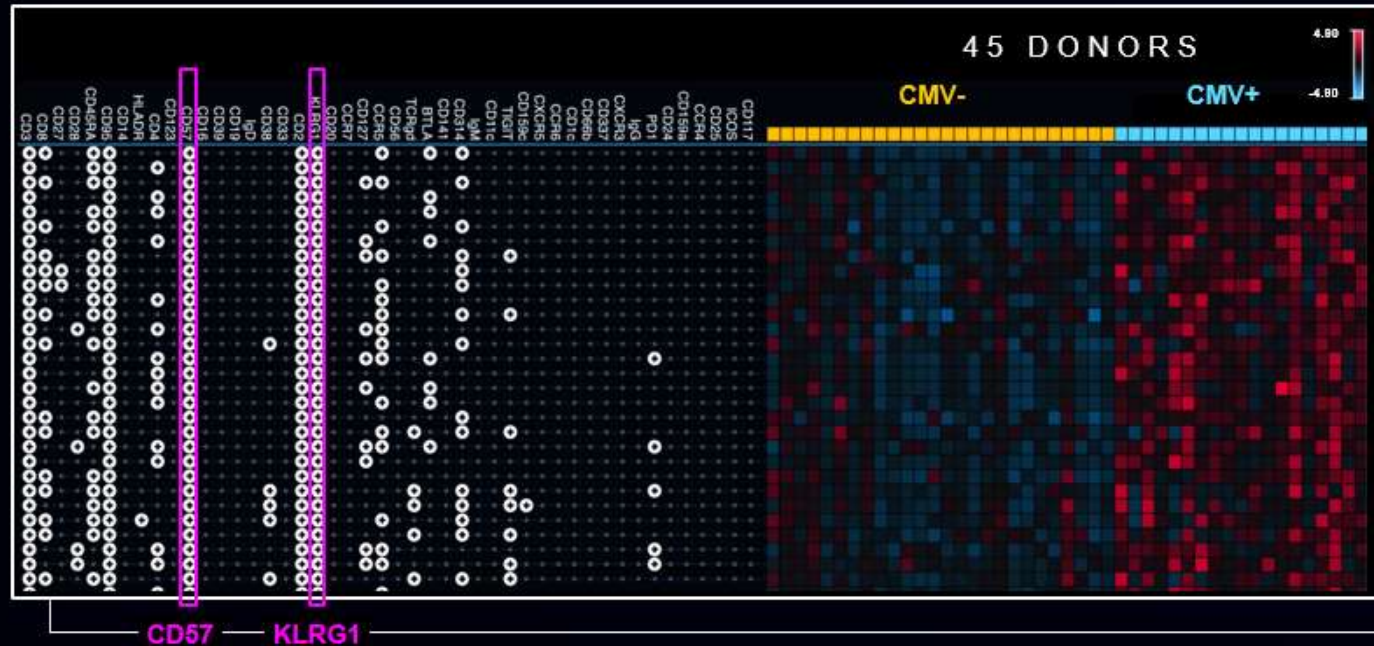
ozette discovery™



Full cell phenotype annotation

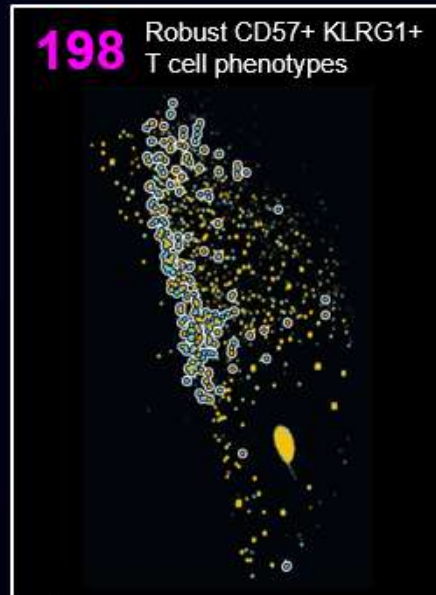
CMV⁺ donors have a higher abundance of T cell phenotypes considered less favorable for allogeneic CAR T cell manufacturing

Phenotype abundance
(stratified by CMV status)

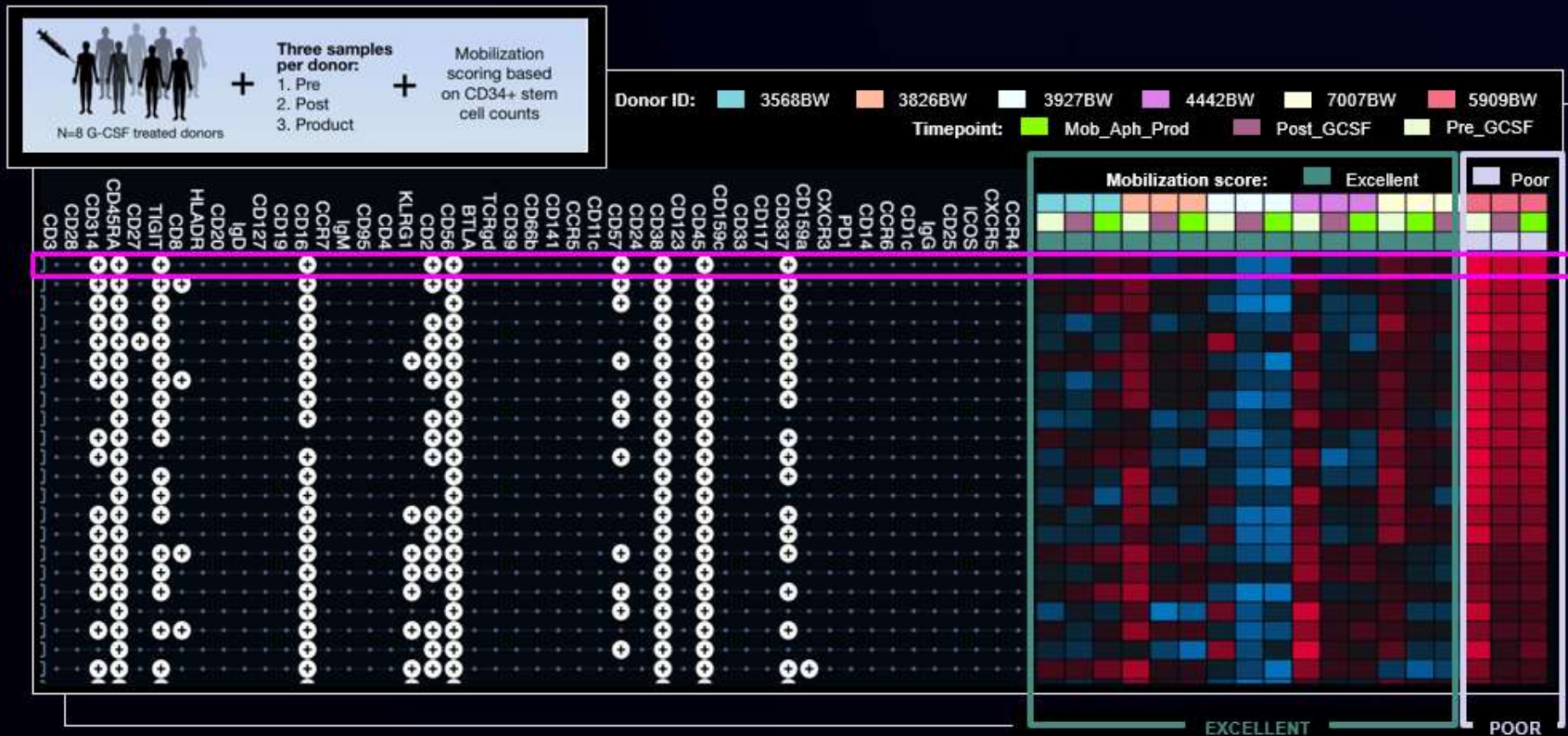


CD57 & KLRG1 are markers associated with chronic viral infection, terminal differentiation & reduced replicative potential, & therefore are considered unfavorable for manufacturing

Single-cell embedding
(colored by CMV status)



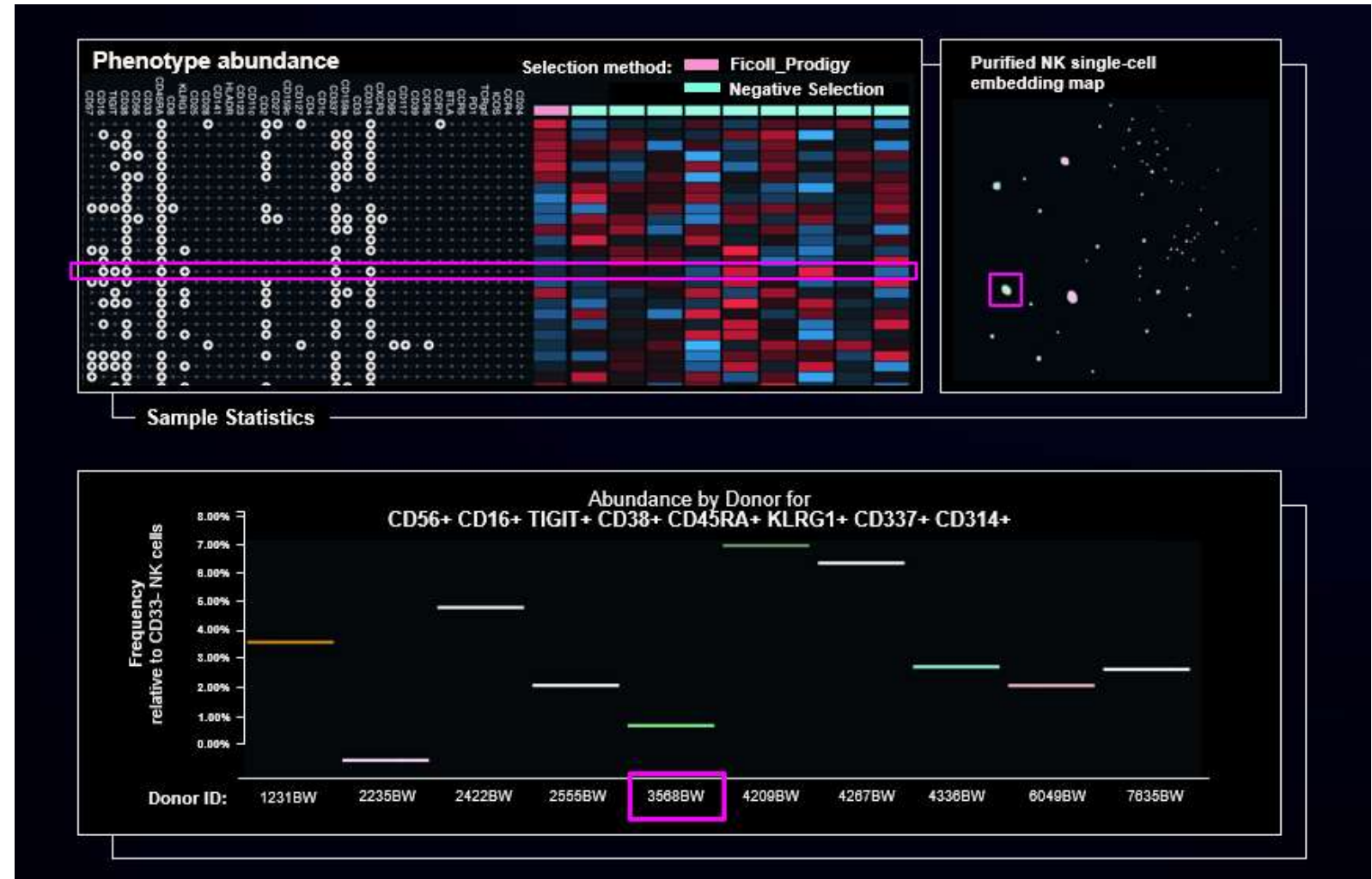
Suboptimal mobilizers have a higher abundance of highly differentiated NK cell phenotypes in their starting material



Ozette identified higher abundance of highly differentiated NK cell phenotypes in suboptimal mobilizer starting material

Distinct and robust NK cellular states are discovered across purified products from allogeneic donors

- Heterogeneity of NK cell phenotypes varies by donor and purification strategy
- NK cell product was purified from the starting material of donor 3568BW, an excellent mobilizer and CMV-
- This signature—comprising classic mature NK markers (CD56⁺, CD16⁺) alongside activation, homing, and checkpoint molecules serves as a distinct cellular feature distinguishing poor vs. excellent stem cell mobilizers



Key takeaways

- Identification of key donor attributes and a comprehensive assessment of immune status may predict whether a patient is a good candidate for cell therapy product development.
- Ozette Endpoints™ and Discovery™ rapidly identifies relevant scientific findings, particularly cellular states and phenotypes that may otherwise go undiscovered.
- Pairing accelerates the analysis of complex datasets and bolsters our ability to provide suitable starting material to researchers and drug developers.

High-Dimensional Immunophenotyping in a Single Tube

Tracking the interplay between therapeutic cells and bystanders requires an immense number of markers. Spectral flow allows for panels exceeding **40+ colors simultaneously**, enabling you to capture a complete profile of both populations at once:

- **Killer/Therapeutic Lineage:** Target antigens, CAR/TCR expression, and exhaustion markers (e.g., PD-1, TIM-3, LAG-3)
- **Bystander Architecture:** Endogenous CD4+ / CD8+ T cells, regulatory T cells (Tregs), NK cells, and myeloid-derived suppressor cells (MDSCs).
- **Functional State:** Cytokine production (IFN- γ , TNF- α), proliferation (Ki-67), and cytotoxic molecules (Granzyme B, Perforin) across both groups.

Scaling it globally: From single site to global clinical trials

Implementing complex characterization strategies across global trials introduces operational and scientific challenges that must be solved deliberately, not left to chance.

01



Assay standardization

Harmonized protocols and reference materials so results are comparable across every testing site.

02



Data integration

Unified data structures that connect flow, genomic, and BioA outputs into one interpretable dataset.

03



Global deployment

Coordinated logistics, training, and lab networks to maintain consistency across regions.

04



Consistency and scalability

Quality systems that hold up as programs scale from early-phase to global pivotal trials.

Thank you

For more information, contact:
Kirsty Maclean
kmaclean@cerbaresearch.com



cerbaresearch.com