Whitepaper

Cerba Research

CAR+ T Drug Development

The critical role of Flow Cytometry

About Cerba Research

Cerba Research is a leading global provider of specialty laboratory and central laboratory solutions for clinical trials, with over 40 years of experience supporting pharmaceutical, biotechnology, and public health partners.

Cerba Research. Science that matters. Solutions that deliver.

We offer integrated laboratory capabilities

across all phases of drug development, including Bioanalysis, Flow Cytometry, Histocytopathology, and Next-Generation Sequencing. Our global lab network enables consistent, high-quality data generation and efficient trial execution across multiple regions and complex protocols.

From protocol inception through development and to market, our scientific and operational teams deliver customized diagnostic testing and biomarker-driven insights to accelerate timelines and improve patient outcomes. With recognized expertise in Oncology, Immunology, Anti-infectives and vaccine, and Cell & Gene Therapy, we help sponsors design and execute trials with greater precision and flexibility.

As part of the Cerba HealthCare Group, Cerba Research operates on five continents, combining global scale with scientific depth to drive innovation in clinical research and advance global health.

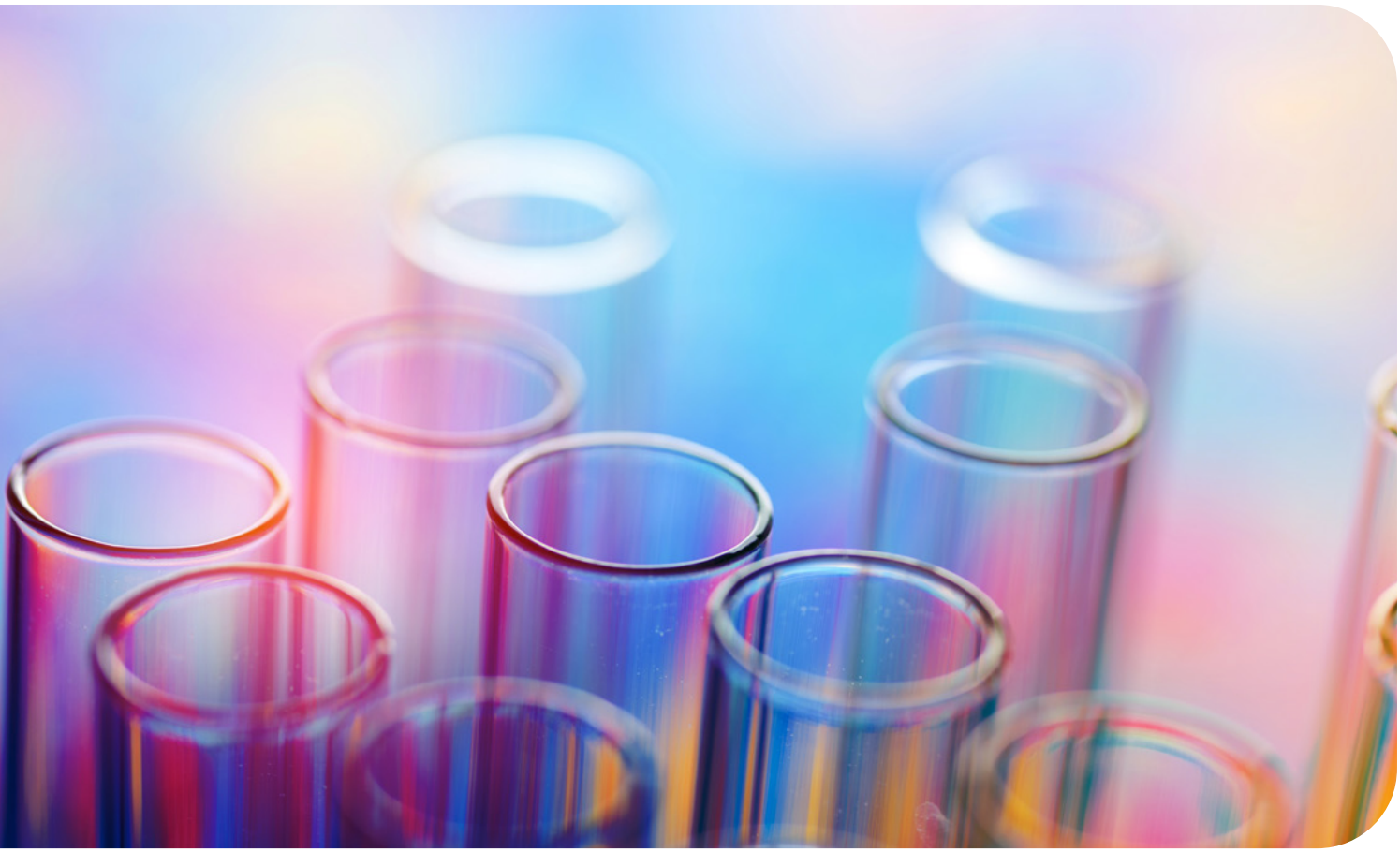
About the authors

Nithianandan Selliah

Nithianandan Selliah has a Ph.D. in Immunology from the University of Kentucky, USA. In 2019, he joined Cerba Research as a Global head of Flow Cytometry. At Cerba Research, he led many assay development and validation projects for global clinical trials.

Rowan Claeys

Rowan Claeys worked in various hospital laboratories after graduating as a medical doctor and clinical pathologist from the University of Louvain. In these roles, he was head of medical validation in laboratory hematology. He joined the Flow Cytometry department as a clinical pathologist at Cerba Research in 2020.



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Chapter 1

The importance of flow cytometry in CAR+ T-cell clinical research, what assays can be used, and an introduction to characterization.

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The importance of flow cytometry in CAR+ T-cell clinical research

Being one of the leading technologies for cellular analysis, flow cytometry generates simultaneous high-throughput enumeration and individual cell characterization data.

With the breakthrough of cellular immune therapies, such as CAR+ T-cell therapy, flow cytometry became a critical platform, not only for the clinical laboratories, but also for drug developers and manufacturers.

Flow cytometry plays a crucial role throughout the CAR+ T-cell therapy process. During manufacturing, it is used to assess transduction efficiency, product purity, and phenotypic characterization of the CAR+ T-cell product prior to infusion. Post-infusion of CAR+ T-cells, flow cytometry is used to monitor CAR+ T-cell expansion, persistence, and functional activity, as well as to assess minimal residual disease (MRD) in treated patients.

Flow cytometry assays in CAR+ T-cell trials

Flow cytometry can be used to monitor CAR+ T-cells and endogenous immune cells, as well as circulating malignant cells.

To monitor CAR+ T-cells, two types of flow cytometry assays are commonly employed: **cellular pharmacokinetic assay (CAR+ T-cell PK assay,** enumeration of CAR+ T cells) and **characterization assay (immunophenotyping of CAR+ T cells).** CAR+ T-cell PK assay is designed to enumerate CAR+ T-cells. While qPCR was initially used for this purpose, since the worldwide standardization of flow cytometry testing was made easier with current cytometers, and high sensitivity can be reached using a high-affinity anti-CAR reagent, flow cytometry can now be used for global PK testing in clinical trials.

Absolute counts for CAR+ T-cell PK assay

Flow cytometry allows for the measurement of both relative percent and absolute cell counts from fresh specimens. Absolute counts can be obtained using two main approaches: **the single-platform and the dual-platform methods.**

In the single platform method, the absolute counts are calculated based on commercial quantification beads (example Trucount tube from BD Biosciences) or the capability of volumetric acquisition (capable only in specific instruments, example Cytek Aurora). While this method offers several advantages—such as reduced variability and improved standardization - it also has limitations. The presence of beads in the tube precludes post-staining wash steps (used with limited markers and lyse/no wash process), and volumetric acquisition is only feasible in certain flow cytometers.

Therefore, the dual platform might be considered for complex multi-color flow assays. The dual platform combines absolute counts of parent cell populations, typically provided by a hematology analyzer, with flow cytometric data – ideally using the same specimen collection tube for consistency. Absolute counts of the population of interest are then calculated by the relative percentage obtained by the flow cytometry assay and the absolute count value from the hematology-analyzer. However, it should be kept in mind that these absolute counts should always be interpreted as an approximation as the ratio of populations within the leukocyte population might differ between the two platforms.

Therefore, as a solution for complex assays, Cerba Research recommends obtaining the absolute count of a parent population from a companion tube with quantification beads. In this tube, the sample is stained with a basic panel in a Lyse/No Wash format.

Immunophenotyping of CAR+ T-cells is designed to characterize CAR+ T cells to assess different subset of T-cells, such as memory phenotype, and to assess activation and exhaustion status. A well-designed assay provides drug developers with critical insight into the in vivo behavior of CAR+ T-cell cells post-infusion and their effects on the patient's endogenous immune system. The assay composition depends on the cell types and biomarkers the investigator wants to explore.

Further, Minimal Residual Disease (MRD) assay can be performed to evaluate treatment efficacy by measuring tumor clearance. These MRD assessments can be further enhanced by phenotyping assays that identify specific biomarkers on malignant cells and monitor antigen loss throughout the course of treatment.

Cerba Research developed CAR+ T-cell characterization assay to primarily focus on the memory differentiation, activation and/or exhaustion status of both expanded CAR+ T-cell cells and endogenous T-cells.

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Chapter 2

Developing a CAR+ T-cell trial: key aspects for assay development and validation.

The key aspects of developing a CAR+ T-cell assay

A well-designed CAR+ T-cell assay reports an accurate set of data for its intended use. The intended use and regulatory requirements define the validation parameters of the assay, which is based on a fit-for-purpose principle.

Having defined the intended use, the next pillar in CAR+ T-cell assay development is the selection of the specimen type. This can be whole blood, bone marrow aspirate or PBMC. The choice is dependent on a range of factors, including the feasibility of local acquisition, the need to run in batches, the need for absolute counts, the presence of the population of interest and the stability of the parameters. PBMC is preferable in instances when stability does not allow global shipment of fresh material, or in cases where batch testing is required. Additionally, PBMC preparations allow for cell enrichment which is particularly valuable in monitoring CAR+ T-cell cells in heavily lympho-depleted patients, which is commonly seen in allogeneic CAR+ T-cell cell therapy. On the other hand, PBMC will not allow the assessment of absolute count.

Another crucial aspect of any assay development is the selection of reagents particularly in CAR+ T-cell PK assays, where **the performance of the anti-CAR detection antibody is crucial for achieving high sensitivity.**

Given that anti-idiotype monoclonal antibodies have a high affinity for CAR+ T-cells and exhibit a low nonspecific binding, they are highly recommended.

A major drawback is that these reagents require customized development. The combination of a long manufacturing period, the risk for inter-lot variability and unpredictable elements within the sample forecast makes it challenging to manage reagent stock in global trials. Therefore, commercial antibodies, such as target fusion proteins or anti-tag monoclonal antibodies, are operationally seen as an easier alternative.

Another aspect to keep in mind when designing the gating strategy is the nature of the CAR+T-cell product itself. There are two types, autologous and allogeneic CAR+ T-cell therapies. Autologous CAR+ T-cell therapy involves collecting T-cells from the patient, genetically modifying (transducing) them to express the CAR construct, and then reinfusing them back into the same patient. These CAR+ T-cell products typically retain **CD3 expression,** allowing for standard **CD3+ T-cell gating.** Allogeneic CAR+ T-cell therapies involve T cells from a healthy donor, which are genetically engineered to knock out the CD3 T-cell receptor in order to reduce the risk of graft-versus-host disease (GvHD). As a result, these CAR+ T-cell products lack CD3 expression, necessitating an alternative gating strategy based on other markers such as CAR expression or alternative lineage markers.

It is essential to minimize the risk of interference from antibody-based therapies targeting markers of interest included in flow cytometry panels. Treatments such as anti-CD38 antibodies (e.g., daratumumab) or PD-1/PD-L1 inhibitors can bind to surface antigens and block detection by conventional clones. Therefore, reagents and antibody clones used in assays must be carefully selected to ensure compatibility with ongoing or prior therapies, allowing for accurate immunophenotyping and reliable data interpretation.

How has assay development and validation been approached in Cerba Research's laboratories?

Cerba Research offers customized panel design and fit-for-purpose assay validation Our approach is truly a scientific collaboration between ourselves and the sponsor with open communication and full transparency regarding validation data, SOPs, and reporting. Once validated in one of the labs (Europe or US), the assay is transferred and implemented to support clinical trials globally.

Cerba Research offers global flow cytometry capabilities with operations across Europe, North America, Africa, Australia, Taiwan and China. We have access to BD FACSLyric instruments on all sites, allowing us to offer global data transparency. We also offer Spectral flow cytometry (Cytek Aurora instrument) to assess high parameter panels for complex immunophenotyping and exploratory research.

In addition to instrument standardization, we use global standardized validation procedures, acquisition and analysis templates and SOPs across all regions. In addition, patient data analysis and review are centralized to ensure consistency in data interpretation.

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Chapter 3

The added value of flow cytometry in multiple myeloma, minimal residual disease (MRD) assessments, and multiple myeloma phenotype.

An overview

Immunophenotyping plasma cells in CAR+ T-cell clinical trials offers significant added value, particularly when performed using flow cytometry. Standardized assay such as from EuroFlow (next generation flow or NGF) provides a highly standardized approach across all stages — from sample preparation and data acquisition to analysis and reporting — employing a refined set of backbone markers to achieve greater sensitivity.

At diagnosis, or during the screening phase prior to CAR+ T-cell therapy, flow cytometry is utilized to identify and quantify malignant plasma cells in the bone marrow. This assessment also includes evaluation of the target antigen expression, ensuring suitability for antigen-directed CAR+ T-cell therapy.

CAR+ T-cell therapy and other immunotherapeutic drugs are designed to target specific antigens expressed on the surface of malignant plasma cells. During follow-up treatment, flow cytometry remains essential, as targeted therapies can induce phenotypic shifts in plasma cells, potentially altering the expression of surface antigens. Monitoring these changes is critical to assessing ongoing antigen expression, understanding mechanisms of resistance, and guide future therapeutic decisions.

The disappearance of monoclonal plasma cells serves as evidence of therapeutic efficacy. Flow cytometry can be used to assess MRD, which is increasingly recognized as a critical surrogate marker for progression-free and overall survival in clinical trials.

How is immunophenotyping of plasma cells in multiple myeloma performed?

In multiple myeloma, immunophenotyping of plasma cells is performed with a set of recommended markers. Plasma cell populations are identified based on the expression of CD45, CD38, and the plasma cell–specific marker CD138.

The expression of a set of other plasma cell characterizing markers is also used. Malignant plasma cells tend to be either CD19 negative and CD56 positive and additionally tend to have a weaker or absent expression of CD27 and CD81. It is only within a small part of multiple myeloma patients that CD117 is expressed on malignant plasma cells.

Finally...

Intracellular staining is used to assess the monoclonality of the malignant plasma cells for kappa and lambda light chains. Ideally, this allows for clear discrimination between normal (polyclonal) and abnormal (monoclonal) plasma cell populations.

In addition to the recommended markers, several other informative markers can be incorporated into multiple myeloma panels to enhance characterization and monitoring. These markers could be predictive of disease progression, such as CD28 and CD200.

It is known that CD138 is a relatively unstable marker, and its expression diminishes between 8 and 24 hours after the sample has been taken. In situations where delayed sample processing is anticipated, it is recommended to incorporate stable surface markers such as CD319 (SLAMF7) and CD229 (Ly9) into the flow cytometry panel. These markers demonstrate a more robust and consistent expression on plasma cells, even in suboptimal or time-delayed conditions.

Their inclusion could enhance the accuracy and reliability of plasma cell identification and phenotyping, ensuring data integrity despite logistical constraints.

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Chapter 4

Focus on CAR+ T-cell therapy: patient treatment, targeted antigens, and solutions for minimal residual disease (MRD) assessment and multiple myeloma phenotype assessment.

Treating patients who undergo CAR+ T-cell therapy

Most patients undergoing CAR+ T-cell therapy for multiple myeloma will have previously received anti-CD38 treatment, such as daratumumab. This therapy can alter the expression of CD38 on plasma cells, making standard CD38-based detection by flow cytometry unreliable.

To ensure accurate identification of plasma cells in this setting, it is recommended to incorporate alternative markers into the immunophenotyping panel. These may include surface markers such as CD229 and CD319, cytoplasmic markers like VS38c, and CD38-specific reagents designed to recognize unaffected or multiple epitopes, including nanobody-based and multi-epitope CD38 antibodies.

In addition, evaluating the expression of the therapeutic target antigen prior to initiating CAR+ T-cell or other antigen-specific therapies is strongly advised. This helps confirm target presence and supports appropriate treatment selection.

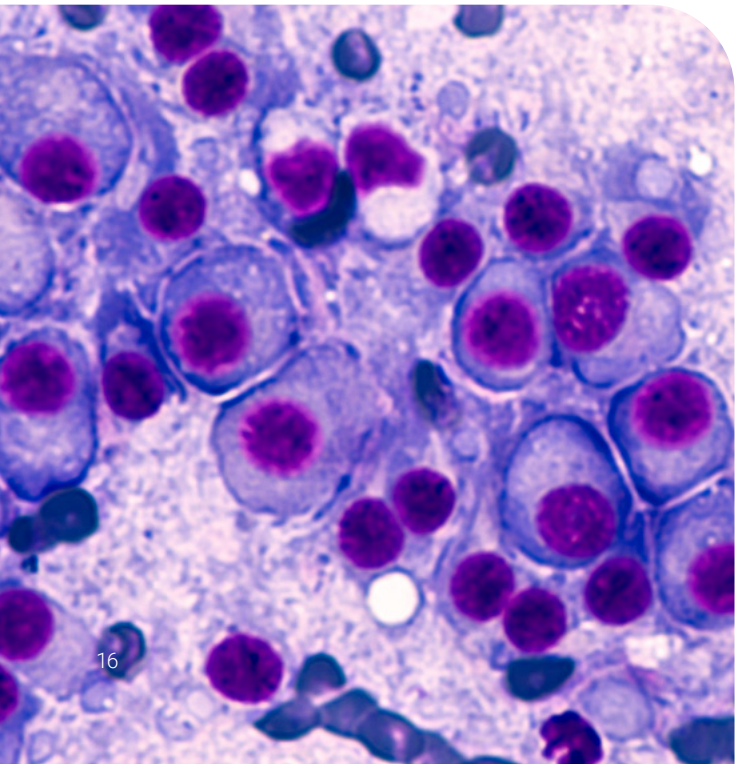
There are a range of other antigens currently being investigated to develop a CAR+ T-cell therapy towards those targets, which includes CD229, CD44, Lewis and other antigens. A number of these antigens are now under investigation in clinical trials.

The impact of immunotherapeutic drugs on the phenotype of the plasma cells for current treatments

Daratumumab which binds to CD38, and plasma cell targeted therapy have impact on detection of plasma cells in patients.

Flow cytometry using CD38 as a primary marker to identify plasma cells can be directly affected by CD38 receptor occupancy following treatment with daratumumab. This therapeutic antibody binds to CD38 on plasma cells, potentially blocking epitope recognition and leading to reduced or undetectable CD38 expression during flow cytometry analysis.

CD38 antigen loss is another effect of treatment with daratumumab, which may either be due to a transient phenomenon or a temporary downregulation of the CD38 antigen on the plasma cells. This can last up to six months after the last infusion of the drug.



To overcome this limitation and maintain the reliability of plasma cell identification, the use of alternative markers and specialized reagents is recommended:

- CD229 (Ly9) and CD319 (SLAMF7) are stable surface markers expressed on plasma cells that are not affected by anti-CD38 therapy
- Multi-epitope CD38 antibodies are engineered to recognize unblocked epitopes on the CD38 molecule
- CD38 nanobody (VHH, clone JK36), developed by Beckman Coulter, binds to a unique epitope unaffected by therapeutic anti-CD38 antibodies
- VS38c is a cytoplasmic marker targeting an endoplasmic reticulum-associated protein, enables the identification of plasma cells independent of surface antigen expression, offering a valuable tool in heavily pre-treated or antigen-negative cases

It is important to note that reduced or absent CD38 expression on plasma cells may not only result from therapeutic interference, but also from biological mechanisms of resistance. One such mechanism is the genetic selection of CD38-negative plasma cell clones, allowing malignant cells to escape daratumumab-mediated targeting. Additionally, trogocytosis, a process in which monocytes or granulocytes physically extract membrane fragments from plasma cells, may contribute to the loss of CD38 and nearby surface antigens. This phenomenon has also been described for other markers such as CD56 and CD44, underscoring the potential for antigen loss in the tumor microenvironment following targeted therapies.

What is the most frequently targeted antigen for CAR+ T-cell therapy?

B cell maturation antigen, or BCMA or CD269 is currently the most frequently targeted antigen for CAR+ T-cell therapy. BCMA is highly expressed on malignant plasma cells, shows low to moderate expression on normal plasma cells, and is largely absent from B-cell precursors cells.

Gamma-secretase removes the BCMA from the plasma cells and these can then be retrieved in the serum of the patients as soluble BCMA.

BCMA expression is positively correlated with disease progression in plasma cell disorders. It is typically low in patients with monoclonal gammopathy of undetermined significance (MGUS) and becomes progressively higher in patients with smoldering myeloma and active multiple myeloma. This gradual increase reflects the expanding malignant plasma cell population and underscores BCMA's relevance as a therapeutic target in advanced disease stages.

Following treatment with anti-BCMA CAR+ T-cell therapy, a reversible downregulation of BCMA expression on plasma cells is likely to occur. In some cases, however, there may also be clonal selection of BCMA-negative or BCMA-low plasma cells, serving as an immune escape mechanism. These resistant plasma cell clones can persist and potentially proliferate, contributing to disease relapse and therapeutic resistance in multiple myeloma.

BCMA antigen loss can occur through multiple mechanisms. Structural aberrations on chromosome 16, where the BCMA gene (TNFRSF17) resides, can lead to reduced or lost BCMA expression. Additionally, gamma-secretase activity can cleave and remove BCMA from the plasma cell surface, resulting in lower detectable antigen levels.

These mechanisms—alongside adaptive downregulation and clonal selection—can lead to relapse with BCMA-low or BCMA-negative plasma cells, posing a significant challenge for ongoing or repeated BCMA-targeted therapies such as CAR+ T-cells or bispecific antibodies.

Trogocytosis may also occur in the context of CAR+ T-cell therapy, with CAR+ T-cells themselves extracting BCMA from plasma cells. This can result in BCMA expression on the CAR+ T-cells, making them targets for other CAR+ T-cells in a process known as fratricide. This self-targeting phenomenon may contribute to reduced CAR-T cell persistence and diminished therapeutic efficacy.

GPRC5D (G protein-coupled receptor, class C group 5 member D) is an emerging target in multiple myeloma and is gaining attention in the context of flow cytometry, particularly in clinical trials involving GPRC5D-targeted therapies such as CAR+ T-cells and bispecific antibodies. It is highly expressed on malignant plasma cells, with limited expression in normal tissues, and it serves as an alternative or complementary target to BCMA, especially in relapsed/refractory MM or in patients with BCMA antigen loss.

As with BCMA, GPRC5D antigen escape, downregulation and trogocytosis are critical resistance mechanisms that can impact the durability of response to GPRC5D-targeted therapies. Flow cytometry is a useful tool to detect and respond to these phenotypic shifts.

Using flow cytometry to look for rare plasma cells in patients' bone marrow aspirate

Response criteria established by the International Myeloma Working Group (IMWG) define complete response (CR) as having <5% plasma cells in the bone marrow, disappearance of any soft tissue plasmacytomas, and negative immunofixation in both serum and urine. Using these criteria, more than 50% of treated patients can achieve a complete response following therapy.

Unfortunately, the majority of multiple myeloma patients will eventually relapse, even those who achieve a stringent complete remission. To detect residual disease that persists below the threshold of conventional response criteria, high-sensitivity techniques are essential. Recognizing this, the IMWG has incorporated MRD negativity—assessed by flow cytometry, molecular methods, or advanced imaging—into the response criteria, as an additional layer beyond complete remission.

Flow cytometry can be employed to assess bone marrow in treated multiple myeloma patients.

How else does MRD relate to clinical outcomes?

Minimal residual disease (MRD) is the most powerful predictor of clinical outcomes in multiple myeloma, including both progression-free survival (PFS) and overall survival (OS). Its prognostic value is independent of disease stage at diagnosis, cytogenetic risk profile, and the use of autologous stem cell transplantation (ASCT).

Currently, therapeutic decisions are not routinely guided by MRD status in clinical practice; however, multiple ongoing trials are exploring the role of MRD in optimizing the timing of ASCT, selecting consolidation strategies, and determining treatment duration.

Given its strong predictive value, MRD negativity is also being considered as a surrogate endpoint for accelerated drug approval. Although regulatory agencies such as the FDA and EMA have not yet formally accepted MRD negativity as a standalone endpoint, negotiations and data-driven validation efforts are ongoing.

In multicenter clinical trials, MRD assessment is gaining increasing importance and is being incorporated as a key endpoint. Both the FDA and EMA have acknowledged MRD as a potential surrogate marker for progression-free survival (PFS) in regulatory frameworks, particularly in the context of clinical trial design and drug development.

While not yet universally accepted as a primary endpoint for approval, MRD is being used to support early efficacy signals and guide therapeutic evaluations across studies.

The challenges of using the assessment of MRD

One such area is the lack of harmonization between regulatory agencies. For example, the FDA currently recommends MRD assessment only in patients who have achieved complete or stringent complete response, whereas the EMA also permits assessment in patients with a very good partial response. Additionally, there is no consensus on optimal time points for MRD assessment or the recommended duration of MRD monitoring in clinical trials or routine care.

Nevertheless, both agencies appear aligned to adopt the IMWG criteria for sustained MRD negativity, which requires at least one year of MRD negativity. They also seem to agree on using a sensitivity threshold of 10^{-5} as a minimum standard for MRD detection.

To comprehensively assess residual disease and avoid false-negative results due to extramedullary involvement, advanced imaging techniques are essential adjuncts to bone marrow–based MRD testing. While liquid biopsy approaches such as flow cytometry in peripheral blood have shown limited utility so far, cell-free DNA (cfDNA)–based methods are emerging as a more promising and non-invasive alternative for systemic MRD detection.

Moreover, further risk stratification is needed for patients who remain MRD-positive during clinical trials. Identifying high-risk subgroups within this population is essential to guide treatment intensification strategies and to better understand differential outcomes.

Currently, only two techniques offer sufficient sensitivity to reliably assess MRD in multiple myeloma: **flow cytometry and next-generation sequencing (NGS).** Each method has distinct advantages and limitations, and the choice between them often depends on clinical context, sample availability, and logistical considerations.

Next Generation Sequencing (NGS) vs Flow Cytometry

NGS allows for delayed analysis, as it does not require a fresh bone marrow sample. This logistical advantage makes it particularly suitable for multicenter trials and retrospective assessments.

It is important to note that flow cytometry can be performed without the need for a diagnostic baseline sample, whereas NGS requires one to identify and track the patient-specific clonal sequence. In flow cytometry, the presence of non-plasma cells can provide valuable insight into sample quality and potential hemodilution, which may affect the reliability of MRD results.

Both techniques offer complementary information. NGS provides molecular insights, including clonal evolution and potential pharmacogenomic associations, while flow cytometry allows for detailed immunophenotypic characterization of both normal and abnormal plasma cells, including antigen expression profiles.

Additionally, NGS requires the analysis of fewer cells compared to flow cytometry, which often necessitates acquisition of several million events to achieve comparable sensitivity, adding to the technical demands of flow-based MRD assessment.

The two-tube, eight-color EuroFlow panel and the 10-color panel developed by Memorial Sloan Kettering Cancer Center (MSKCC) are among the most widely used and standardized flow cytometry panels for MRD assessment in multiple myeloma. Both panels offer very high sensitivity, often reaching detection thresholds of 10^{-5} , and have been validated across multiple clinical settings.

In addition, alternative panels developed by French and German flow cytometry groups provide valuable tools for MRD detection. While these are scientifically robust and clinically informative, they are not yet adopted as international standards, limiting their widespread implementation in multicenter trials or regulatory frameworks.

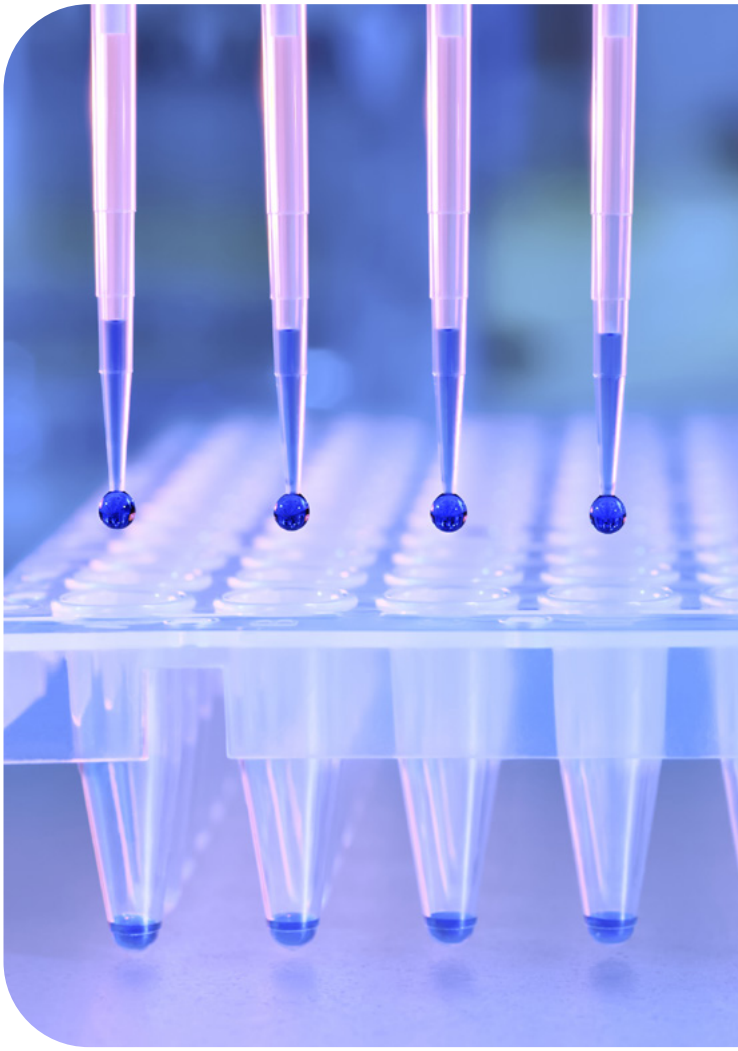
However, the choice between NGS and flow cytometry depends on the clinical context.

- Flow cytometry is advantageous when a baseline sample is unavailable, when genetic aberrancies are absent or not detectable due to technical limitations (5-10% of cases), or when rapid turnaround is needed
- NGS is better suited for clinical trials, longitudinal monitoring, and evaluating deep treatment responses due to its molecular precision and clonal tracking capability

Cerba Research's solutions for MRD assessment?

Cerba Research currently provides the Euroflow panel on the BD FACS Lyric instrument for MRD assessment.

Cerba Research can develop expanded flow cytometry panel that incorporate additional plasma cell and prognostic markers. These enhancements aim to address antigen modulation and therapy-induced phenotypic changes, improving the accuracy and clinical relevance of MRD assessment.



Should MRD and CAR+ T-cell persistence be monitored simultaneously after the CAR+ T-cell infusion?

Currently, there are no standardized guidelines for monitoring CAR+ T-cell therapy in multiple myeloma. Recent recommendations suggest assessing both the residual immune system and the persistence and functionality of CAR+ T-cells in parallel.

Relapse and MRD-positivity can occur for multiple reasons. In up to 50% of cases, relapse is attributed to lack of CAR+ T-cell persistence, while in others, it may result from loss or downregulation of target antigens on plasma cells. Therefore, before considering CAR+ T-cell reinfusion, it is essential to confirm antigen expression and examine plasma cell phenotype, ideally using high-sensitivity flow cytometric assays capable of detecting rare events.

Is NGS assay able to identify more MRD positive patients than flow cytometry assays?

Whether NGS detects more MRD-positive cases than flow cytometric assays remains a subject of investigation. While both techniques can reach similar sensitivities (typically around 10^{-5}), direct comparison data are limited. A notable study by the EuroFlow group compared their two-tube, eight-color Next Genetation Flow (NGF) assay with the LymphoTrack® NGS platform, both validated at a sensitivity of 10^{-5}.

In this study of 105 patient samples, 10 discordant cases were identified - all of which were NGS-positive and flow cytometry-negative, with MRD levels below 10^{-5}. However, only three of these patients eventually relapsed, raising the possibility that NGS may yield false-positive results at very low MRD levels, especially near the limit of detection.

Another important consideration is that flow cytometry and NGS are typically performed on separate aliquots of bone marrow, introducing the risk of sample-related variability, particularly in the presence of hemodilution. This pre-analytical factor may contribute to discordant results and should be carefully controlled in comparative MRD assessments.

The EuroFlow MM MRD assay (NGF) is a highly standardized, flow cytometry-based method developed for sensitive MRD detection in multiple myeloma. Under optimal conditions - using high-quality, non-hemodiluted bone marrow and analyzing at least 10^{7} events (10 million cells) - it could achieve a sensitivity of up to 10^{-6}. However, in routine clinical settings, a validated sensitivity of 10^{-5} is more commonly reached, depending on sample quality and cell yield.

NGS-based assays are currently considered the most sensitive and standardized methods for MRD assessment in multiple myeloma. While LymphoTrack® is typically validated at a sensitivity of 10^{-5} due to DNA input and assay design, ClonoSEQ® can achieve sensitivity up to 10^{-6}, offering deeper detection of MRD when sufficient DNA is available.

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Summary

1. **Flow cytometry is a cornerstone technology in CAR+ T-cell clinical research,** playing a critical role across the therapy lifecycle—from product manufacturing to post-infusion monitoring.
2. **Two main types of flow cytometry assays are used in CAR+ T-cell trials:**
   a. **Pharmacokinetic (CAR+ T-cell PK)** assay to quantify CAR+ T-cell cells.
   b. **Characterization assay (immunophenotyping of CAR+ T-cells)** to evaluate phenotype, activation, memory, and exhaustion status of CAR+ T-cell and endogenous T-cells.
3. Flow cytometry is also essential for **plasma cell immunophenotyping**, enabling detection of **MRD** and monitoring of target antigen expression—both prior to and during treatment. This supports patient selection and helps detect antigen escape or phenotypic changes.
4. **MRD can be assessed using two high-sensitivity methodologies: Flow cytometry and Next-Generation Sequencing (NGS)**. Flow cytometry allows for rapid, phenotype-based detection without needing a baseline sample and is particularly valuable when genetic aberrancies are absent or undetectable NGS, on the other hand, provide deeper clonal tracking and molecular precision. Both approaches are increasingly used as surrogate markers for clinical outcomes, such as progression-free and overall survival.
5. **At Cerba Research, CAR+ T-cell characterization assays are tailored to the intended use**, leveraging fit-for-purpose panel design and advanced flow cytometry platforms, including the **BD FACSLyric™ instrument and Cytek Aurora spectral flow cytometry**. This enables flexible, high-dimensional immune profiling across global clinical trials.