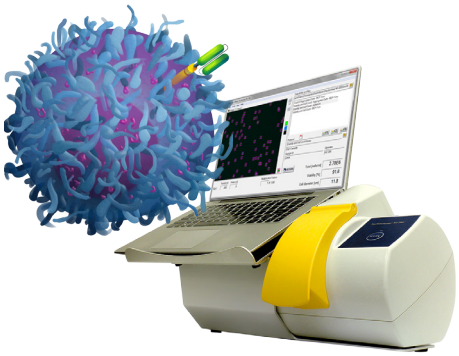
NucleoCounting CAR-T Cells

Clinical manufacturing of CAR-T cells for adoptive therapy using NucleoCounter® instruments

The NucleoCounter® NC-200™ provides robust and accurate determination of cell count and viability of T cells during adoptive CAR-T cell manufacturing. The unique Via1-Cassette™ combines cell sampling, staining and counting chamber loading into a single step, followed by automatic image acquisition and analysis by the NucleoCounter® for increased robustness.

Accurate detection of T cells, even in the presence of erythrocytes, beads or formulation reagents are facilitated by Acridine Orange and DAPI, which are already pre-loaded into the Via1-cassette™. All in all, the NucleoCounter® system is ideal for adoptive CAR-T cell manufacturing.



Adoptive immunotherapies using engineered CAR-T cells

One of the most promising approaches in cellular therapies is immunotherapies using autologous T cells. These patient-specific T cells can be engineered to express either T cell receptors or chimeric antigen receptors (CAR) to enhance the specificity and toxicity of T cells in response to different diseases, like graft-versus-host disease and cancer. Adoptive CAR-T cell therapy involves the collection of peripheral blood mononuclear cells (PBMC) from the patient, followed by enrichment of the desired T cell subset and genetic modification by viral transfection to create engineered CAR-T cells. The engineered CAR-T cells are then expanded in large-scale and infused back into the patient. With the NucleoCounter® NC-200™ it is possible to continuously monitor every step of the entire process of manufacturing CAR-T cells under GMP (Figure 1).

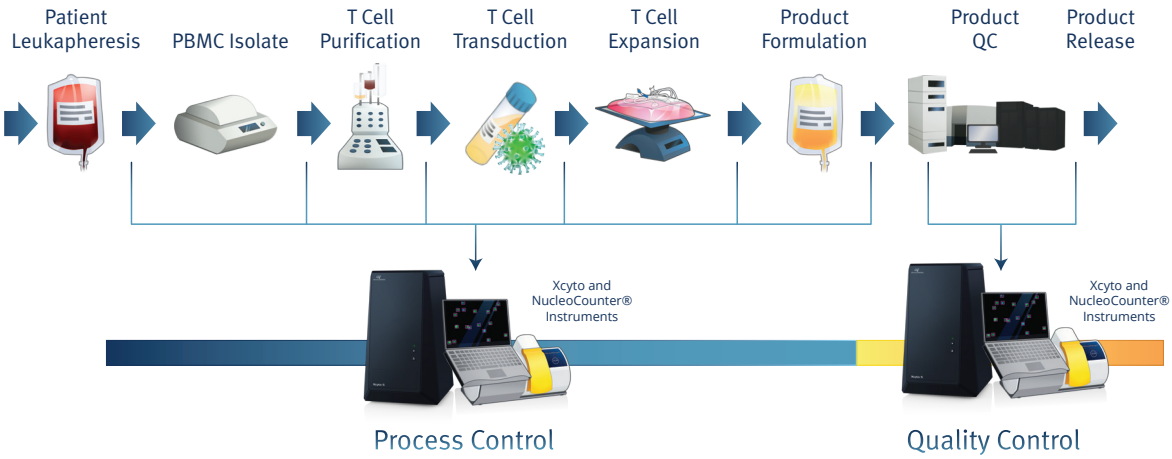


Figure 1: Monitoring the whole process from leukapheresis to the formulated product.
With the NucleoCounter® NC-200™ it is easy to monitor all different steps of the purification, expansion and formulation of CAR-T cells using the same instrument to ensure precise and reliable results.

Monitoring the whole CAR-T manufacturing process with

The NucleoCounter® NC-200™

Easy and user-adaptable protocols allow performing cell counting directly from leukapheresis as well as from purified and expanded CAR-T cells using the same instrument. The Via1-Cassette™ is pre-loaded the two dyes DAPI and Acridine Orange, which avoid some of the problems that lead to huge inaccuracies when bright field based counting is used. When counting samples containing erythrocytes, pre-treatment of the sample with an erythrocytes lysing buffer avoids the quenching effect of hemoglobin.

Afterwards, the total cell count and the dead cell count are respectively determined by cell staining with Acridine Orange and DAPI using the Via1-Cassette™. The analysis excludes cell fragments and artifacts like micelles as well as undersized events such as platelets, giving a highly accurate result. During isolation of T cells, magnetic beads may be used. These beads will have no influence at all on the cell counting with the NucleoCounter® NC-200™. In conclusion, the NucleoCounter® NC-200™ can be used for every step of the cell manufacturing process.

Superior Data Management and Presentation

The NucleoView™ software allows visual inspection of the fluorescence image and the opportunity to verify the counting (Figure 2). Specific event populations can be selected in the scatter plots and examined visually to determine the validity of their inclusion or exclusion from the final counting results. The adapted protocol can be then saved for future measurements, increasing reproducibility.

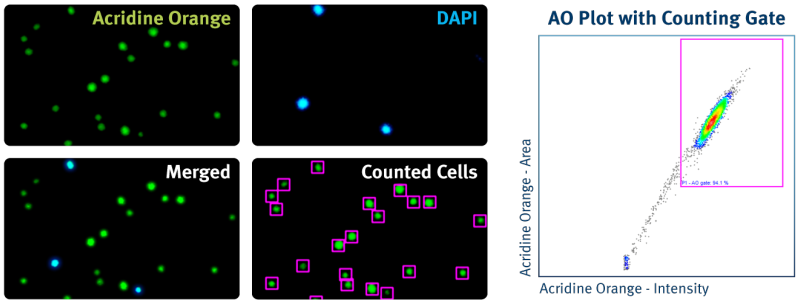


Figure 2: Superior data management and presentation using the accompanying NucleoView™ software. *The software allows for easy coupling between the obtained image and plots showing quantitative fluorescence for precise control of the analysis.*

Calibrated and GMP-Ready for 21 CFR Part 11

The mounting success of CAR-T clinical trials now requires the development of GMP manufacturing of clinical-grade CAR-T cells in order to safely commercialize this promising personalized therapy. As the Via1-Cassette™ minimizes variabilities during the cell counting process, the NucleoCounter® NC-200™ can easily be implemented in GMP and 21 CFR part 11 compliant laboratories. The built-in IQ/OQ/PQ protocols ensure control of consistent operation. Standardized PDF reports, user control and audit trails allow for adequate usage documentation (Figure 3).

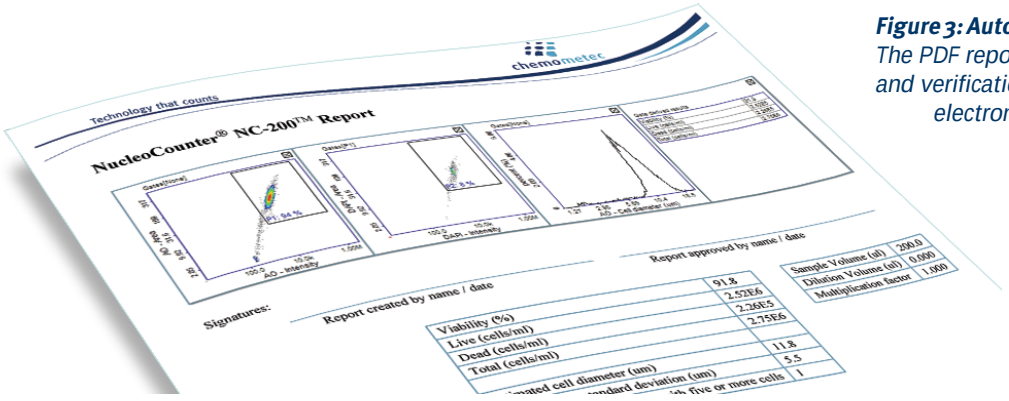


Figure 3: Automate and standardize PDF reports. *The PDF reports ensure accurate documentation and verification of the obtained results by using electronic approvals and signatures.*

For more information, please visit
www.chemometec.com/clinical-manufacturing-car-t-cells-adoptive-immunotherapy/

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