WHITE PAPER SUMMARY

Advanced flow cytometry for cell therapy manufacturing QC

The Cell and Gene Therapy Catapult (CGT Catapult) brought together leading companies to help accelerate technology development, and lower costs in cell and gene therapy manufacturing. The multi-partner, international consortium assessed multiple novel process analytical technologies (PAT) to drive advancement of an optimal CAR-T cell bioprocess.

In cell therapy analytics, **multiparameter flow cytometry** is a powerful analytical tool that can:

- Provide in-depth characterisation of cell-based medicinal products
- Establish a target product profile (TPP) defining product identity, safety and potency
- With a relatively quick turnaround time, flow cytometry can critically inform manufacturers on:
    - Quality of product batches
    - Consistency of manufacturing process[1,2,3]

Harnessing the potential of advanced flow cytometry for cell therapy manufacturing requires:

- Optimisation and standardisation of workflows
- Automation of data acquisition and analysis

A **critical step** in the consortium's bioprocess analytics was the use of the BD FACSLyric™ Flow Cytometer and a novel 12 colour antibody panel to monitor the phenotype of the T cells during and post bioprocessing.

An efficient and standardised QC process could help to reduce variability and improve quality ensuring the manufacture of products with reliable performance. Ultimately, this will help to reduce costs, making advanced therapy medicinal products (ATMPs) more accessible to patients.

BD

CGT Catapult in partnership with BD

CGT Catapult partnered with BD to develop a **12 colour flow cytometry panel** to monitor T cell phenotype and functionality during their exemplar **8 day CAR-T cell bioprocess,** providing detailed characterisation of complex medicinal cell products.

Good flow cytometry practice was employed, using[4,5,6]

- Titrated antibodies on specific target cell populations
- A defined hierarchical gating strategy
- Appropriate controls to reduce error spreading and improve detection sensitivity

The **BD FACSLyric™ Flow Cytometer** was used for acquisition and the **BD FACSuite™ software** was used for data analysis.

BD shared expertise to help develop and optimise a novel 12 colour T cell panel to characterise cells. Monitoring the T cell phenotype, wherein the T cell differential marker expression distinguishes between naïve, effector and memory subsets, alongside their activation and exhaustion status was of critical importance to compare and baseline the data generated by the novel PAT. It was essential that the panel was robust and provided high-quality phenotypic data. Defining these phenotypes in T cell manufacture is crucial to determine quality target product profile (QTPP) and critical quality attributes of a final T cell-based medicinal product.

The results

From three independent experiments, using three different healthy donors, the panel showed:

- Clear resolution of individual cell populations throughout the manufacturing process
- Consistent patterns of surface marker expression at each timepoint

The findings:

- Demonstrated the panel as fit for purpose to apply to the PAT Consortium's dataset[7]
- Highlighted the panel's reliable performance in identifying T cell phenotypes
- Supports the use of the panel for QC guidance in CAR-T cell processing

The full article, which includes the panel used and the results generated, is published by Cell and Gene Therapy Catapult on their website and can be accessed here

Learn more about how BD Biosciences can support you in the field of Cellular therapy and CAR-T therapy development.

References

1. Hartmann FJ, Babdor J, Gherardini PF, Amir EAD, Jones K, Sahaf B, et al. Comprehensive Immune Monitoring of Clinical Trials to Advance Human Immunotherapy. Cell Rep. 2019 Jul;28(3):819-831.e4.
2. Campbell JDM, Fraser AR. Flow cytometric assays for identity, safety and potency of cellular therapies. Cytometry B Clin Cytom. 2018 Sep;94(5):725–35.
3. Sommer C, Boldajipour B, Kuo TC, Bentley T, Sutton J, Chen A, et al. Preclinical Evaluation of Allogeneic CAR T Cells Targeting BCMA for the Treatment of Multiple Myeloma. Molecular Therapy. 2019 Jun;27(6):1126–38.
4. Tantalo DG, Oliver AJ, von Scheidt B, Harrison AJ, Mueller SN, Kershaw MH, et al. Understanding T cell phenotype for the design of effective chimeric antigen receptor T cell therapies. J Immunother Cancer. 2021 May 25;9(5):e002555.
5. Sabatino M, Hu J, Sommariva M, Gautam S, Fellowes V, Hocker JD, et al. Generation of clinical-grade CD19-specific CAR-modified CD8+ memory stem cells for the treatment of human B-cell malignancies. Blood. 2016 Jul 28;128(4):519–28.
6. Schmueck-Henneresse M, Omer B, Shum T, Tashiro H, Mamonkin M, Lapteva N, et al. Comprehensive Approach for Identifying the T Cell Subset Origin of CD3 and CD28 Antibody–Activated Chimeric Antigen Receptor–Modified T Cells. The Journal of Immunology. 2017 Jul 1;199(1):348–62.
7. Catapult Cell and Gene Therapy. CGT Catapult concludes the Process Analytical Technologies (PAT) consortium aimed at improving ATMP bioprocessing. https://cgt.ams3.cdn.digitaloceanspaces.com/White-Paper_Advanced-Flow-Cytometry-for-Cell-Therapy-Manufacturing_v1.0_May-2023.pdf

BD Flow Cytometers are Class 1 Laser Products.

CE BD FACSLyric™ Flow Cytometer with the BD FACSuite™ Clinical and BD FACSuite™ Application are in vitro diagnostic medical devices bearing a CE mark.

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