European Biotechnology

Spring 2016

Bioprocessing

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Mastering more patient-centric bioprocessing

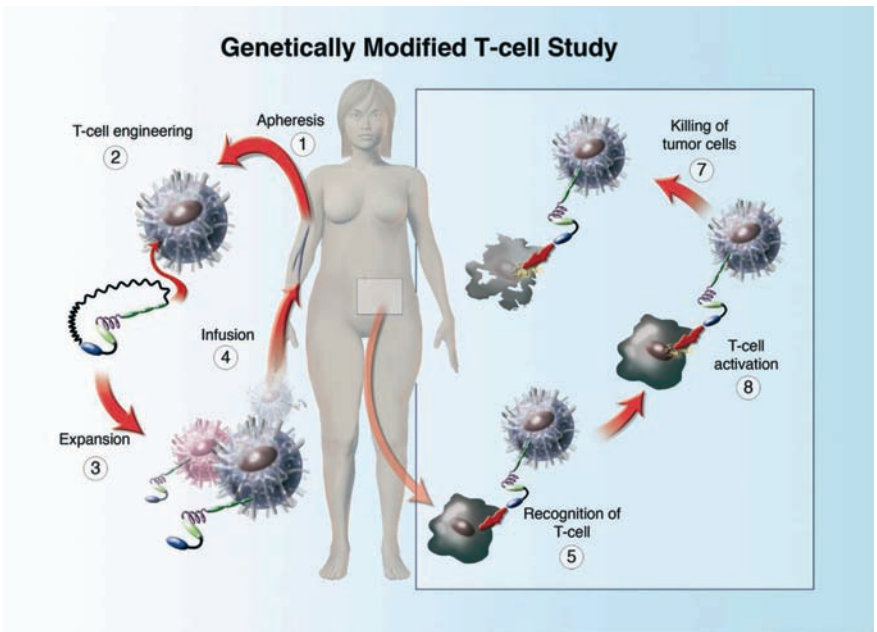
PROCESSING As one-fits-all therapies for large patient groups are increasingly being displaced by targeted biologics and biosimilars, treatment is evolving to become more patient-centric. The clinical triumph of antibody targeting directly impacts the CMO market. The trend of producing small lots for preselected subpopulations is particularly evident in the field of cancer immune cell therapy.

The past decade has seen a significant shift in the nature of the products being manufactured and sold by the biopharma industry. The portfolio of today reflects increased therapeutic competition, a greater prevalence of large molecule drugs, expansion in the number of personalised or targeted products, and a rise of treatments for many orphan diseases. These trends have given rise to biopharmaceutical products with extremely limited production runs, highly specific manufacturing requirements, genotype-specific products and patient-near cell therapy approaches. This fundamental shift in the overall product mix and a focus on continuing to improve the efficiency and effectiveness of production is spurring an evolution in the technologies and processes needed to support advanced biopharmaceutical manufacturing.

Innovation in bioprocessing is helping to drive improved economics, flexibility and quality while potentially benefiting patients both directly and indirectly. Manufacturers have been boosting investments in the following areas:

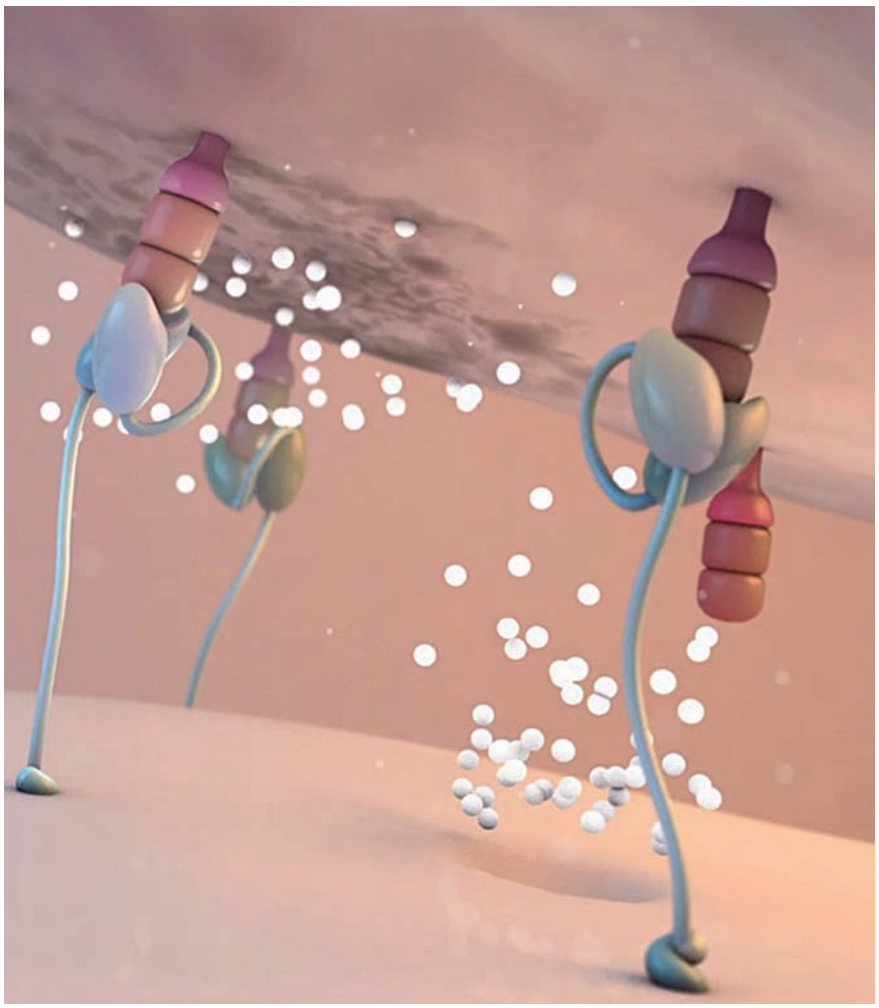
- Continuous manufacturing to improve scalability and facilitate time to market, while lowering capital and operating costs and enhancing quality
- New process analytical tools to improve process robustness, accelerate scale-up to commercial production and drive more efficient use of resources
- Single-use systems to increase flexibility and reduce production lead times, while lowering capital investment and energy requirements
- Alternative downstream processing techniques to improve yields while lowering costs, reduce waste, and new vaccine and therapy production methods to increase capacity, scalability, and flexibility.

Additionally, new types of products are coming to market. Albeit in early clinical development, adoptive T cell therapies have attracted significant attention due to the fact that CD19-targeting blood cancer therapies achieved response rates of up to 89%. However, clinical supply of T killer cells transduced with genes encoding artificial T cell receptors or chimeric antigen receptors (CARs) is currently limited due to difficulties in standardising these autologous therapies. CAR-T cell therapy manufacturers face multiple challenges:



GMP-compliant ex vivo manufacture of T cells, engineered to express a tumour-selective chimeric antigen receptor (CAR, 2) is currently limited to few institutions worldwide, which can provide the aseptic infrastructure and skills required for the laborious cleanroom preparation and expansion of patient-derived CAR-T cell treatments. Artificial T cell receptors

Picture: The Penn Ovarian Cancer Research Center



T-cell (above) docking to a tumour cell (below) antigen (most often CD19) with the single chain antibody fragment of its artificial T cell receptor. The hinge region of the scFv might contain binding site for a small molecule or a suicide switch used to control the immune reaction.

- No step – from T cell harvesting over transduction of the T cells (with genes encoding a targeting single chain antibody fused to T-cell-activating costimulatory molecules) –is currently being automated, which boosts the probability for errors or contaminations.
- most processes are based on engineering of patient-derived (autologous) T-cells, and thus can't be carried out in a central facility producing frozen off-the-shelf products
- supply of materials is often limited to few specialist providers

At the end of January, six companies and two university clinics kicked off the development of an automated and scalable process in a closed system aimed at producing GMP-compliant, autologous CAR-Ts. The budget of the EU consortium dubbed CARAT (Chimeric Antigen Receptors for Advanced Therapies) over the next four years is €6m.

CARAT: towards a gold standard

The plan is to automate the currently laborious steps of cell manufacture by carrying out all processing steps in bags with closed tubing pathways and connections, which would allow producers to work in Class 100,000 instead of the costly Class 100 sterile environments.

Following isolation of appropriate T cell subpopulations, all steps – including cell preparation, enrichment, activation, transduction with lentiviral vectors, expansion to final formulation – will be carried out in a closed, single use tubing set with automated in process control and documentation by flow cytometry and subsequent barcoding. Usage of such unit-based systems would allow multiple production processes to be carried out in parallel, without the danger of cross-contamination.

Off-the shelf allogenic products

While process automation holds great promise for autologous CAR-T cell therapies in several blood cancers. i.e. developed by Novartis, Kite Pharma, Juno, or Bellicum, Paris-based Cellectis SA and British Adaptimmune follow a different approach. Limiting off-target effects as well as autoimmune reactions by Cellectis' proprietary TALEN-based genome editing technology, they claim to be capable of using allogenic T cells without inducing a severe auto-immune reaction (host-versus-graft HvG, or graft-versus host, GvH, reaction). In contrast to the autologous approach, the allogenic off-the shelf-approach does not require precise logistics, proximity between production facilities and the bedside or long waits for hospitalised patients due to the long time it takes to generate appropriate T cells for therapy.

Prevention of GvH is achieved by a targeted knock-out of the TCRab within the CAR-T cell genome, while HvG is addressed by using lymphodepleting regiments such as purine nucleotide analogues (PNA). The allogenic CAR-T cells are designed to have a higher tolerance to PNAs by TALEN-mediated inactivation of the gene that encodes deoxycytidine kinase (dCK). dCK catalyses the reaction that make the PNA prodrugs toxic. At the end of December, Cellectis applied to carry out a first-in-man trial with its blood-cancer candidate UCART19 in the UK. ■

Picture: Cellectis

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BIOMANUFACTURING Supported by long-term state funding, Poland is poised to become a technology transfer and product development hub for globally active biotechs. Gdansk-based Polpharma Biologics provides all the skills and capabilities needed to succeed in global biopharmaceutical markets.

Beyond 2020, the Polish government plans to invest heavily into building scientific partnerships, expanding technology transfer and boosting advanced manufacturing capacities. The city of Gdansk is determined to become Poland's precision medicine hub by robust investments being made in the country's leading biobank and the rapid construction of an ultra-modern university hospital.

Polpharma Group – a regional powerhouse and partner of choice

Polpharma Biologics, an emerging leader in biopharmaceutical outsourcing, is a name to remember for your business development agenda. Today, the Polpharma Group, with its 80-year heritage in developing and manufacturing fine medicines, is a pharmaceutical powerhouse. Commercial operations stretch across the region and the company's development and manufacturing network is designed as a hub and spoke product supply framework poised for rapid growth. Tomorrow the company will be a principal partner in Central and Eastern Europe for the development and manufacture of biopharmaceuticals.

Want to outsource with confidence?

Polpharma Biologics offers early mover advantage in a way which has the ability to offset risk and make budgets go further. Contract Research, Development & Manufacturing supports rapid market access, drives efficiencies in development programmes and moreover tech transfer may qualify for external development financing. Flexibility is a valuable ingredient and there is a thirst for new technologies, which you can turn into opportunity.

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Polpharma Biologics is an ideal partner for international biotech companies looking for flexible, small-scale cell culture development (up to 1,000 litres) and commercial scale manufacture. Regulatory, development and manufacturing processes are fully compliant to EMA and FDA approval. Global players have already placed their trust in the company and are jointly develop-

Picture: polpharma

ing products with Polpharma for global markets. And so can you.

Outsource with upside – make your budgets go further

Polpharma Biologics offers outsourced development and manufacturing with a plus, particularly:

- Flexible outsourcing of global R,D & M programmes to drive efficiency – time and cost advantage
- Using local D & M as a route to market in Europe, CIS & MENA – accelerate growth
- Transferring technology to benefit from financial incentives.

For companies looking to enter this region with their brands, the company's footprint confers real advantage in terms of market access where local manufacturing is an important key to market access and success. In the future, the company will be offering large scale volume manufacture and is also evaluating bacterial production.

It is worth remembering that partnerships are at the core of the company's success. The company has an important role to play in global development programmes where flexibility and efficiency play a vital role – beyond established markets. Polpharma Biologics is a preferential licensing partner in the new emerging markets of continental Europe, CIS & MENA where biotech is poised to take off and nearshoring is a nascent, yet rapidly growing trend.

Why Polpharma Biologics?

- Flexible, biopharmaceutical outsourcing for EU, CIS & MENA – regulatory, development and manufacturing – an approach driven by quality, efficiency and cost advantage
- A focused and dynamic team of international professionals with significant experience in the regulation, development and manufacture of biopharmaceuticals gained in leading global companies
- A powerful licensing partner for global biotech companies looking for serious growth in new export markets.

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Picture: polpharma

Highly automated protein characterisation

LAB-ON-THE-CHIP Timely and precise characterisation is crucial when it comes to the analysis of critical quality attributes of postranslationally modified monoclonal antibodies during DOE experiments. The LabChip GXII platform provides a rapid alternative to current time-consuming techniques such as SDS-Page, CE or HPLC.

› By Rick Bunch, PerkinElmer, Hopkinton, US

Monoclonal antibody (MAb) products are inherently heterogeneous because of post-translation modification that often occurs during the fermentation process. Therefore, thorough characterisation of MAb products is required in order to assess their critical quality attributes (CQAs). In early stage process development, Design of Experiments (DOE) studies or Factorial Experimental Design (FED) examinations are performed to understand the relationships between the process parameters and product quality in order to define a process design space around the variability in the process parameters. The increased number of variables to be examined in a DOE study of cell culture conditions or purification process produces large number of samples that need to be processed. Current methods of monitoring protein product quality include SDS-PAGE and HPLC. SDS-PAGE is both labour intensive and difficult to automate and has been supplanted by CE-based separation in modern analytical laboratories. CE and HPLC analysis require separation times of 15-60 minutes per sample, which can limit DOE studies.

In the past several years, microfluidic-based assays for sizing, quantification, and purity assessment of proteins are finding wide use because they address the limitations of SDS-PAGE, HPLC, and CE methods. Chen et al. studied the use of the LabChip technology for screening MAb product quality attributes. They analysed MAbs from crude CHO cell culture supernatants and purified samples under reducing and non-reducing conditions. They showed similar resolution and sensitivity as conventional CE-SDS on a 20 cm capillary but on a time scale of ap-

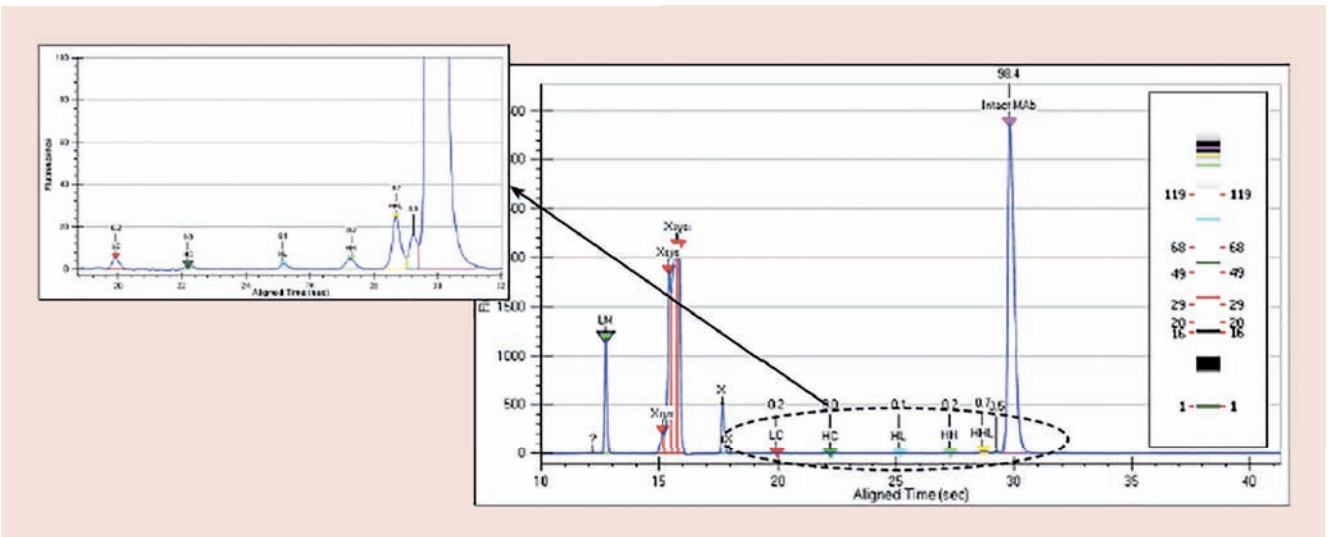

Figure 1: Non-reduced MAb electropherogram indicating the percentual purity of the fragments and intact MAb. The peak marked X at approx. 17.5 seconds is lysozyme spiked into the antibody and used as an internal standard. LM is the lower marker and Xsys are the system peaks.

Picture: PerkinElmer

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The LabChip GXII platform provides automated rapid analysis of proteins and nucleic acids. Sampling directly from a 96- or 384-well plate, the LCGXII offers characterisation of proteins for purity, glycan profile, and charge heterogeneity. Each individual sample can be analysed in 41 seconds or less, enabling a 96-well plate to be processed in slightly longer than one hour.

SDS-PAGE on-a-chip

The protein sizing on the microfluidic chip is achieved by integration of the main features of one-dimensional SDS-PAGE: these include the separation, staining, destaining, and detection steps. Denatured proteins are brought onto the chip directly from a microtiter plate through a capillary sipper. The samples are then electrokinetically loaded and injected into the 14mm long separation channel that contains a low viscosity matrix of entangled polymer solution. The protein-SDS complex and free SDS micelles in the sample plug are immediately stained by dye present in the sieving matrix. At the end of the separation channel, the sample is diluted to reduce the SDS concentration below its critical micelle concentration in order to reduce the background fluorescence so that protein-SDS-dye complexes can be detected.

Separate and quantify MAbs quickly

The LabChip microchip-based assays are used to separate and quantify monoclonal antibodies and their fragments with minimal sample preparation artifacts, including crude samples. Figure 1 is an example of an electropherogram for a non-reduced MAb at a concentration of 1 mg/mL. The inset plot shows that the low level fragments, such as, the light chain, heavy chain, heavy-light chain, heavy-heavy chain, heavy-heavy-light chain, and intact MAb

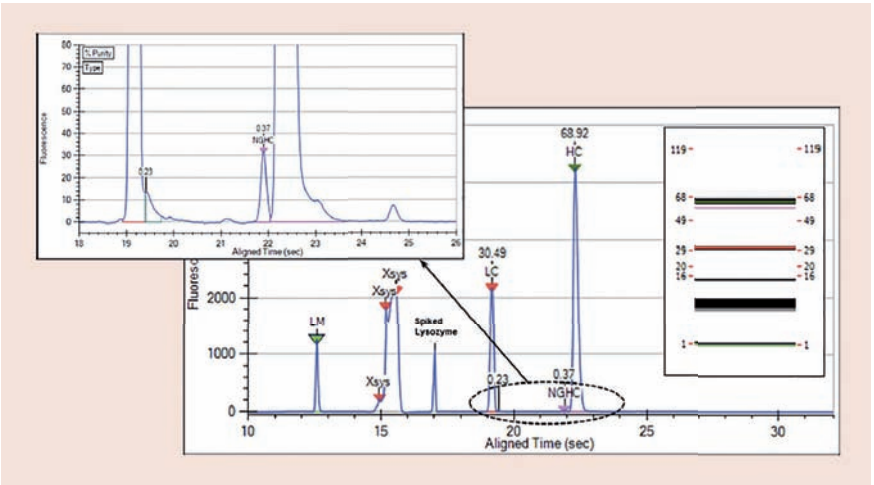

Figure 2: Reduced MAb electropherogram

are all well resolved. The electropherogram for the reduced MAb is shown in Figure 2, illustrating sufficient resolution of the non-glycosylated heavy chain (NGHC) from the heavy chain for accurate quantification..

Inter-assay precision is another parameter that is important for the application of instrumentation to protein product purity assessment. An example of the precision of the LabChip Protein Express assay is provided by reviewing the results of the following experiment. Three different operators did eight sample preparations for each MAb under reducing and non-reducing conditions. Each operator then ran the samples on separate chips and instruments. The relative standard deviation (RSD) of the intact MAb was ≤ 0.5%. The heavy chain was ≤ 2%, while the light chain and non-glycosylated heavy chain (NGHC) ≤ 4%.

Inter-assay precision and reproducibility

Another important assay parameter is sensitivity. In order to consistently assess the amount of small peak areas associated with partially assembled, or partially degraded MAbs, an assay capable of quantitating not only intact components but also multiple small 1% or 2% amounts of various components is required. To demonstrate the utility of the Protein Express assay for determining small component concentrations, the following experiment was run. Three MAbs were diluted to an expected concentration of 1,000 µg/mL. Lysozyme was used as an internal standard and spiked into the sample buffer at 10 µg/mL. The time-corrected area of the antibody peak was normalised to the time corrected area of the lysozyme peak for each sample. As in the assay precision determination, three operators did eight sample preparations and ran the samples on separate chips and instruments. The RSD for the quantification was ≤ 2% for intact MAb1 and MAb3 and ≤ 5% for intact MAb2.

Finally, in order to demonstrate the detection of low-level impurities, lysozyme was spiked into the sample at 1% of the total protein concentration. The MAb was run under reducing conditions at 1mg/mL. The small lysozyme peak was resolved with a signal to noise ratio of 9:1.

The HT Antibody Analysis 200 assay on the LCGXII can be used as a high throughput automated alternative to SDS-PAGE and conventional CE-SDS for screening antibody product quality attributes including quantification, fragmentation, and purity analysis. It offers a quick time to result (41 seconds per sample) with ≤ 1% precision. Good linearity is achieved for assays over a concentration range of 8 to 2000 µg/mL. The assays have the ability to detect low level impurities with a signal to noise ratio of 9:1. ■

Picture: Perkin Elmer

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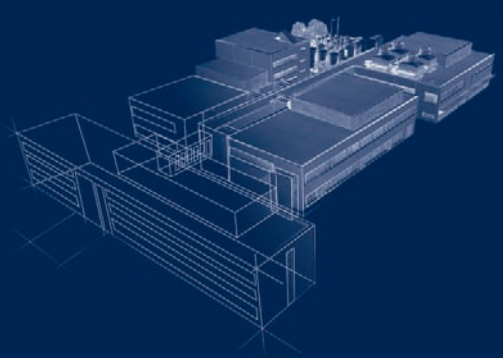

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