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BioPharm INTERNATIONAL®

Biopharmaceutical Process Development

Considerations for Smarter and Faster Process Development

This custom ebook is sponsored by Cytiva and presented in partnership with *BioPharm International*.

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Introduction

Smart process development uses tools and methodologies to improve process outcomes and shorten time to market. New modalities entering the pipelines often lack platform process solutions, so working smartly in process development may become a key differentiator.

For example, an evolution in process development methodology has process developers moving away from studying one factor at a time toward multivariate data analysis. This analysis involves statistical models, such as design of experiments (DoE), and, more recently, mechanistical models based on mathematical equations.

In addition, the use of parallelization and automation for experimental setup, known as high throughput process development (HTPD), increases the amount of information available and reduces the sample amount used.

All these tools and methodologies enable accelerated process development and improve general process understanding, robustness, and performance.

In the following articles, we share some industry drivers to go smarter and faster in process development as well as insights on how to make both upstream and downstream process development workflows more efficient.

–Henrik Ihre, Ph.D.
Director, Strategic Downstream Technologies
Cytiva

What is driving the need to go smarter and faster in biopharmaceutical process development?

Henrik Ihre, Director of Strategic Downstream Technologies at Cytiva outlines some trends in the process development world.

The biopharmaceutical industry experienced a decade of tremendous success with more than 1600 commercially available drugs approved for therapeutic use. At the time of writing in mid-2022, more than 300 antibodies and recombinant proteins are in late-stage development, and a great number are expected to enter late-stage development in the coming years (1).

A constantly diversifying pipeline

Monoclonal antibodies (mAbs) continue to have a strong position in the drug pipeline. At the same time, molecule diversity is constantly increasing thanks to medical innovation. For example, nearly a quarter (24%) of mAbs and antibody fragments in the pipeline are novel (e.g.,

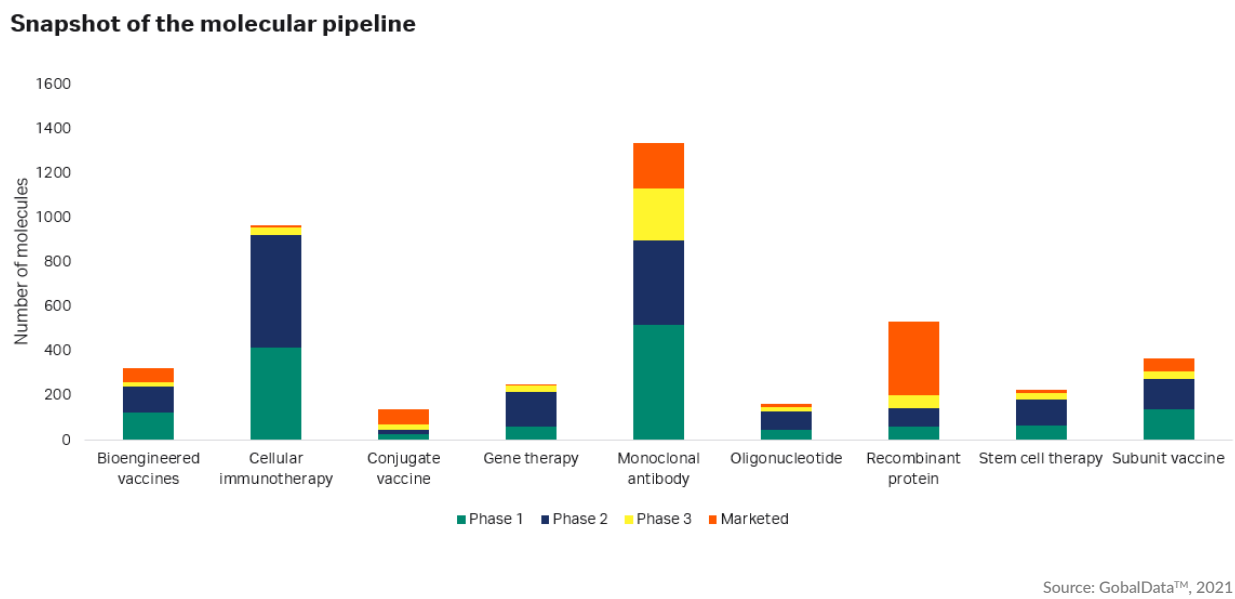
bispecific antibodies, antibody fragments, and antibody drug conjugates). And the diversity expands beyond mAbs. The new class of mRNA vaccines is just one of several examples of that diversity, which also includes plasmids, oligos, cell and gene therapies, and other novel vaccines (FIGURE 1).

INTERVIEW

Top challenges to overcome when developing AAV processes

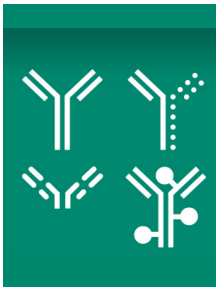
For these new modalities, one key challenge is the lack of established platforms compared with the now well-established processing protocols for traditional mAbs. In addition, these new modalities have molecular properties that often differ from conventional biomolecules. Therefore, existing tools may not always offer the best solutions. New products and formats may have to be developed to offer novel and flexible platform solutions going forward. However, smart process development combined with existing solutions will enable a good starting point also for these new modalities.

FIGURE 1: The molecular diversity is constantly increasing, expanding beyond mAbs, due to medical innovation.



The need to reduce development timelines and costs and secure process robustness

Historical drug development timelines used to be eight to ten years (FIGURE 2). Now however, with accelerated clinical development, the overall development timeline can be shortened significantly, with many companies demanding that new molecules be developed in only three to five years. Consequently, process development activities have become critical factors affecting the time to market with several activities performed in parallel with clinical trials.



WEBCAST

Navigating the diverse antibody pipeline with chromatography innovations

However, shortened time to market is not the only important goal. Equally important is to design, with a quality by design spirit, a manufacturing process that is productive, scalable, and robust. Designing such a process is enabled by smart process development ways of working.

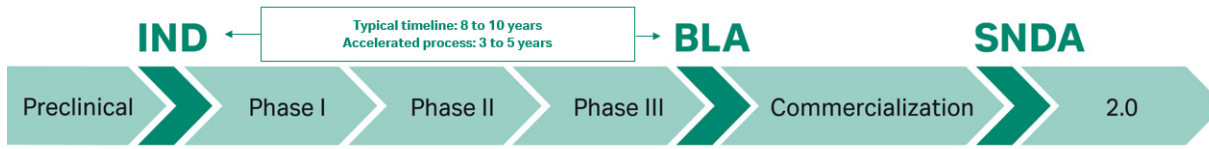
INTERVIEW

Insights and strategies for successful development of scalable AAV processes

Reference:

1. GlobalData [Online.] https://www.globaldata.com/. Accessed 17 May 2022.

FIGURE 2: The typical timelines between investigational new drug (IND) and biological license application (BLA) have historically taken eight to ten years, but accelerated processes can shorten it to three to five years.



Insights and approaches to increasing efficiency during upstream process development

Andreas Castan, Ph.D.

Andreas Castan, Ph.D., the Director of Strategic Upstream Technologies at Cytiva, recently answered some questions about process development and scaling.

Q: What does the workflow for process development look like, and on what important areas should a process developer focus?
AC: Process development creates the basis for a sustainable manufacturing process. The starting point for process development is the knowledge of the properties of the product. Is it stable, unstable, or toxic for cells? This knowledge will help you decide between two major process modes: fed-batch development or continuous.

Process-development work starts with cell line development. A good, high-producing cell line that is stable and produces a product with the desired quality attributes are key for your process.

Let me walk you through a typical workflow from cell line development through process development to pilot-scale production of material for toxicological studies (tox supply). FIGURE 1 illustrates what activities are performed, the analytical focus, how many clones can be carried into the next phase of process development, and what culture volumes are applied.

After single-cell cloning, the clones are mainly assessed for titer in micro-titer format. The best clones are transferred to the next stage in deep-well plates. Product quality may also be screened. Next, a relevant number of clones are transferred into a small-scale fed-batch development to assess the platform fit, e.g., the lactate profile, feed compatibility, and sheer tolerance. This stage can be performed in micro-bioreactors. Only a few clones are taken further into full process development where the full product quality is assessed in stirred tank bioreactors. The full process development phase studies parameters such as pH, feeding strategies, aeration strategies, etc.

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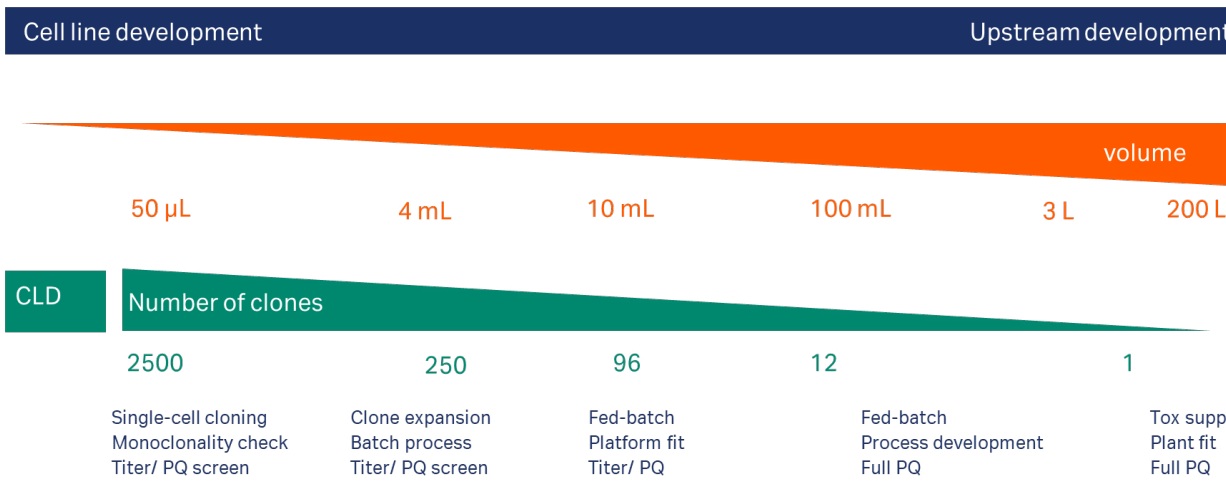
Optimized process efficiency in upstream manufacturing

FIGURE 1: Workflows in cell line development and upstream process development.



You should consider process intensification at an early stage in the development. Process intensification should focus on the bottlenecks in the process. Examples for process intensification are N-1 perfusion, high cell density and high-volume cell banks, and hybrid processes in the production bioreactors. Your objective is to always improve the volumetric productivity of the process, i.e., making more product in smaller bioreactors in shorter time.

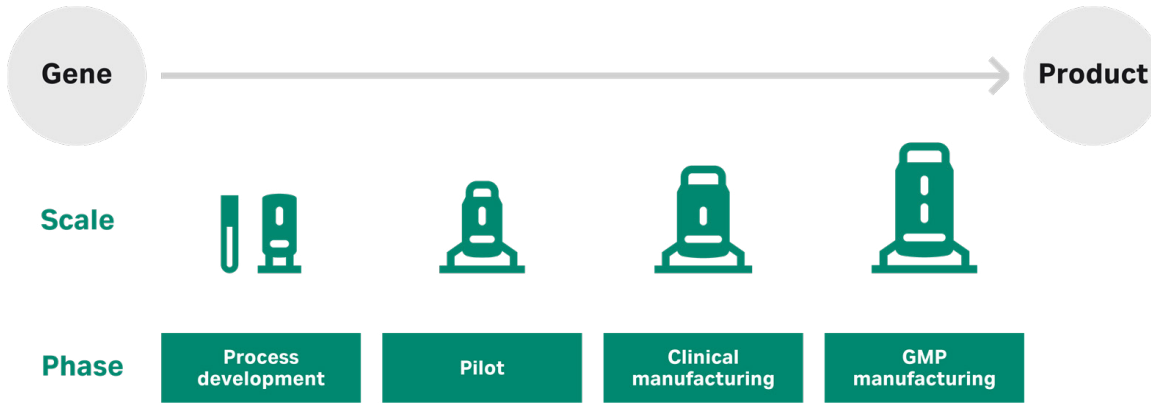
The result of the full process development is one fully developed clone. This clone is used to produce the master cell bank (MCB) and is scaled up to pilot scale to produce the material used for toxicological studies.

Apart from cell line and process development, the choice of cell culture media and feeds is key to a high-producing process. My advice is to focus your process-development activities on the requirements coming from the target molecule and leverage platform technologies, e.g., media platforms for fed-batch and perfusion, that accelerate your development.

Q: What are the major goals of moving from a small-scale culture to larger processes? And what challenges should be considered?

AC: When starting a small-scale development activity, you need to keep the end in mind. At an early stage, you need to have information about the pilot, good manufacturing practices (GMP), and the commercial plant. This information is related to the plant equipment and the staff. You should also get early visibility of the manufacturing strategy, e.g., will production be in-house or outsourced to a contract manufacturing organization (CMO)? FIGURE 2 illustrates a product moving from early research to process development to manufacturing.

FIGURE 2: Process scale-up is a given step in all projects going from PD to commercial manufacturing.



When moving from process development to pilot or clinical manufacturing, there is a huge difference whether you run a fed-batch or a perfusion process. The media preparation capability is also an important factor to consider. Furthermore, you need to plan how the upstream process connects to the downstream train. For example, you need to know in what cadence harvest material is produced. Knowing your projected material needs helps to determine the scale of clinical and commercial manufacturing.

Other things you should consider when designing a process in process development include:

- The strategic choice you make in process development will impact manufacturability for a long time.
- The complexity of the process design should be appropriate to the skills of your staff. Design the process as simple as possible.
- The scalability and transferability of sensor technology and automation to a GMP environment is important. Select technologies that work in manufacturing.
- The factors affecting manufacturability such as the number of feeds, the process duration, and plant scheduling considerations, should be taken into account.
- The qualities of the cell culture media system, such as stability and ease of preparation and storage are also important factors for the ease of manufacturing.

"The strategic choice you make in process development will impact manufacturability for a long time."

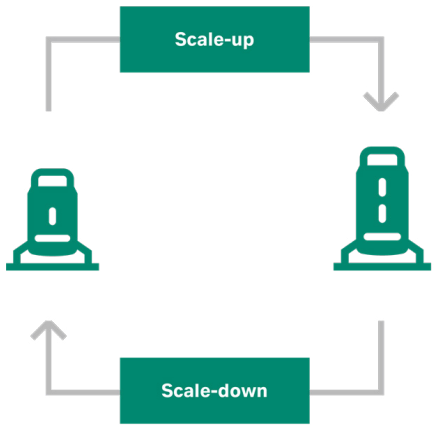
Q: What strategies and tools would you consider for scaling to bioreactors?

AC: With bioreactors, you need to find suitable operating parameters for the larger scale to ensure sufficient oxygen transfer and CO_2 removal. This process is called a scale-up strategy. Think about the capabilities and limitations in your large-scale bioreactor that you need to consider during process development. What are the capabilities and limitations in your large-scale bioreactor that you need to consider in process development? In other words, how can you develop a small-scale process in a design space that overlaps with the larger-scale design space? Ensuring an overlap of the small-scale and large-scale design spaces requires an established scale-down model (FIGURE 3).

ARTICLE

Bioreactor scale up and scale down: applications and tools

FIGURE 3: Using an established scaling model helps both scale-up and scale-down.



Scale-up

Find operating parameters for the next scale the process is transferred to

Scale-down

Develop a process on a small scale that can run with the existing limitations in mass transfer in the pilot or production scale bioreactor

Both the scale-up and scale-down models need to investigate in a multidimensional design space, which is described by the agitation, sparger geometry, and gas-flow rates. Tools are needed to ensure sufficient oxygen transfer and CO_2 removal across scales. Some companies base their scaling strategies on experience. Other companies use spreadsheet-based tools to guide them. Cytiva™ has developed a bioreactor scaler that helps you establish sound scaling strategies.

Q: What do you recommend to people developing new processes?

AC: Again, it is important to keep the end, i.e., the final manufacturing process, in mind. Start with the implications of the type of product you have and what type of process can you run in your plant or at a CMO. The cell line determines the total productivity — invest in it in the cell line development process and take several clones with you to final process development. Use platform technologies where available, such as media and feeds, single-use bioreactors, and automation solutions. Consider process intensification options at an early stage where it makes sense for your process and plant. Develop a process and establish scale-down models that will facilitate later scale-up. With all that in mind, you are in a good position to develop a successful manufacturing process.

This content is based off an interview with Andreas Castan, Ph.D., that can be viewed here.

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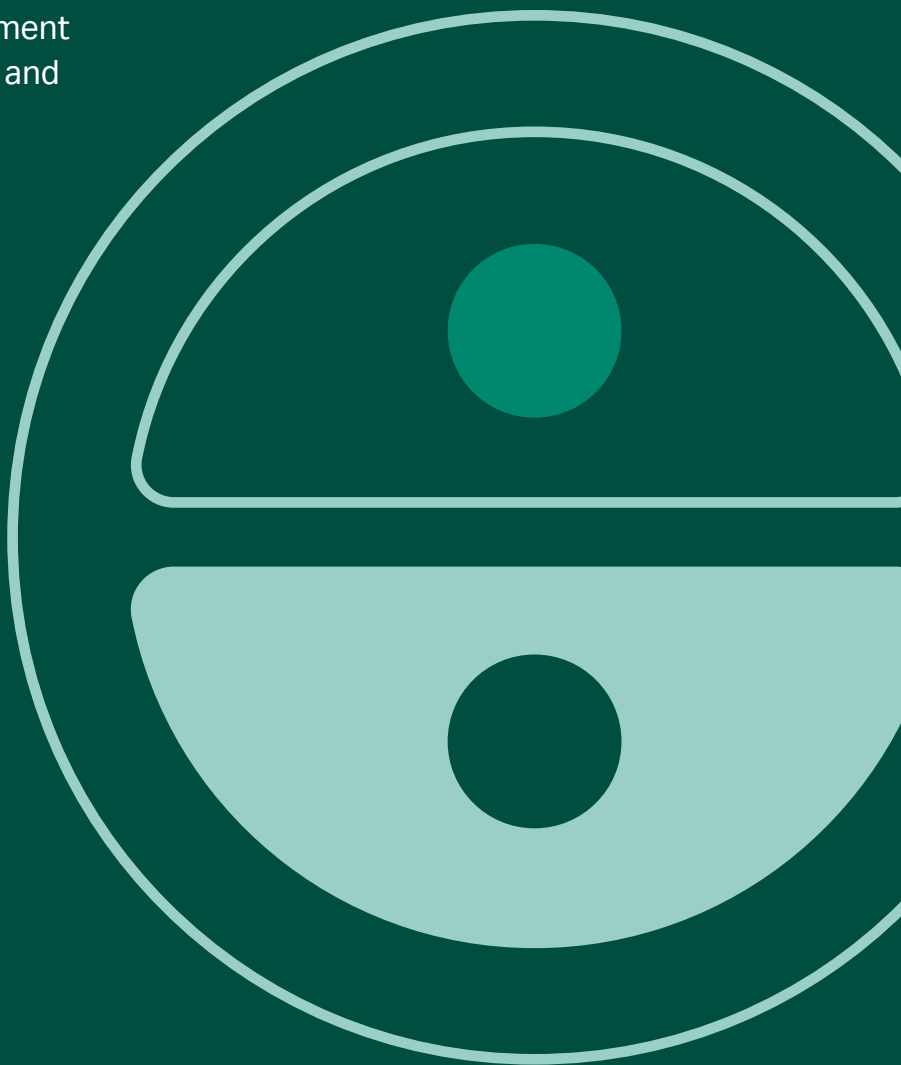

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Tools and methodologies for smarter downstream process development

John Scibetta

John Scibetta, Advanced Chromatography Specialist at Cytiva, explains how to make chromatography process development smarter and faster.

EVOLUTION OF DOWNSTREAM PROCESS DEVELOPMENT METHODS: FROM OFAT TO MECHANISTIC MODELING

If you are experienced in biopharmaceutical process development, you know that the technology available to you has changed dramatically over the years. Initially, process developers used to depend on one factor at a time (OFAT) methods, which required many experiments to develop workable processes. It was time consuming.

Design of experiments introduces randomization

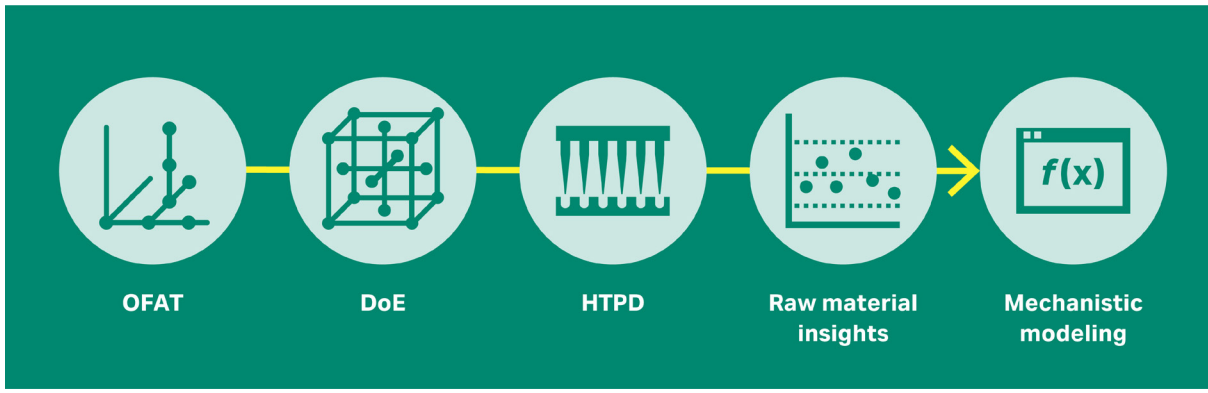
Thankfully, process development scientists developed smarter methods and tools that enabled time and cost saving (FIGURE 1). For example, they recognized that interactions between factors could be evaluated with data mining approaches such as multivariate data analysis using statistical modeling to help direct, focus, and reduce screening efforts. So they employed design of experiments (DoE), which is a systematic approach to varying several experimental parameters simultaneously to obtain greater information.

An important method made possible by DoE was the principle of randomization. To avoid biased results, combinations of running parameters (such as pH, mass load, and conductivity) from the total population they are meant to represent (i.e., the eventual operating space, or design space), are tested in a stochastic order. DoE, combined with the use of statistical methods, transformed the framework of modern experimental design.

DoE, combined with the use of statistical methods, transformed the framework of modern experimental design.

Another benefit of DoE is that it allows you to identify important interactions that could have been missed if you used only OFAT methods. DoE minimizes the number of experiments in a controlled fashion and delivers information more quickly — all while increasing process understanding. As a result, you need fewer resources and save time and cost.

FIGURE 1: The evolution of process development methodologies.



High-throughput process development allows parallel processing

The next remarkable advance happened in the late 2000s: high-throughput process development (HTPD) using multi-well plates. With HTPD, you can evaluate a wide range of experimental conditions in parallel. HTPD shortens the development time while it increases the amount of information available during early process development. You can use HTPD to characterize a design space and define the process parameters that need to be monitored and controlled. "What would take months with multiple scientists could now be done in weeks by a single scientist," recalls chromatography expert John Scibetta.

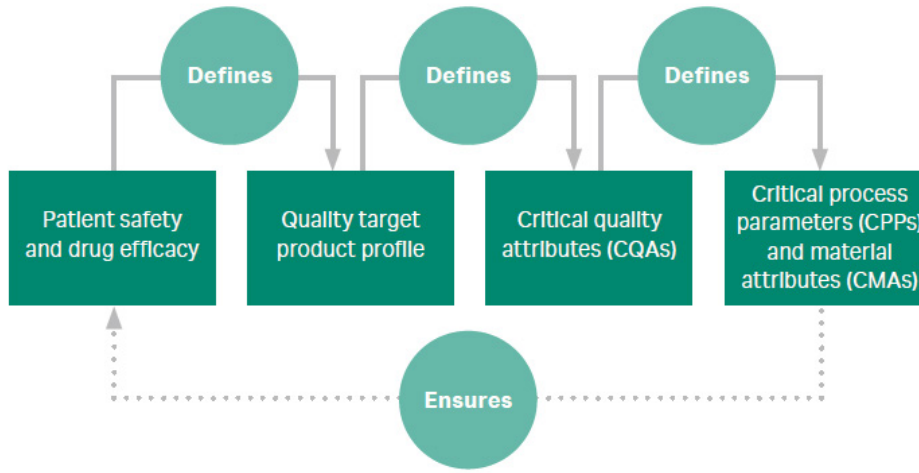
"What would take months with multiple scientists could now be done [with HTPD] in weeks [by a single scientist]."

– JOHN SCIBETTA

Quality by design drives the need for smarter process development

Another great shift in process development was the practice of quality by design (QbD). QbD was introduced in 2002 by the United States Food and Drug Administration (FDA) and formally accepted in the US and Europe in 2009. Other countries implemented it in the years that followed (1). Regulatory guidance toward QbD began with the recognition that increased testing does not improve product quality. Quality must be built into the product.

FIGURE 2: The QbD principles.



QbD is a systematic approach to product design, development, and manufacture. It begins with predefined objectives and emphasizes product and process understanding and process control. QbD principles lead to better process outcomes and, as a result, creates processes that will inherently help you make quality products (FIGURE 2).

Smart process development tools and methods are important components of QbD-compliant process development. QbD is based on sound science and quality risk management, which smart process development strategies elucidate. "QbD is driving the FDA, Cytiva, and manufacturers to develop technologies that support different new methodologies and ways of working," says Scibetta.

Raw material insights support process robustness

"QbD seeks to develop a process that will be resilient in manufacturing," he continues. Raw material insights are an important component. "The critical material attributes of the incoming raw materials, such as chromatography resins, are a key aspect of QbD. The question to answer is: How does the interplay between critical material attributes (CMA) of the resin, such as ligand density, and critical process parameters (CPP) represent a risk of process variation?" This question of resin variability is typically addressed during process characterization phase. If a risk is identified, a control strategy can be implemented to secure process robustness.

Mechanistic modeling: The future is now

Years ago, modeling lab work on a computer may have seemed like something out of a science fiction movie. But it's now a reality.

Mechanistic modeling is the most recent smart process development tool. Mechanistic models give you the ability to create computer simulations of chromatograms based on physiochemical phenomena known to occur in chromatography. It is a revolutionary, breakthrough methodology that gives even more process understanding.

For example, this methodology uses differential equations that describe how molecules move between resin beads and inside the bead pores. It also uses adsorption isotherms to quantify how molecules compete for ligands when binding.

"Right now, we're able to look at the physiochemical, first-principle interactions within a column or within a resin bead and then make predictions *in silico* using

INTERVIEW

Insider recommendations for getting started with mechanistic modeling of chromatography

a modeling software. We can do the equivalent of thousands of runs in the software with significantly less wet work in the lab," explains Scibetta (FIGURE 3).

By using computer simulations, mechanistic models decrease the number of experiments you need to do during process development. It also increases the design space you investigate. You gain the following advantages:

- Cost reduction and time saving: More data from fewer experiments, which accelerates your process development
- Improved robustness and efficiency to fulfill regulatory demands on quality by design

"In the end, what we get are more resilient, flexible, and enduring processes," Scibetta says. "We are reducing the energy and effort required to gain this knowledge, and [we're] achieving better outcomes because we're coming closer to true understanding."

TOOLS FOR SMARTER DOWNSTREAM PROCESS DEVELOPMENT

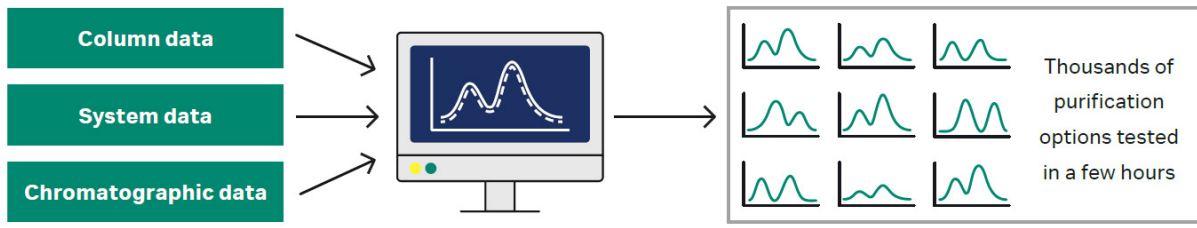
To benefit from smart process development, you need the right tools. Cytiva has you covered (FIGURE 4).

Accelerating process development with high-throughput development tools

Parallelization and automation are key parts of HTPD, and multiwell plates and robotic minicolumns are the smart PD tools that will help you do both.

Cytiva's PreDictor™ 96-well plates can be used with a robot or manually with a multichannel pipette. Their versatility allows you to easily develop results with a minimal amount of investment of energy and capital. "They come in packages of four because you would work it in triplicate to account for pipetting errors. So, with four 96-well plates, you can do 128 experiments in triplicate", says Scibetta.

FIGURE 3: Mechanistic modeling creates computer-simulated chromatograms.



Multiwell plates can be used only in static conditions. So, if you want to look at dynamic flow characteristics and minimize volume, Scibetta recommends minicolumns. Cytiva's PreDictor™ RoboColumn™ units can be used as an automated HTPD tool using a robotic liquid handler.

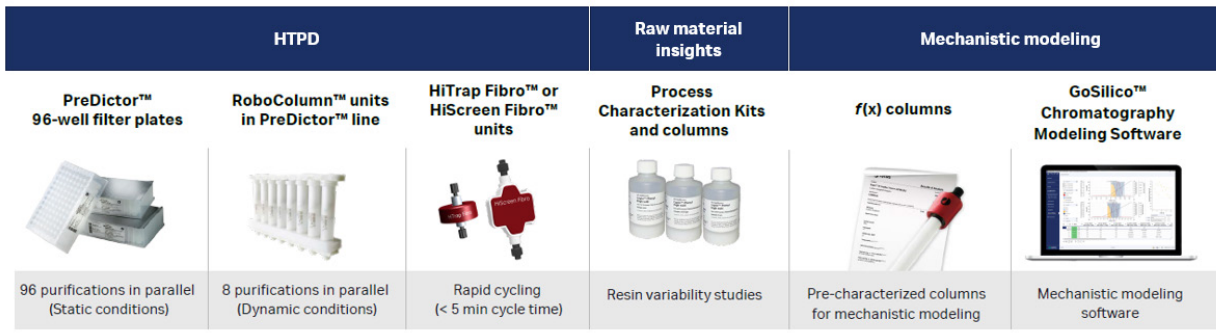
Another HTPD technology that you could use is Cytiva's recently developed Fibro™ adsorber. The adsorber is an electrospun cellulose fiber matrix with an open pore structure. Mass transfer in the matrix is governed by convective flow rather than diffusive flow observed in beads. This structure allows high binding capacities at very short residence times. Using Fibro™ rapid cycling chromatography, your cycle times will be in minutes instead of hours.

Developing robust processes using Process Characterization Kits

If you're a Phase III process developer, you need to perform process characterization and process validation (PC/PV) studies. Cytiva's Process Characterization Kits can help with those studies. Of course, the earlier in your process development that you investigate your resin characteristics, the greater the compounded benefits you will see.

Because chromatography resins are always produced within a specification interval, you need to investigate whether the inherent variability within a manufacturer's given specification range impacts process outcomes, such as yield or productivity. Your process should be robust, so that it can handle these normal variations.

FIGURE 4: Cytiva tools for smarter downstream process development.



"We worked with customers to look at their CPPs and how they interact with the resin CMAs. Porosity and bead size can be considered, but ligand density is most critical," Scibetta explains. Quantifying these interactions is important for process understanding. They make a process that is resilient.

Cytiva's Process Characterization Kits allow you to study the potential impact that resin ligand density might have on the process outcome. For a given chromatography resin, the kit consists of three 25 mL bottles with different ligand densities that represent low, average, and high values in the manufacturing envelope. You can obtain critical insights on resin variation within the ligand density specification interval and develop a solid control strategy to obtain a robust and scalable downstream chromatography process (FIGURE 5).

Adding mechanistic modeling to your PD with Cytiva modeling software, columns, and support

With mechanistic modeling, you can move from empirical work to *in silico* computational work. GoSilico™ Chromatography Modeling Software democratizes computer simulation and gives you the ability to build your own mechanistic models. This software creates digital twins of downstream processes. It can be used for a wide range of molecules and applications from early process development to late-stage scale-up, troubleshooting, and chemistry, manufacturing, and controls.

FIGURE 5: Studying how the interplay of process parameters and resin variability impacts process outcome during process characterization (left). Process parameter target values can be defined to minimize the impact of resin variability with a control strategy (e.g., shifting process parameter target value) (right).

To design a resilient process, you must first characterize your chromatography column in the lab. Performing column characterization experiments takes time and requires expert knowledge to ensure that accurate parameter values are produced. "Characterization is exacting work — somewhat art and a lot of technique. To unburden the end-user, Cytiva has created a series of precharacterized *f*(x) columns," explains Scibetta. With *f*(x) columns, your parameter values are instantly available to provide faster and more reliable mechanistic modeling results. Specific values are given for column parameters such as total porosity, interstitial porosity, ionic capacity, specific adsorber surface area, and ligand density. These parameters are plugged into the GoSilico™ Chromatography Modeling Software to begin the process of creating a mechanistic model.

INTERVIEW

Using mechanistic modeling in process development for advanced therapeutics

To help you get started using chromatography modeling software, Cytiva offers consulting, training, and contract modeling services to guide you on your mechanistic modeling journey. "The time to learn how to model is time well spent," says Scibetta. He estimates that a nascent modeler could become comfortable in less than a year with support from Cytiva. "I think mechanistic modeling is worth trying out. It's the closest thing you get to truly understanding what's going on in the column," he says.

"With the *f*(x) column and the software, I've seen one person — a single process developer — create a model with some support from our team. He never had any modeling or mathematics experience, and he was able to create a model for an adeno-associated virus (AAV) purification with a little help. These are some powerful tools. Users will be amazed at how quickly they develop expertise and excellence."

CURRENT AND FUTURE APPLICATIONS FOR MECHANISTIC MODELING

Scibetta suggests that a key application for modeling is cation exchange chromatography for mAb processes. "Cation exchange is a very important part of the mAb platform process. Although it's relatively well-characterized and understood, I've seen a lot of customers having a lot of success with mechanistic modeling."

Other important applications are anion exchange chromatography and polishing for new therapies, such as AAV or virus-like particles (VLP) which do not have established platforms. "The good news is that a virus is relatively easy to model because of its characteristic morphology and charge regularity," says Scibetta.

"I think the greatest knowledge and understanding of making commercial processes for these will be effectuated through mechanistic modeling."

"Chromatographers are going to enjoy being in the lab more than ever because of the ability to do modeling."

– JOHN SCIBETTA

"I think mechanistic modeling is the future. It's a 21st century tool for chromatography," concludes Scibetta.

"I don't think it will supplant empirical models. Rather, HTPD, DoE, and statistical models will support the outputs from a mechanistic model. I think that chromatographers are going to enjoy being in the lab more than ever because of the ability to do modeling."

This article was based on a video interview with Cytiva's John Scibetta, which can be viewed at http://www.processdevelopmentforum.com/articles/smart-process-development-approach/.

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1. FDA Guidance for Industry, Q8(R2) Pharmaceutical Development, Nov 2009 https://www.ich.org/page/quality-guidelines.

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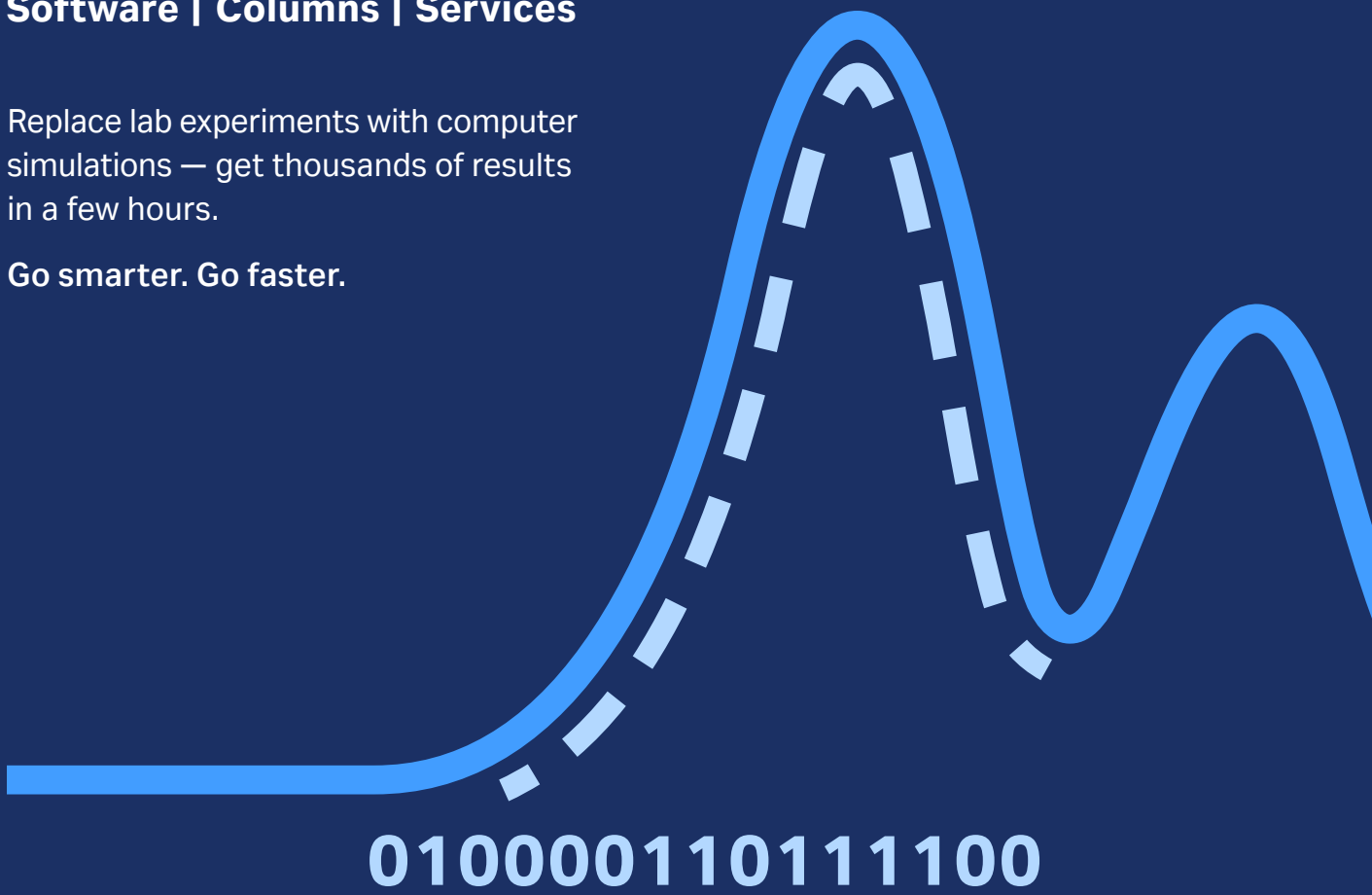

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