Optimum process chain: Jennifer Tummes (left) and Philipp Ehrsam check a pH sensor on the fermenter in which cells produce biologics.

Photos: Peter Ginter/Bayer AG (5)

OPTIMUM CONDITIONS FOR THERAPEUTIC PROTEINS

The medicine of giant molecules

Biologics provide new ways for treating diseases like cancer, inflammation and hemophilia. However, manufacturing the complex protein molecules requires highly specialized process engineering. Bayer researchers have now commissioned a complete facility to produce these new beacons of hope in medicine.

They are giants, yet only visible to those who view the world through the eyes of a molecular biologist. Dr. Karl-Heinz Schneider, a cell biologist at Bayer HealthCare, does just that every day. Together with his team, he is working on developing these new stars of future medicine: biologics, optimized protein molecules derived from natural sources. Compared to classic, small, low-molecular-weight medicinal substances like acetylsalicylic acid, the active ingredient in Aspirin™, biologics are the giants of the molecular world. Furthermore, they support entirely new therapies and are regarded as a great source of hope for patients suffering from diseases like multiple sclerosis or cancer. "Many cancer patients are already being given antibodies, a special variant of biologics, in combination with chemotherapy today," explains Dr. Simone Kardinahl, Head of Global Biological Development at Bayer HealthCare. But the dimensions of these therapeutic proteins pose major challenges for scientists like Schneider and Kardinahl. "Biologics are so large that they can no longer be chemically synthesized in precisely defined reaction steps. Only living organisms can help us manufacture these complex biomolecules," Schneider explains.

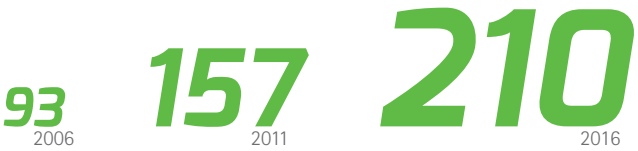
Cell expert: before bacteria or mammalian cells end up in a fermenter for the production of drug products, they are first selected for this task in the laboratory by Dr. Beate Müller-Tiemann and then given new properties, optimized for production and further developed.

Molecules so large they can only be produced by living organisms

The Head of the Cell Culture Pilot Plant is standing in a new Bayer HealthCare production facility in Wuppertal, inspecting hoses from purification and separation systems, and taking the time to show the finished product: just a few milliliters of a liquid containing dissolved protein molecules, the efficacy of which is now to be tested in clinical trials on humans. But before Schneider could hold the finished product in his hand, all the participants in its development, including biochemists, biotechnologists and pharmacists, had to run a metaphorical relay race, full of hurdles and water pits. Antibodies are originally part of the human immune system, where they help track down and fight unwanted intruders such as viruses and bacteria. Because an antibody can only bind to a very specific molecular structure, the human body produces billions of different antibody molecules, which helps to protect it against pathogens. This invention of the human immune system is reproduced in the laboratory by the researchers: accordingly, when searching for new molecular candidates, they can choose from a wide variety of antibodies. "We can call on an entire molecular library to find a matching candidate," says Kardinahl.

So the researchers first have to sift through an enormous portfolio to find one new, promising antibody. "A lot of times, it is minuscule differences in molecular structure that are decisive," says Dr. Beate Müller-Tiemann, Head of Cell & Protein Sciences at Bayer HealthCare. In one of their search runs, conducted using automated screening technologies, Müller-Tiemann's team found an antibody that binds specifically to mesothelin, a protein expressed on the cell surface of a variety of tumors. "If the antibody is coupled with a chemically active substance, it can first identify cancer cells and then hinder their growth," Müller-Tiemann explains.

Global spending on biologics is growing in billion U.S. dollars



Source: IMS Health

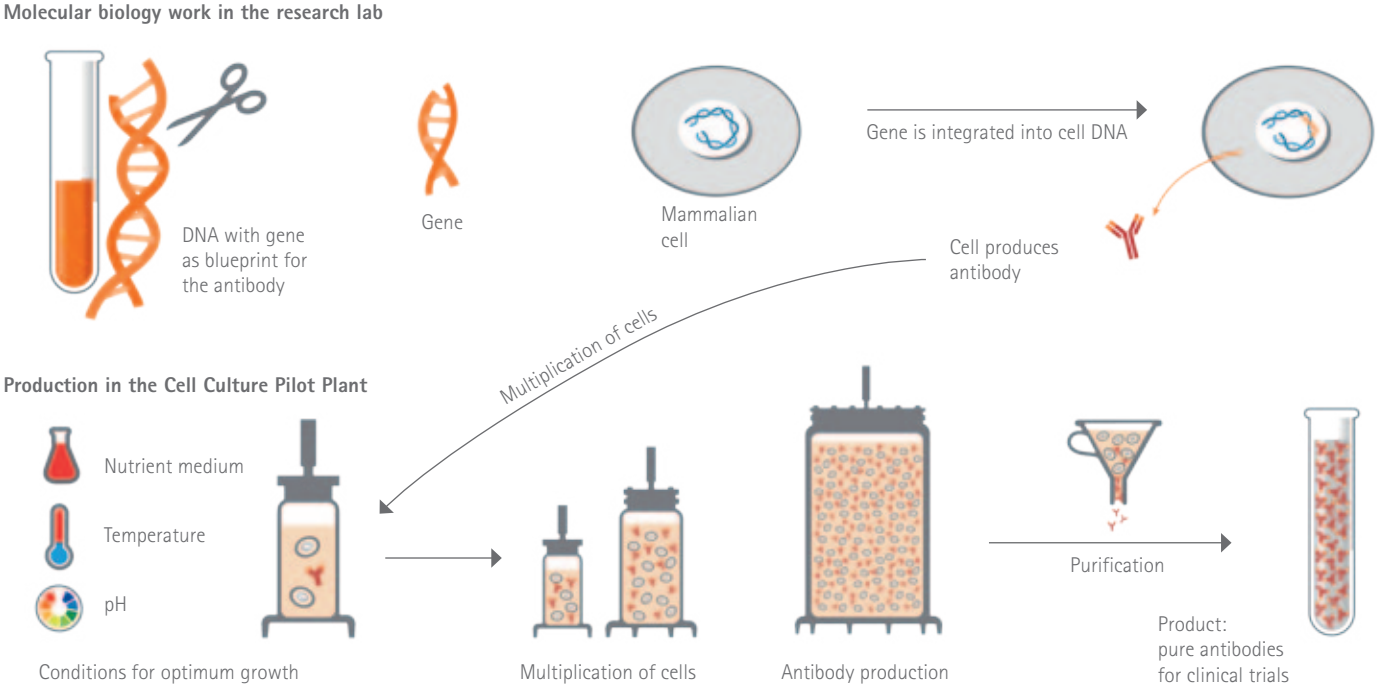
Clean work: at the sterile bench, Jennifer Tummes (photo, left) takes small samples of the protein solution and then analyzes them in clean-room conditions. Even the tiniest impurities such as small quantities of bacteria or fungal hyphae could make the entire batch unusable. Thomas Lettner (photo, right) works in a sterile isolator glove box when preparing the pure antibody solution for clinical trials.

"In this way, the substance exerts a very targeted effect, with the potential to protect healthy tissue."

Once the suitable candidate has been found, it is then optimized. "For instance, we replace individual amino acids – the building blocks of an antibody – to prevent adverse reactions from the immune system," says Müller-Tiemann. The stability of the protein and how it binds to target structures like mesothelin are also improved. For the researchers, giving an antibody this finishing treatment is the run-up to the next hurdle: the clinical trials. For these tests, they must produce the biologic drug in large quantities and at high levels of purity. While a relatively simple protein like insulin can be made from yeasts or bacteria, the researchers need mammalian cells to produce antibodies. For this purpose, the antibody gene – the blueprint for the final

From gene to finished antibody therapy

To manufacture a new antibody, first of all the blueprint for the antibody – a gene – is inserted into the DNA of a mammalian cell in the laboratory. Once the scientists have determined that the cell is producing the antibody correctly, they work out the ideal production conditions and multiply the living miniature factories in ever-larger reaction containers called fermenters. After growing for six weeks in the largest fermenter, the cells have produced sufficient quantities of antibodies which are then purified and ready to be tested for efficacy in clinical trials.



active substance – is incorporated into numerous cells at random locations in the DNA. "The position does, however, influence the quantity and quality of the subsequent product," says Müller-Tiemann. Using automated testing, the researchers select the cell line in which the gene is in the optimum position and also has stable and robust growth and production properties.

At this point, it is time to pass the baton. The researcher hands the cell line over to her co-workers in the new Cell Culture Pilot Plant, where the live production machines grow and work for several weeks in special vessels called fermenters. However, the few milliliters that Schneider gets are not enough to fill the roughly 4-meter tank. "We first have to multiply the cells significantly before we can fill up our 2,500-liter fermenter," the biologist explains. That requires great care, and most importantly strictly sterile conditions. "If even just a few contaminants – tiny amounts of bacteria or fungal hyphae would be enough – get into the tank, we have to discard the entire batch, entailing unnecessary costs," Schneider says.

From molecule candidates to clinical trials: a long relay race with hurdles

The fermenter tank is the heart of the facility that was completed in Wuppertal in mid-2013. Its miles of gas and water pipes, purification units and water treatment systems extend over five floors. All these systems ensure that the cells meet with optimum conditions. Only then do they produce enough high-quality antibodies. To this end, the Bayer researchers test different nutrient media and feeding methods, in other words which nutrients should be added at which intervals and in what quantities. And to ensure that the cell factories are not asphyxiated in the sealed fermenter, Schneider's facility must also continuously feed a mixture of air and oxygen into the tank, which likewise must be free of germs. An agitator stirs the gas mixture, liquid nutrient medium and cells. "But it must run very slowly, because it would otherwise literally rip these sensitive organisms apart," Schneider explains. Parameters such as pH and temperature must be monitored at all times and corrected as necessary.

One last step separates production in the fermenter from the finished product: the mammalian cells secrete large amounts of the desired antibodies into the nutrient solution. Bayer's experts have to filter this solution and purge the active substance of DNA and cell residue. Each protein requires different cleaning and filtration methods, which means that the apparatus has to be reconfigured repeatedly. "The entire system is of a modular design, and we can flexibly combine the individual components," Schneider explains. The technology is constantly evolving; Bayer Technology Services is currently developing basic ideas for the next-generation production plant as the lead company in a biotech consortium named MoBiDiK. MoBiDiK (see text box) utilizes today's facility knowledge and then integrates anticipated future needs.

Schneider's pilot plant is still not the finish line for the pure antibodies. If the active substance is as successful as hoped in the clinical trials, the researchers pass the baton on to the Product Supply team. And just like in the real track-and-field event, Schneider runs a bit further after the handover, sharing his experiences with the industrial production experts, because production of the drug must take place under precisely the same conditions as in his fermenter. To avoid unwanted quality fluctuations, you need a trained eye to handle the giant molecules of medicine.

Optimizing production

Focus on a new concept: project manager Dr. Andrea Vester is collaborating with partners on cost-effective, flexible solutions for biologics productions.

Many therapeutic proteins are produced from animal cells in "fed-batch processes": the product, such as an antibody for treating cancer, is produced, isolated and purified step by step. Bayer researchers are collaborating with industry and university partners in the MoBiDiK (Modular Bioproduction – Disposable and Continuous) research project to develop a next-generation production concept. With this method, liquid is removed continuously from a cell suspension and the product isolated from it. The cells flow with fresh nutrient solution back into the reactor. Devices, such as filters or purification units, are no longer made of stainless steel, but are disposable systems. "New, sterile elements are used for every production run," explains Dr. Andrea Vester, Project Manager at Bayer Technology Services. That cuts costs, increases the flexibility in the production set-up, and makes it possible to run production for relatively small patient groups. The size of the facility is also shrinking: the team is targeting the same production capacities in a 200-liter fermenter as with a 2,500 liter fermenter. The concept is to be tested in a demonstrator in Leverkusen. The facility is currently under construction, and the first production runs are scheduled to begin in 2014.

www.research.bayer.com/biologics
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