



We Innovate Healthcare

Biotechnology - new directions in medicine



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Cover picture

The Roche Group, including Genentech in the United States and Chugai in Japan, is a world leader in biotechnology, with biotech production facilities around the globe. The cover photo shows a bioreactor at Roche's Penzberg facility and conveys at least a rough of idea of the sophisticated technical know-how and years of experience required to manufacture biopharmaceuticals.

Published by
F. Hoffmann-La Roche Ltd
Corporate Communications
CH-4070 Basel, Switzerland

© 2006

Second, revised edition

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This brochure is published in German (original language) and English.

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English translation: David Playfair
Layout: Atelier Urs & Thomas Dillier, Basel
Printers: Gissler Druck AG, Allschwil
7 000 728-1

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Over the past few decades biotechnology – sometimes described as the oldest profession in the world – has evolved into a modern technology without which medical progress would be scarcely imaginable. Modern biotechnology plays a crucial role both in the elucidation of the molecular causes of disease and in the development of new diagnostic methods and better targeted drugs.

These developments have led to the birth of a new economic sector, the biotech industry, associated mostly with small start-up companies. For their part, the more established healthcare companies have also been employing these modern techniques, known collectively as biotechnology, successfully for many years. By studying the molecular foundations of diseases they have developed more specific ways of combating diseases than ever before. This new knowledge permits novel approaches to treatment, with new classes of drug – biopharmaceuticals – attacking previously unknown targets. Increasing attention is also being paid to differences between individual patients, with the result that in the case of many diseases the goal of knowing in advance whether and how a particular treatment will work in a given patient is now within reach. For some patients this dream has already become reality.

Diagnosis and treatment are thus becoming increasingly intertwined. When a disease, rather than being diagnosed on the basis of more or less vague signs and symptoms, can be detected on the basis of molecular information, the possibility of successful treatment depends largely on what diagnostic techniques are available. To the healthcare industry this represents a major development in that diagnosis and treatment are growing ever closer together, with clear benefits for companies that possess competence in both these areas. To patients, progress in medical biotechnology means one thing above all: more specific, safer and more successful treatment of their illnesses. To the healthcare industry it represents both an opportunity and a challenge. For example, more than 40% of the sales of Roche's ten best-selling pharmaceutical products are currently accounted for by biopharmaceuticals, and this figure is rising.

This booklet is intended to show what has already been achieved via close cooperation between basic biological research, applied science and biotechnologically based pharmaceutical and diagnostic development.

Beer for Babylon



For thousands of years human beings have used microorganisms to make products – and in so doing have practised biotechnology. Just as in the past the development of beer, bread and cheese were major breakthroughs, another revolution is now about to overtake medicine: compounds produced using biotechnological methods are opening up entirely new possibilities in medical diagnostics and therapy, and in so doing are bringing about a major restructuring of markets.



5000–2000 BC

Fermentation processes are used in Egypt, Babylon and China to make bread, wine and beer. Wall painting from an Egyptian tomb built during the Fifth Dynasty (c. 2400 BC).

500 BC

The antibiotic effect of tofu mould cultures is discovered and used for therapeutic purposes in China.

From knowledge to science: the history of biotechnology

Babylonian biotechnologists were a highly regarded lot. Their products were in demand among kings and slaves and were exported as far as Egypt. They are even mentioned in the Epic of Gilgamesh, the world's oldest literary work – the Babylonian brewers, with their 20 different types of beer. Their knowledge was based on a biological technology that was already thousands

of years old – fermentation by yeast.

Though it may sound strange, the brewing of beer is an example of biotechnology. Likewise, so is the baking of bread. Wine, yogurt, cheese, sauerkraut and vinegar are all biotechnological products. Biotechnology is practised wherever biological

Terms

Biopharmaceuticals drugs manufactured using biotechnological methods.

DNA deoxyribonucleic acid; the chemical substance that makes up our genetic material.

Genes functional segments of our genetic material that serve mostly as blueprints for the synthesis of proteins.

Genome the totality of the DNA of an organism.

Gene technology scientific work with and on the genetic material DNA.

Recombinant proteins proteins obtained by recombining DNA, e.g. by introducing human genes into bacterial cells.

processes are used to produce something, whether Babylonian beers or monoclonal antibodies. The only thing that is relatively new about the biotechnology industry is its name.

Stone Age, Iron Age, Age of Biochemistry

The term 'biotechnology' was first used in a 1919 publication by Karl Ereky, a Hungarian engineer and economist. He foresaw an age of biochemistry that would be comparable to the Stone Age and the Iron Age in terms of its historical significance. For him, science was part of an all-embracing economic theory: in combination with political measures such as land reform, the new techniques would provide adequate food for the rapidly growing world population – an approach that is just as relevant today as it was in the period after the First World War.

AD 100

Ground chrysanthemum seeds are used as an insecticide in China.

800–1400

Artificial insemination and fertilisation techniques for animals and plants improve reproduction rates and yields in the Middle East, Europe and China.

1595

Hans Janssen, a spectacle maker, builds the first microscope.



© Rijksmuseum van Oudheden, Leiden, The Netherlands

Ereky's vision is all the more astonishing given that at that time the most important tools of modern biotechnology were yet to be discovered. Until well into the second half of the 20th century biologists worked in essentially the same way as their Babylo-

1665

Examining a thin slice of cork under the microscope, Robert Hooke discovers rectangular structures which he names 'cells'. Two years later Antoni van Leeuwenhoek becomes the first person to see bacterial cells.

C. 1830

The chemical nature of proteins is discovered and enzymes are isolated.

C. 1850

The cell is identified as the smallest independent unit of life.

nian predecessors: They used the natural processes that occur in cells and extracts of plants, animals and microorganisms to produce the greatest possible yield of a given product by carefully controlling reaction conditions.

Thanks to newly developed methods, however, the biotechnology of the 20th century was able to produce a far greater range of such natural products and at far higher levels of purity and quality. This was due to a series of discoveries that permitted the increasingly rapid development of new scientific techniques:

- In the first half of the 19th century scientists discovered the basic chemical properties of proteins and isolated the first enzymes. Over the following decades the role of these substances as biological catalysts was elucidated and exploited for research and development.
- The development of ever more sophisticated microscopes rendered the form and contents of cells visible and showed the importance of cells as the smallest units of life on Earth. Louis Pasteur postulated the existence of microorganisms and believed them to be responsible for most of the fermentation processes that had been known for thousands of years. This was the birth of microbiology as a science.
- From 1859 Charles Darwin's theory of evolution revolutionised biology and set in train a social movement that led ultimately to a new perception of mankind. For the first time the common features of and differences between the Earth's organisms could be explained in biological terms. As a result, biology changed from a descriptive to a more experimental scientific discipline.
- The rediscovery of the works of Gregor Mendel at the end of the 19th century ushered in the age of classical genetics. Knowledge of the mechanisms of inheritance permitted targeted interventions. Cultivation and breeding techniques that had been used for thousands of years now had a scientific foundation and could be further developed.

1859

Charles Darwin publishes his revolutionary theory of evolution.

1866

The Augustinian monk Gregor Mendel discovers the rules governing the inheritance of traits in peas. It will be 35 years before his work receives the recognition it deserves and lays the foundations of modern genetics.

1869

Friedrich Miescher, working in Tübingen, isolates a substance from white blood cells in purulent bandages that he refers to as 'nuclein'. His description leads later to use of the term 'nucleic acids'.

These developments changed the face of biochemistry and biotechnology. In addition to the classical, mostly agricultural, products, more and more new products entered the marketplace. Enzymes were isolated in highly purified form and made available for a wide variety of tasks, from producing washing powder to measuring blood glucose. Standardised biochemical test methods made their entrance into medical diagnostics and for the first time provided physicians with molecular measuring instruments. The structures and actions of many biomolecules were elucidated and the biochemical foundations of life thereby made more transparent. Biochemistry progressed from basic research to a field of development.

Gene technology spurs innovation

However, it was only with the advent of gene technology that biology and biotechnology really took off. From 1953, when James Watson and Francis Crick presented the double helix model of DNA, work on and with human genetic material took on the attributes of a scientific race. As more was discovered about the structure of DNA and the mechanisms of its action, replication and repair, more ways of intervening in these processes presented themselves to researchers. Desired changes in the genetic makeup of a species that previously would have required decades of systematic breeding and selection could now be induced within a few months.

For example, newly developed techniques made it possible to insert foreign genes into an organism. This opened up the revolutionary possibility of industrial-scale production of medically important biomolecules of whatever origin from bacterial cells. The first medicine to be produced in this way was the hormone insulin: in the late 1970s Genentech, an American company, developed a technique for producing human insulin in bacterial cells and licensed the technique to the pharmaceutical company Eli Lilly. Hundreds of millions of diabetics worldwide have ben-

1878

While searching for the organism responsible for anthrax, Robert Koch develops techniques for the cultivation of bacteria that are still used today.

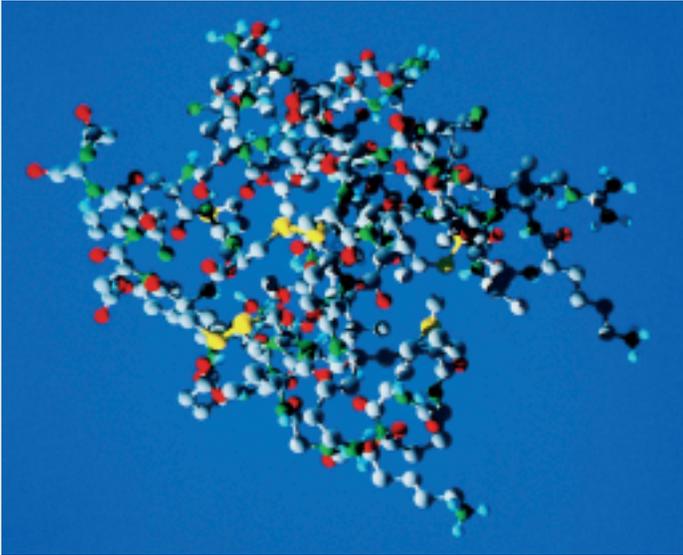
1879

Walther Fleming describes the 'chromatin' present in cell nuclei; this will later be identified as DNA.

1913

In studies on the fruit fly *Drosophila melanogaster*, Thomas Hunt Morgan discovers more rules of inheritance.

Gene technology: human insulin from bacteria



In 1982 human insulin became the world's first biotechnologically manufactured medicine. This hormone plays a central role in glucose metabolism in the body. In diabetics the body either has lost the ability to produce insulin in sufficient quantity (type 1 diabetes) or else no longer responds adequately to the hormone (type 2 diabetes). All people with type 1 diabetes and most people with type 2 diabetes require regular doses of exogenous insulin.

Until 1982 insulin was isolated from the pancreas of slaughtered animals via a complex and expensive process – up to 100 pig pancreases being required per diabetic patient per year. In its day, this classical biotechnological method itself represented a major medical breakthrough: until 1922, when medical scientists discovered the effect of pancreatic extracts, a diagnosis of type 1 diabetes was tantamount to a death sentence. The hormone obtained from cattle and pigs differs little from the human hormone. However, some patients treated with it develop dangerous allergic reactions.

In 1978 the biotech company Genentech developed a method of producing human insulin in bacterial cells. Small rings of DNA (plasmids), each containing part of the gene for the human hormone, were inserted into strains of *Escherichia coli*. The bacteria then produced one or the other of the two insulin chains. These were then separately isolated, combined and finally converted enzymatically into active insulin. The pharmaceutical company Eli Lilly acquired an exclusive licence for this method from Genentech and introduced the medicine in 1982 in the USA and later worldwide – thus firing the starting gun for medical biotechnology.

Some 200 million diabetics worldwide now benefit from the production of human insulin. Without gene technology and biotechnology this would be impossible: in order to meet current demands using pancreatic extract, around 20 billion pigs would have to be slaughtered annually.

1919

Karl Ereky, a Hungarian engineer, coins the term 'biotechnology'.

1922

Frederick Banting, Charles Best and James Collip observe the beneficial effect of a pancreatic extract on diabetes; the hormone insulin is discovered.

1928

Alexander Fleming discovers the antibiotic effect of penicillin.

efited from this, the first biotechnologically manufactured medicine, since its introduction in 1982 (see box, p. 12).

A new economic sector arises

This technology laid the foundation for a new industry. The early start-up biotech companies joined forces with large, established pharmaceutical companies; these in turn used biotechnology to develop high-molecular-weight medicines.

Rapid expansion and stock market boom

In the early 1980s very few companies recognised the medical potential of the rapidly expanding field of biotechnology. One such visionary company was Genentech. This company, which can lay claim to being a founder of the modern biotech industry, was formed in 1976 by Herbert Boyer, a scientist, and Robert Swanson, an entrepreneur, at a time when biochemistry was still firmly grounded in basic research. However, Genentech did not remain alone for long. From the late 1970s, and even more after the introduction of recombinant human insulin, more and more companies that aimed to exploit the scientific success of gene technology for the purposes of medical research and development were formed, especially in the USA. Even today, nine of the ten biggest companies devoted purely to biotechnology are based in the USA (see box, p. 16).

At first these young companies worked in the shadow of the pharmaceutical giants. This was true both in relation to sales and number of companies and also in relation to public profile. The situation changed abruptly, however, when biotech products achieved their first commercial successes. In the 1990s progress in gene technological and biotechnological research and development led to a veritable boom in the biotech sector. Within a few years thousands of new biotech companies sprang up all over the world. Many of these were offshoots of public or

1944

Oswald Avery, Colin MacLeod and Maclyn McCarthy identify DNA as the chemical bearer of genetic information.

1953

On the basis of Rosalind Franklin's x-ray crystallographic analyses, James Watson and Francis Crick publish a model of the genetic substance DNA.

From 1961

Various researchers unravel the genetic code.

private research institutes whose scientists hoped to obtain financial benefit from their findings. Fuelled by expectations of enormous future profits, the burgeoning biotechnology industry became, together with information technology, one of the driving forces behind the stock market boom of the final years of the 20th century.

Measured on the basis of their stock market value alone, many

young biotech companies with a couple of dozen employees were worth more at that time than some established drug companies with annual sales running into hundred of millions of dollars. While this 'investor exuberance' was no doubt excessive, it was also essential for most of the start-ups that benefited from it. For the development of a new drug up to the regulatory approval stage is not only



This life-size bronze sculpture of Genentech's founders is on display at the company's research centre in South San Francisco.

extremely lengthy, but also risky and hugely expensive. The main reason for this is the high proportion of failures: only one in every 100,000 to 200,000 chemically synthesised molecules makes it all the way from the test tube to the pharmacy.

Biotechnological production permits the manufacture of complex molecules that have a better chance of making it to the market. On the other hand, biotechnological production of drugs is more technically demanding and consequently more expensive than simple chemical synthesis. Without the money generated by this stock market success, scarcely any young biotech company could have shouldered these financial risks.

For this reason many smaller biotech companies – just like Genentech in 1982 – are dependent on alliances with major drug

1973

Stanley Cohen and Herbert Boyer use restriction enzymes and ligases to recombine DNA.

1975

Georges Köhler and César Milstein publish their method for the production of monoclonal antibodies.

1976

Herbert Boyer and Robert Swanson found Genentech, the first modern biotechnology company.

The first modern biotechnology company: Genentech

It took courage to found a biotechnology company in 1976. At that time the business world considered the technology to be insufficiently developed and the scientific world feared that the search for financial rewards might endanger basic research. It was scarcely surprising, therefore, that the respected

biologist Herbert Boyer had intended to grant the young venture capitalist Robert Swanson only ten minutes of his time. Yet their conversation lasted three hours – and by the time it ended the idea of Genentech had been born. Further developments followed rapidly:

1976 On 7th April Robert Swanson and Herbert Boyer founded Genentech.

1978 Genentech researchers produce human insulin in cloned bacteria.

1980 Genentech shares are floated at a price of USD 35; an hour later they have risen to USD 88.

1982 Human insulin becomes the first recombinant medicine to be approved for use in the USA; the drug is marketed by the pharmaceutical company Eli Lilly under licence from Genentech.

1985 For the first time, a recombinant medicine produced by a biotech company is approved for use: Protopin, produced by Genentech (active ingredient: somatrem, a growth hormone for children).

1986 Genentech licenses Roferon-A to Roche.

1990 Roche acquires a majority holding in Genentech and by 1999 has acquired all the company's shares.

1987–97 Major new drug approvals: Activase (1987; active ingredient: alteplase, for dissolving blood clots in myocardial infarction); Actimmune (1990; interferon gamma-1b, for use in chronic immunodeficiency); Pulmozyme (1992; dornase alfa, for use in asthma, cooperative project with Roche); Nutropin (1993; somatropin, a growth hormone); Rituxan (1997; rituximab, for use in non-Hodgkin's lymphoma, cooperative project with Idec).

1998 The humanised monoclonal antibody Herceptin (trastuzumab) is approved for use against a particular type of breast cancer.

1999 *Fortune* magazine rates Genentech as one of the 'hundred best companies to work for in America'; Roche refloats Genentech on the New York Stock Exchange (NYSE).

2002 The journal *Science* rates Genentech as the most popular employer in the field of biotechnology and pharmaceuticals.

2003–2004 Approval of Xolair (omalizumab, for use in asthma); Raptiva (efalizumab, for use in psoriasis); Avastin (bevacizumab, for the treatment of cancer).



1977

Walter Gilbert, Allan Maxam and Frederic Sanger present their method for sequencing DNA.

1982

Human insulin becomes the first medicine to be produced using gene technology, ushering in the age of modern biotechnology.

1983

Kary Mullis and coworkers develop the polymerase chain reaction (PCR).

World's largest biotech companies by sales in 2003, in million USD

1	Amgen (USA)	8360
2	Genentech (USA)	3300
3	Serono (Switzerland)	2000
4	Biogen Idec (USA)	1850 ¹
5	Chiron (USA)	1750
6	Genzyme (USA)	1570
7	MedImmune (USA)	1050
8	Invitrogen (USA)	780
9	Cephalon (USA)	710
10	Millenium (USA)	430

Source: company reports

¹ comparative figure after the merger of Biogen and Idec in Nov. 2003

Many of the major healthcare companies are now also involved in the biotech sector. If these too are taken into account, the following picture emerges:

World's largest healthcare companies by sales of biotech products in 2003, in million USD

1	Amgen	7866
2	Roche Group including Genentech and Chugai	6191
3	Johnson & Johnson	6100
4	Novo Nordisk	3561
5	Eli Lilly	3043
6	Aventis	2075
7	Wyeth	1870
8	Schering-Plough	1751
9	Serono	1623
10	Baxter International	1125
11	Biogen	1057
12	Schering AG	1035
13	Genzyme	879
14	MedImmune	780
15	GlaxoSmithKline	729
16	Bayer AG	563
17	Pfizer	481
18	Abbott Laboratories	397
19	Akzo Nobel	375
20	Kirin	355

Source: Evaluate Service

companies or the services of contract manufacturers. As a result of the changed stock market conditions after 2000 some of these alliances evolved into takeovers: the market value of most biotech companies collapsed as abruptly as it had risen, and access to additional capital via the stock market was mostly impossible. The modern biotechnology sector is therefore now in the middle of its first wave of consolidation.

From 1984

Genetic fingerprinting revolutionises forensics.

1990

The Human Genome Project is launched; the German gene technology law is passed.

1994

The first genetically modified tomatoes are marketed in the USA.

Europe: Pharma enters the biotech sector

This development did not, however, occur in exactly the same way all over the world. Unlike its counterpart in the USA, the European biotechnology industry soon came to be dominated by established companies founded on classical biochemistry, chemistry and pharmacology. The United Kingdom, Germany, France and Scandinavia, in particular, have vibrant biotechnology sectors, while Serono, the European market leader, is a Swiss company. However the motors driving development in the world's second most important biotech region are derived almost exclusively from the classical industrial sectors.

Boehringer Mannheim (BM) provides a good example of this trend. As a supplier of laboratory equipment for use in biochemical research and medical diagnostics, this German company had possessed an abundance of expertise in developmental and manufacturing processes for the biotechnology sector since its very inception. As early as the 1940s BM had engaged in classical biotechnology, first in Tutzing and later in Penzberg, near Munich (see box, p. 19). It made the transition to modern biotechnology during the 1980s with the introduction of a number of recombinant (i.e. genetically engineered) enzymes.

In 1990 BM introduced its first genetically engineered medicine, NeoRecormon (active ingredient: erythropoietin, or EPO). In a more recently developed form, this drug still plays an important role in the treatment of anemia and in oncology. This makes it one of the world's top-selling genetically engineered medicines – and an important source of income for the company, which was integrated into the Roche Group in 1998.

Roche itself has been a pioneer of biotechnology in Europe. Like BM, Roche had had an active research and development programme in both therapeutics and diagnostics for decades. It began large-scale production of recombinant enzymes as long ago as the early 1980s. In 1986 it introduced its first genetically en-

1997

For the first time a eukaryotic genome, that of baker's yeast, is unravelled.

1998

The first human embryonic cell lines are established.

2001

The first draft of the human genome is published.

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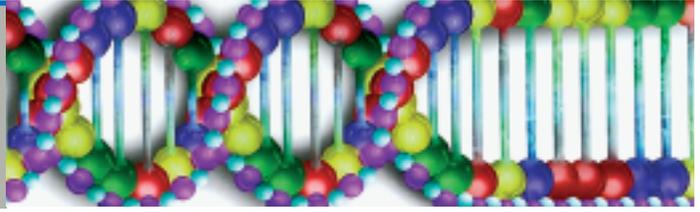
gineered medicine, Roferon-A, containing interferon alfa-2a. This product for use against hairy cell leukemia was manufactured under licence from Genentech. After its takeover of Boehringer Mannheim, Roche developed the Penzberg site into one of Europe's biggest biotechnology centres.

Following its acquisition of a majority stake in Genentech in 1990, Roche's takeover of BM was the Group's second major step into biotechnology. Finally, its acquisition of a majority stake in the Japanese pharmaceutical and biotechnology company Chugai in 2002 put the Roche Group close behind the world market leader Amgen in terms of biotech sales.

Roche thus provides a good example of the development of European biotechnology. Its competitors have followed a similar course, though in some cases later or with different focuses.

2003

The sequencing of the human genome is completed.



'Big biotech' at the foot of the Alps: Penzberg

Research could scarcely be more picturesque: one of Europe's biggest biotech sites is situated 40 kilometers south of Munich at the foot of the Bavarian Alps. For over 50 years researchers at Boehringer Mannheim, working first in Tutzing and later in Penzberg, developed biochemical reagents for biological research and medical diagnostics and therapy. Since Roche took over BM in 1998, Penzberg has become the Group's biggest biotechnological research and production site.

1946 Working with a small research group, Dr Fritz Engelhorn, a departmental head at C. F. Boehringer & Söhne, undertakes biochemical work in the former Hotel Simson in Tutzing.

1948 The amino acid mixtures 'Dymal', 'Aminovit' and 'Laevohepan' become BM's first biotechnologically produced pharmaceuticals.

1955 Under the brand name 'Biochemica Boehringer', BM supplies reagents for research and enzyme-based diagnostics throughout the world.

1968 The isolation of polynucleotides launches research into molecular biology.

1972 BM acquires a disused mining site in Penzberg and builds a new production plant there for its rapidly expanding biochemical and diagnostics product lines.

1977 First work in gene technology at Tutzing.

1980 Establishment of a laboratory for the production of monoclonal antibodies at Tutzing.

1981 Large-scale production of recombinant enzymes begins at Penzberg.

1985 Roche is awarded German Industry's Innovation Prize for Reflotron, an analytical device for determining blood parameters.

1986 Process development work for BM's first recombinant medicine, NeoRecormon (active ingredient: erythropoietin) begins.

1990 NeoRecormon is approved for use in the treatment of anemia.

1996 Rapilysin (active ingredient: tissue plasminogen activator, for the treatment of myocardial infarction) becomes the first recombinant drug to be discovered, developed and produced in Germany.

1998 The Roche Group takes over BM; over the following years Roche develops the Penzberg site into one of Europe's biggest and most modern biotechnology centres.



No newcomer to biotech: the Roche Group

Roche's line of biotechnological products dates back to the 1940s. The resulting expertise has paid off: The Roche Group is now the world's second largest biotechnology company and has a broader product base than any of its biotech competitors. Its three best-selling medicines are biopharmaceuticals, and almost half the sales of its top ten pharmaceutical products are accounted for by biopharmaceuticals. Roche's Diagnostics Division supplies over 1700 biotechnology-based products. PCR technology alone generates annual sales of 1.1 billion Swiss francs. Key milestones on the way to this success are listed below:

1986 Fritz Hoffmann-La Roche founds the pharmaceutical factory F. Hoffmann-La Roche & Co. in Basel.

1933 Industrial production of vitamin C begins; within a few years Roche becomes the world's largest producer of vitamins.

1968 With its Diagnostics Division, Roche opens up a forward-looking business segment; Roche establishes the Roche Institute of Molecular Biology in Nutley, USA.

1971 The Basel Institute for Immunology is set up and financed by Roche.

1976 Georges Köhler (a member of the Institute from 1976 to 1985) begins his work on monoclonal antibodies.

1980 Cooperation with Genentech begins; over the following decades alliances with biotech companies become a central feature of the Roche Group's corporate philosophy.

1984 Niels Kaj Jerne and Georges Köhler of the Basel Institute for Immunology are awarded the Nobel Prize for Physiology or Medicine jointly with César Milstein; their colleague Susumu Tonegawa (a member of the Institute from 1971 to 1981) is awarded the Nobel Prize in 1987.

1986 The alliance with Genentech leads to the development of Roferon-A (active ingredient: interferon alfa-2a), Roche's first genetically engineered drug; Roche introduces an HIV test.

1991 Roche acquires worldwide marketing rights to the polymerase chain reaction (PCR) from Cetus Corporation; only two years later this technology forms the basis of the HIV test Amplicor, the first PCR-based diagnostic test.

1992 Hivid, Roche's first AIDS drug, is introduced.

1994 Roche takes over the US pharmaceutical company Syntex and in 1995 converts it into Roche Biosciences.

1998 Roche takes over the Corange Group, to which Boehringer Mannheim belongs. Cooperation with deCODE genetics begins.

1999 Following its complete takeover of Genentech, Roche returns 42% of the company's shares to the stock market; the monoclonal antibody Herceptin is approved for use in breast cancer.

2000 The Basel Institute for Immunology is transformed into the Roche Center for Medical Genomics.

2001 The merger of Nippon Roche and Chugai results in the formation of Japan's fifth largest pharmaceutical manufacturer and leading biotech company.

2002 Pegasys (active ingredient: peginterferon alfa-2a, for use against hepatitis C) is approved for use in Europe and the USA; Roche sells its Vitamins and Fine Chemicals Division to DSM.

2003 Cooperation with Affymetrix on the production of DNA chips begins; AmpliChip CYP 450, the world's first pharmacogenomic medical diagnostics product, is introduced.

2004 New biotechnological production plants are built in Basel and Penzberg.



Japan: potential in biotechnology

Compared to their counterparts in Europe, the pharmaceutical companies of the various Asian countries – which are otherwise so enthusiastic about new technology – were slow to recognise the potential of this new industrial sector. This despite the fact that the Japanese pharmaceutical market is the world's second largest, after that of

Number one in Japanese biotechnology: Chugai Pharma



When the Japanese set themselves a goal, their competitors have a hard time of it. A few years ago the Japanese pharmaceutical company Chugai set its sights on joining the first rank of the world's biotech companies. Since then it has been catching up at an astonishing rate and is now at the top of the Japanese market, at least. Since its merger with Nippon Roche, Chugai has become not only the fifth largest pharmaceutical company, but also the largest modern biotechnology company, in Japan. A brief chronology follows:

1925 Juzo Uyeno founds a small pharmaceutical company in Tokyo that becomes increasingly important nationally over the coming decades.

1986 The present-day company Chugai Pharma Europe takes up headquarters in London.

1989 Chugai acquires Gen-Probe, an American biotech company and diagnostics manufacturer.

1990 Epogin (active ingredient: erythropoietin, a growth factor) becomes the first genetically engineered drug produced by Chugai to be approved for use in Japan.

1991 Granocyte (active ingredient: rHuG-CSF, for promoting the growth of white blood cells) is approved for use in Japan and later also in Europe, Australia and China.

1993–96 Chugai enters into a number of alliances for the discovery, development and marketing of drugs.

1995 The Chugai Research Institute for Molecular Medicine is founded.

1997 Chugai Diagnostics Science is formed.

2002 Chugai and Nippon Roche merge to form Japan's fifth largest pharmaceutical company.

the USA; in scarcely any other country are so many drugs prescribed, an eighth of worldwide pharmaceutical sales being accounted for by Japan alone. Moreover, two Japanese companies, Takeda and Sankyo, rank among the 20 largest pharmaceutical companies in the world.

In the 1990s Japan set out on the road to catch up, in particular via large-scale support programmes and targeted alliances. The result is that Japanese pharmaceutical companies are now at least on a par with their counterparts in most European countries in terms of sales of biopharmaceutical products. However, the country still lags behind in terms of the number of biotech companies based there, the period of rapid expansion in the 1990s having largely passed Japan by. As yet, Japanese companies devoted exclusively to modern biotechnology have an even smaller slice of the world market than their European competitors.

Japanese biotechnology is largely in the hands of representatives of classical branches of industry such as the brewery Kirin, the food manufacturer Takara, the chemical manufacturer Kyowa Hakko and various pharmaceutical companies. The market leader in modern biotechnology in Japan is Chugai Pharmaceutical

Co., Ltd., a company with an 80-year tradition and one of the first companies in Japan to invest in gene technology.

Milestones along this company's development in this area were its acquisition of the American biotech company Gen-Probe in 1989 and, a year later, the granting of regulatory approval for its first genetically engineered drug, Epogin (active ingredient: erythropoietin, for use in anemia). Access to the worldwide market for these products is provided by the Roche Group, which acquired a majority stake in Chugai in 2002.

The merger between Nippon Roche, Roche's Japanese subsidiary, and Chugai in 2002 led to the formation of Japan's fifth-largest pharmaceutical company and largest biotech company. Chugai operates as an independent member of the Roche Group and is listed separately on the stock exchange. It is responsible for the sale of all Roche products in Japan and also benefits from the Group's worldwide sales network; for its part, Roche has licensee rights to all Chugai products marketed outside of Japan or South Korea.

**Prospects:
biotechnology in
transition**

As seen from the example of the Roche Group, small, innovative biotech companies are increasingly entering into alliances with big pharmaceutical companies. At the same time, the big companies have expanded their portfolios by acquiring majority stakes in biotech companies listed separately on the stock exchange and by entering into alliances in this area. And an impetus to change is arising from biotech companies themselves: by engaging in takeovers and opening up new business segments, they too are investing beyond their established areas of operation.

As a result of this development, most biotechnologically manufactured drugs are marketed by pharmaceutical companies. And this trend is likely to become even more pronounced in the future. Thus, Roche is currently the world's second biggest supplier of biotechnological products and, with more than 50 new drug projects under way at present, has the world's strongest early development pipeline in this area. Aventis and Glaxo-SmithKline, each with 45 drug candidates, share second place in this ranking. Amgen, currently the world's largest biotech company, had about 40 drug candidates in the pipeline in 2004.

At the same time, worldwide growth in the biotechnology market shows no sign of slackening. Thus, at present 40% of the

sales of Roche's ten best-selling pharmaceutical products are accounted for by biopharmaceuticals, and this figure is rising. The many young biotech companies with drug candidates now approaching regulatory approval are also banking on this growth. Both in Europe and in the USA, many such companies formed at the time of the stock market boom in biotechnology will soon be marketing their first drug or drugs. Sales of these will support their development pipelines – and thereby also intensify competition in this field.

At present the world's ten largest biotech companies account for about 85% of the approximately 37 billion US dollars of sales of biotechnological products worldwide. A comparison of the development pipelines of the big companies with those of the generally smaller companies that are devoted exclusively to biotechnology suggests that this concentration is likely to become even greater in the coming years, though given the spectacular growth rate of this sector, the possibility of surprises cannot be ruled out. What is clear is that biotechnology has had a decisive influence on the pharmaceutical market – and that the upheaval is not yet at an end.

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Drugs from the fermenter



Biotechnological production of drugs confronts pharmaceutical research and development with new challenges. For example, complex biomolecules such as proteins can only be produced by living cells in complex fermentation plants, yet they have the potential to open up entirely new directions in medicine.

Biopharmaceuticals transform medicine

Though you might not think so at first glance, modern biotechnology and traditional drug development have much in common. The aim of both, for example, is to develop substances able to cure or prevent disease. To achieve this they both rely on recent findings from the life sciences. For most patients it is a matter of indifference whether a drug is obtained by biotechnological or chemical means. The main thing is that it works. However, beneath the surface there are striking differences between the two kinds of drug product.

Molecules hundreds of times bigger

Almost all traditional drugs are small molecules. They are usually relatively simple organic compounds containing a few functional molecular groups. On the other hand, therapeutic proteins, the largest group of biopharmaceuticals, are quite a different kettle of fish.

They are made up of dozens, sometimes hundreds, of amino acids, each of which is as big as the acetylsalicylic acid molecule of aspirin.

To take an example, the active ingredient in CellCept, currently Roche's top selling traditional drug, is an organic compound made up of 62 atoms with a total molecular weight of 433.5 daltons (one dalton [Da] equals $1.7 \cdot 10^{-27}$ kg). Roche's leading biopharmaceutical, the monoclonal antibody MabThera/Rituxan (rituximab), is nearly 350 times heavier,

weighing in at a hefty 150,000 daltons. No wonder this large molecule poses entirely different challenges for research, development and production. And it also acts differently than conventional drugs in the body.

Terms

Biopharmaceuticals drugs manufactured using biotechnological methods.

Dalton (Da) unit used to express the weight of atoms and molecules; one dalton is equal to $1.7 \cdot 10^{-27}$ kg.

Enzymes biocatalysts; proteins able to facilitate and accelerate chemical reactions.

Eukaryotes organisms whose genetic material is enclosed in a cell nucleus; they include all fungi, plants and animals, including man.

Fermentation a chemical reaction in which biological substances are acted upon by enzymes.

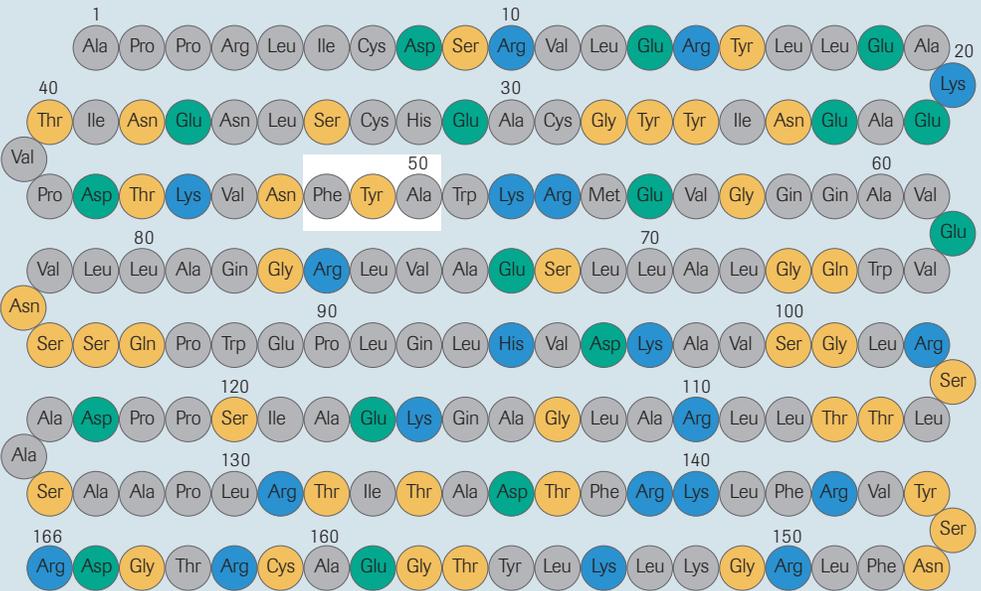
Fermenter also known as a bioreactor; a cultivation and reaction vessel for living cells.

Gene technology scientific techniques for manipulating the genetic material DNA.

Recombinant proteins proteins obtained by recombining DNA, e.g. by introducing human genes into bacterial cells.

Therapeutic proteins proteins used as active agents in pharmaceuticals.

Size comparison: erythropoietin and aspirin



Proven methods for small molecules

The most important consequence of the size difference between traditional and biotechnological drugs relates to their structure. The three-dimensional shape of simple organic molecules, known in chemical parlance as ‘small molecules’, is essentially determined by fixed bonds between the individual atoms. As a result, traditional drugs are usually highly stable compounds that retain their three-dimensional shape in a wide range of ambient conditions. Only drastic changes to the milieu – e.g. the presence of strong acids or bases or elevated temperatures – are able to cause permanent damage to these molecules. Traditional drugs are usually easy to handle and can be administered to patients conveniently in various forms such as tablets, juices or suppositories. It is true that many traditional drugs were originally derived from natural products. For example, healers used an extract of the leaves or bark of certain willow species to treat rheumatism, fever and pain hundreds of years before the Bayer chemist Felix Hoffmann reacted the salicylate in the extract with acetic acid in 1897 to form acetylsalicylic acid, a compound that is gentler on the stomach. Today drugs like these are usually produced chemically from simple precursors. The methods have been tried and tested for decades, and the drugs can be manufactured anywhere to the same standard and in any desired amount. Sterile conditions, which pose a considerable technical challenge, are rarely necessary. On the other hand, preventing the organic solvents used in many traditional production processes from damaging the environment remains a daunting task.

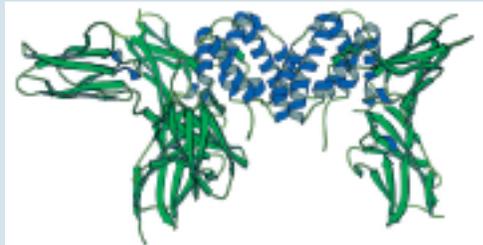
Unstable structure of proteins

Biopharmaceuticals require a far more elaborate production process. Most drugs manufactured by biotechnological methods are proteins, and proteins are highly sensitive to changes in their milieu. Their structure depends on diverse, often weak, interactions between their amino-acid building blocks. These interactions are optimally coordinated only within a very narrow range of ambient conditions that correspond precisely to those in which the organism from which the protein is derived best thrives. Because of this, even relatively small changes in the temperature, salt content or pH of the ambient solution can damage the structure. This, in turn, can neutralise the function of the protein, since this depends on the precise natural shape of the molecule. This applies analogously to therapeutic proteins used in medi-

cine. Most of these molecules act as vital chemical messengers in the body. The target cells that receive and translate the signals bear special receptors on their surface into which the corresponding chemical messenger precisely fits. If the three-dimensional shape of the chemical messenger is even slightly altered, the molecule will no longer be recognised by its receptor and will be inactive.

The situation is similar for another group of therapeutic proteins, the antibodies. In their native state these molecules are components of the immune system. Their function is to recognise foreign structures, for which purpose they have a special recognition region whose shape precisely matches that of the target molecule. Changing just one of the several hundred amino acids that make up the recognition region can render the antibody inactive. It is possible to produce antibodies to target any desired foreign or endogenous substance. Modern biotechnology makes use of the technique to block metabolic pathways in the body involved in disease processes. Like other therapeutic proteins, antibodies must therefore assume the correct molecular arrangement to be effective.

Detecting signals: interferon gamma and its receptor



The signal protein interferon gamma (blue) is recognised by a specific receptor (left and right) located on the surface of its target cells. Interferon gamma as a biopharmaceutical is used to treat certain forms of immunodeficiency.

(Source of illustration:

<http://arginine.chem.cornell.edu/structures/ifncomplex.html>)

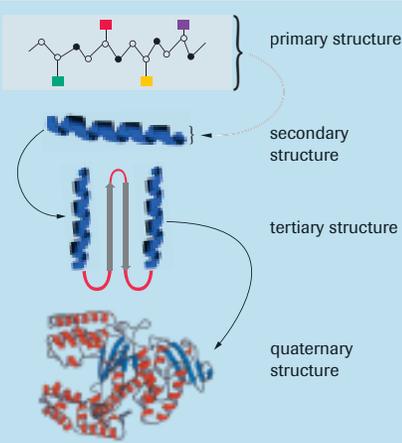
Biopharmaceuticals: biological instead of chemical production

This structural sensitivity also causes problems because proteins do not always automatically assume the required structure during the production process. Long chains of amino acids in solution spontaneously form so-called secondary structures, arranging themselves into helical or sheetlike structures, for example. However, this process rarely results in the correct overall shape (tertiary structure) – especially in the case of large proteins where the final structure depends on the interactions of several, often different, amino acid chains.

During natural biosynthesis of proteins in the body's cells, a series of enzymes ensure that such 'protein folding' proceeds correctly. The enzymes prevent unsuitable structures from being

Diverse and changeable: the structure of proteins

A chain of up to twenty different amino acids (primary structure – the variable regions are indicated by the squares of different colours) arranges itself into three-dimensional structures. Among these, helical and planar regions are particularly common. The position of these secondary structures in relation to one another determines the shape of the protein, i.e. its tertiary structure. Often, a number of proteins form functional complexes with quaternary structures; only when arranged in this way can they perform their intended functions. When purifying proteins, it is extremely difficult to retain such protein complexes in their original form.



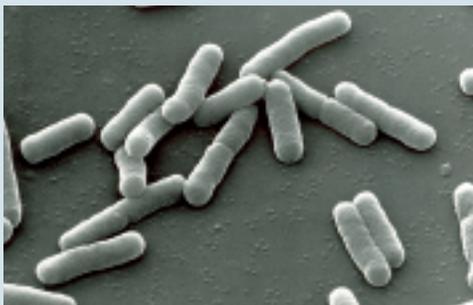
formed in the early stages, separate signal-processing segments from the proteins, add non-protein sections, combine several proteins to form complexes and interlink these as required. These strictly controlled processes make protein production a highly complex process that has so far proved impossible to replicate by chemical means. Instead, proteins are produced in and isolated from laboratory animals, microorganisms or special cultures of animal or plant cells.

Natural sources limited

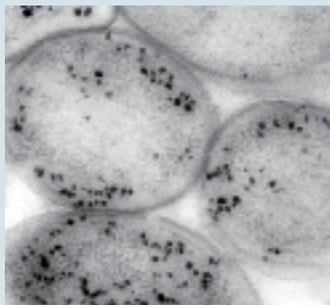
Biological production methods do, however, have several disadvantages. The straightforward approach, isolating natural proteins from animals, was practised for decades to obtain insulin (see article ‘Beer for Babylon’). But the limits of this approach soon became apparent in the second half of the 20th century. Not only are there not nearly enough slaughtered animals to meet global demands for insulin, but the animal protein thus obtained differs from its human counterpart. As a result, it is less effective and may trigger allergic reactions. The situation is similar for virtually every other biopharmaceutical, particularly since these molecules occur in animals in vanishingly small amounts or, as in the case of therapeutic antibodies, do not occur naturally in animals at all.

Most biopharmaceuticals are therefore produced in cultures of microorganisms or mammalian cells. Simple proteins can be

Little helpers: the biological production of drugs



The bacterium *Escherichia coli* is relatively easy to cultivate. However, it can only be used to manufacture simple proteins that require no modifications following biosynthesis.



A cell line that was developed from Chinese hamster ovary cells (CHO cells) is now used in biopharmaceutical production facilities worldwide.

obtained from bacteria. For complicated substances consisting of several proteins or for substances that have to be modified by the addition of non-protein groups such as sugar chains, mammalian cells are used. To obtain products that are identical to their human equivalents, the appropriate human genes must be inserted into the cultured cells. These genetically manipulated cells then contain the enzymes needed to ensure correct folding and processing of the proteins (especially in the case of mammalian cells) as well as the genetic instructions for synthesising the desired product. The responsible gene is then placed under the control of a super-active DNA signal element. In this way a genetically modified cell is obtained which produces large quantities of the desired product in its active form.

Biotech production: each facility is unique

But multiplying these cells poses a technological challenge, particularly when mammalian cells are used to produce a therapeutic protein. Cells are living organisms, and they react sensitively to even tiny changes in their environment. This concerns not only easily controllable factors (e.g. temperature and pressure), as in conventional chemical synthesis. From the nutrient solution to the equipment, virtually every object and substance the cells touch on their way from, say, the refrigerator to the centrifuge can affect them.

High-tech cell cultivation: biotechnological production facility in Penzberg



Large-scale industrial production facilities for biopharmaceuticals are precisely controlled closed systems. The

smallest impurity can render a batch useless. Biopharmaceuticals must be produced under strict cleanroom conditions.

These factors determine not only the yield of useful product but also the quantity of interfering or undesired byproducts and the structure of the product itself. As a result, each biopharmaceutical production plant is essentially unique: Changing just one of hundreds of components can affect the result. In extreme cases it may even be necessary to seek new regulatory approval.

Focus on Chinese hamster cells

Laboratories and manufacturers around the world work with standard cell lines to produce biopharmaceuticals, enzymes and antibodies.

These cell lines are used because they are well researched and, as far as is possible with living organisms, are amenable to standardisation. This allows reproducible results to be obtained worldwide. Important standard organisms used in basic research and the biotech industry include bacteria of the species *Escherichia coli* and eukaryote CHO (Chinese hamster ovary) cells (see figure, p. 31).

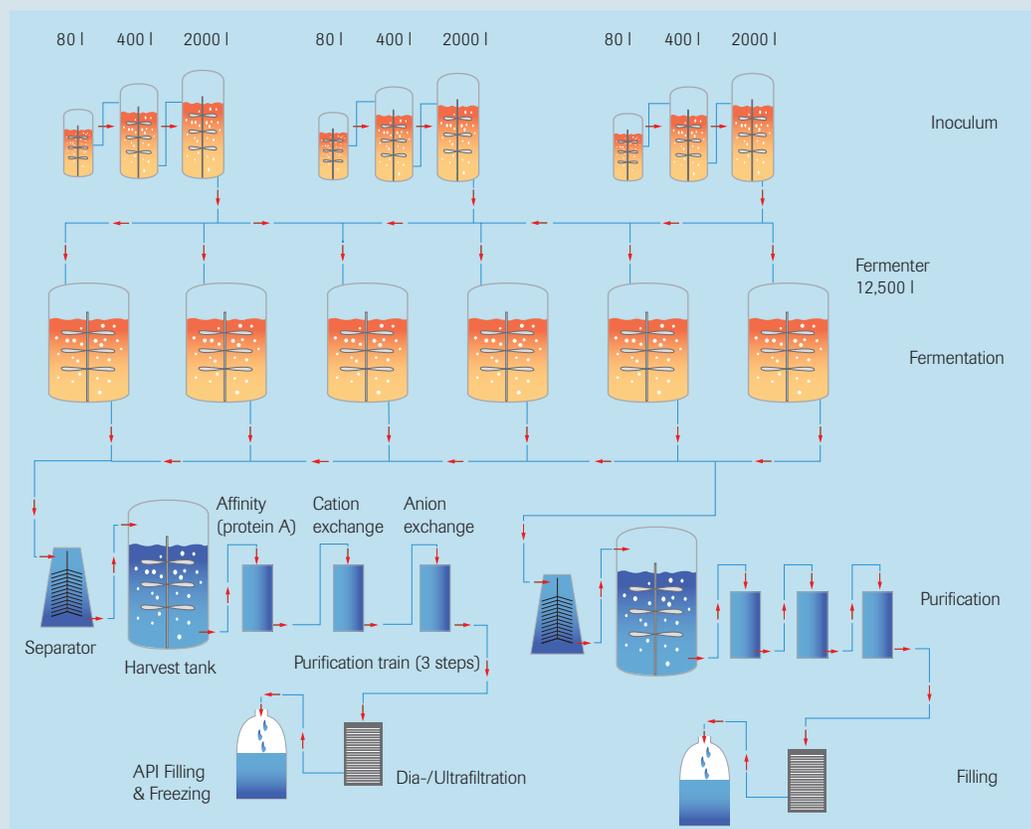
Biotech researchers insert structural and control genes into the cells of these and similar lines to produce the desired pharmaceutical. This establishes a new cell line, which is usually treated as a closely guarded company secret. After all, these cells are the actual factories of the biopharmaceutical concerned. They are allowed to reproduce and are then safely stored at low temperatures in what is known as a master cell bank. If the cells need to

be stored for long periods, they can be kept almost indefinitely in liquid nitrogen at -196°C .

Cells are then drawn from the cell banks and used in biopharmaceutical production. Broadly speaking, the production process is divided into the following steps:

Cultivation: The cells are transferred from the cryogenic cell bank to a liquid nutrient medium, where they are allowed to reproduce. The length of this step depends on the type of cell used. Under favourable conditions bacterial cells such as *Escherichia coli* usually divide once every 20 minutes; thus

Industrial cell cultivation: flowchart of biopharmaceutical production



The production of biopharmaceuticals starts when a nutrient solution is inoculated with cells from a cell bank. These are allowed to reproduce in stages up to a scale of several

thousand liters. The cells secrete the desired product, which is then isolated from the solution, purified and transferred to containers.

one cell gives rise to $4.7 \cdot 10^{21}$ cells within 24 hours. By contrast, mammalian cells such as CHO cells divide about once every 24 hours, and it takes correspondingly longer to obtain a sufficient number of cells. During the growth phase the cell culture is transferred to progressively larger culture vessels.

Fermentation: The actual production of the biopharmaceutical occurs during this phase. The culture medium contains substances needed for the synthesis of the desired therapeutic protein. In total, the medium contains around 80 different constituents at this stage, although manufacturers never disclose the exact composition. The industrial-scale steel vessels in which fermentation takes place have capacities of 10,000 liters or more. There are not only technological but also biological constraints on the size of the reactor vessel: The bigger a fermenter is, the more difficult it becomes to create uniform conditions around all the cells within it.

Purification: In technical terms, the production of biopharmaceuticals in cells is a one-step process and the product can be purified immediately after fermentation. In the simplest case the cultured cells will have secreted the product into the ambient solution. In this case the cells are separated from the culture medium, e.g. by centrifugation or filtration, and the desired product is then isolated via several purification steps. If, on the other hand, the product remains in the cells following biosynthesis, the cells are first isolated and digested (i.e. destroyed), and the cellular debris is then separated from the solution together with the product.

The yield from bioproduction processes is usually much lower than from chemical synthesis. For example, a 10,000-liter fermenter yields only a few kilograms of a therapeutic antibody such as MabThera/Rituxan (rituximab) or Herceptin (trastuzumab). The production steps, including purification, take several weeks. Several more weeks are then needed to test the product: Each product batch is tested for purity to avoid quality fluctuations, and a 99.9 percent purity level is required for regulatory approval. Only then can the finished product be further processed and shipped.

Formulation: The final steps in the production of biopharmaceuticals are also demanding. The sensitive proteins are converted to a stable pharmaceutical form and must be safely packaged, stored, transported and finally administered. Throughout all these steps the structural integrity of the molecule has to be safeguarded to maintain efficacy. At pres-

ent this is only possible in special solutions in which the product can be cryogenically frozen and preserved, though the need for low temperatures does not exactly facilitate transport and delivery. Biopharmaceuticals are therefore produced strictly on the basis of demand – even more so than traditional drugs.

Because of the sensitive nature of most biopharmaceuticals, their dosage forms are limited to injectable solutions. Therapeutic proteins cannot pass the acidic milieu of the stomach undamaged, nor are they absorbed intact through the intestinal wall. Though work on alternatives such as inhalers is in progress (especially for the relatively stable insulin molecule), injection remains the only option for introducing biopharmaceuticals into the body.

Nowadays all the steps in the production of biopharmaceuticals are fully automated. Production staff step in only if problems occur. Because cell cultures react so sensitively to fluctuations in ambient conditions, the window for high-yield production is quite narrow: If the physical and chemical properties of the nutrient medium deviate ever so slightly from the norm, the production staff must take action to restore optimum conditions. Even trace amounts of impurities can spell considerable economic loss, as the entire production batch then has to be discarded and the production process has to be restarted from scratch with the cultivation of new cells.

Advantages in terms of efficacy and safety

Despite their elaborate production process, biopharmaceuticals offer a number of advantages, two of which are uppermost in patients' minds: efficacy and safety. These are determined by the molecular properties of therapeutic proteins.

Thanks to their structure, proteins have a strong affinity for a specific target molecule. Unlike traditional, low-molecular-weight drugs, biopharmaceuticals therefore rarely enter into nonspecific reactions. The result is that interference and dangerous interactions with other drugs as well as side effects are rare. Nor do therapeutic proteins bind nonspecifically to receptors that stimulate cell growth and cause cancer. Biopharmaceuticals are unable to penetrate into the interior of cells, let alone into the cell nucleus, where many carcinogenic substances exert their dangerous (side) effects.

Bio vs. traditional: advantages and disadvantages of biopharmaceuticals

Traditional drugs	Biopharmaceuticals
Unspecific binding	Specific binding
Interactions with other drugs	Interactions rare
Carcinogenic substances possible	Not carcinogenic
Pharmacokinetics difficult	Breakdown is predictable for the most part
Immune reactions rare	Immunogenic effects possible
Theoretically, any target molecule can be reached	Target molecules limited, only outside the cell
6% success rate in phases I–III	25% success rate in phases I–III
Development costs high, production costs low	Development costs low, production costs high

However, this property is also associated with a drawback compared to traditional drugs: The number of possible targets is limited. Ultimately, only substances that occur in an unbound state between cells or on the outer cell surface come into question.

Another ambivalent property is the fact that therapeutic proteins strongly resemble endogenous proteins. On the one hand, this means that their breakdown rate can be readily predicted and varies far less between individuals than is the case with traditional drugs. This makes it easier for physicians to determine the right drug dose for their patients. On the other hand, therapeutic proteins are more likely than small molecules to trigger immune reactions. Simply put, proteins present a larger surface area for the immune system to attack. Moreover, foreign proteins may be interpreted by the immune system as a sign of infection. One way in which researchers are trying to prevent these reactions, for example in the case of monoclonal antibodies, is via the use of ‘humanised’ therapeutic antibodies, which are produced by inserting human antibody genes into cultured cells.

Higher success rates

Overall, the virtues of biopharmaceuticals in terms of their efficacy and safety also mean an economic advantage: The likelihood of successfully developing a new biopharmaceutical is significantly greater than in traditional drug development. Not least because interactions, side effects and carcinogenic effects are rare, 25 percent of biopharmaceuticals that enter phase I of the regulatory process are

eventually granted approval. The corresponding figure for conventional drugs is no more than six percent.

However, the lower risk of failure is offset by an investment risk at the end of the development process. The construction of a biopharmaceutical production plant is so technologically, legally and scientifically demanding – and therefore so time-consuming – that it must be planned even before the phase III studies. From a medical point of view it seems likely that the current success of biopharmaceuticals will continue unabated and that these products, especially those used in the treatment of common diseases such as cancer, will gain an increasing share of the market. However, therapeutic proteins are unlikely ever to fully replace their traditional counterparts. In many applications small molecules will remain the drugs of choice. Examples include lipid-lowering drugs and drugs for the treatment of type 2 (non-insulin-dependent) diabetes. The future also holds promise for hybrids of conventional and biopharmaceutical drugs. The potential of such ‘small molecule conjugates’ is discussed in the following article along with other major areas of research.

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Main avenues of research



Modern medical biotechnology is a relatively new discipline. Nevertheless, new discoveries about the molecular causes of diseases and the influence exerted by our genes on the effectiveness of medicines are already leading to the development of specific diagnostic techniques and better targeted treatment for individual patients.

The changing face of biotechnology ... and of medical science

Few sectors of the economy are as research-intensive as the healthcare industry. Any findings and methods discovered by universities and institutes working in the life sciences usually find their way immediately into the industry's development laboratories. But companies do not just borrow findings from academic researchers. They also invest a great deal of effort and money into their own research. This is true both of biotechnology companies and of major healthcare companies. The exchange between industry and science is vigorous and productive. Just a few examples:

- During the 1990s biology was defined by the fields of human *genetics* and *genomics*. By deciphering the human genome researchers obtained profound new insights into the hereditary basis of the human body. From the mass of genetic information now available researchers can filter out potential

target molecules for new biopharmaceuticals.

- Since the late 1990s *proteomics* has attracted increasing attention both in basic research and in drug development. Because proteins can act either as target molecules or as drug molecules, new findings benefit drug research doubly. In addition, proteins can serve as markers for diagnostic tests.

- *Modifications* of DNA and proteins have recently moved into the limelight. It has been recognised that such modifications in biomolecules – some temporary, some permanent – play a role in many diseases and can therefore serve as useful diagnostic aids. In addition, modifications of therapeutic proteins strongly influence their efficacy and stability.

- In recent years researchers have succeeded in shedding more light on the *key functions of the immune system*. These findings have led to various new diagnostic approaches and more refined methods for developing therapeutic antibodies.

Terms

Chimeric made up of components from two different species or individuals.

Chimeric antibody a modified antibody molecule whose variable segments, which bind to an antigen, are derived from a different antibody than its constant region. The technique led to the production of the first humanised chimeric antibodies, in which variable segments obtained from mouse antibodies are combined with a constant segment from a human antibody.

Copegus (ribavirin) a Roche product used in combination with Pegasys for the treatment of hepatitis C.

Enzymes biocatalysts; proteins able to facilitate and accelerate chemical reactions.

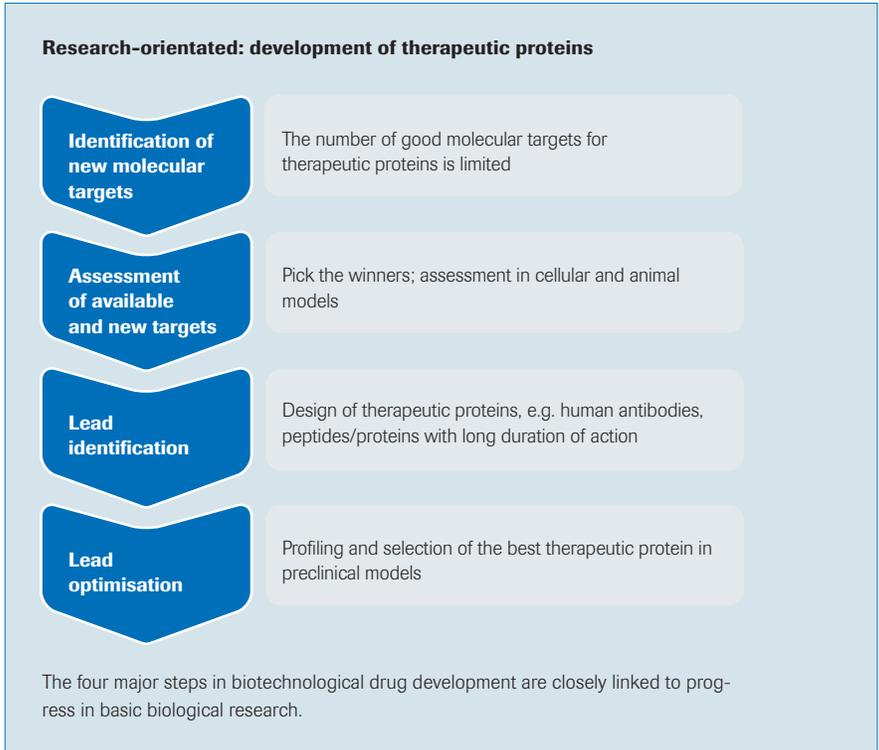
PCR polymerase chain reaction; molecular biological method for copying sections of hereditary material (DNA) millions of times over.

Targets the molecules in our body with which drugs interact; most are proteins.

Therapeutic antibodies antibodies used as agents for the treatment of diseases.

Therapeutic proteins proteins used as active substances in drugs.

- *Techniques* such as the polymerase chain reaction (PCR) and the development of DNA chips have opened up new horizons in the fields of basic research and drug and diagnostic test development.



Most important drug group: therapeutic proteins

Modern medical biotechnology uses a wide range of methods to diagnose and treat diseases – from the biotechnological production of simple natural products to gene therapy. The most important group of biotechnological drugs by far, however, are the therapeutic proteins. Most therapeutic proteins are chemical messengers, enzymes or, especially in recent times, monoclonal antibodies. Some occur naturally in the body. For example, many long-established biotechnological products such as the hormones insulin and erythropoietin (EPO) are natural chemical messengers. Now these molecules can be produced in genetically modified cells that carry the hereditary information for producing the human protein.

In addition, new findings from basic research now allow therapeutic proteins to be coupled with non-protein components to improve their efficacy and duration of action.

A glycoprotein: EPO

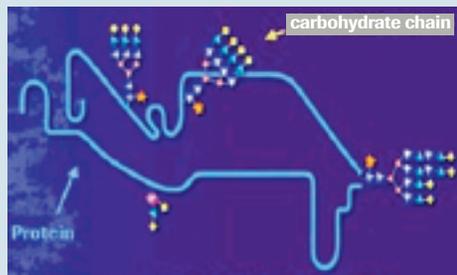
In the human body the hormone erythropoietin (EPO) controls the formation of red blood cells from precursor cells in the bone marrow. Since the substance is produced mainly in the kidneys, patients with renal damage are prone to develop anemia. Those affected – often dialysis patients – generally feel weak and tired, because their red blood cells no longer carry sufficient supplies of oxygen to the body. But the most often unrecognised and severe complication of anemia is rapid progressing congestive heart failure (CCF) as the heart has to pump much more in order to compensate for insufficient red blood cells. CCF is the leading cause of death in patients with anemia of chronic kidney disease.

Anemia can also be due to other causes, e.g. chemotherapy, autoimmune diseases, inflammation associated with cancer, bone marrow transplantation or HIV infection.

Since the early 1990s recombinant erythropoietin has replaced time-consuming, costly and risky blood transfusions, previously the standard treatment for anemic patients. Because the hormone is a glycoprotein (see illustration), it cannot be produced in bacterial or yeast-cell cultures: the erythropoietin molecule has several carbohydrate side chains that slow its breakdown in the body but also modify its intrinsic bioactivity. These side chains can be attached to

proteins only by the synthesising apparatus found in mammalian cells. For this reason, only mammalian cells can be used to produce complex therapeutic proteins. In the case of erythropoietin, researchers have inserted the human EPO gene into Chinese hamster ovary cells, for which reason the product is also known as rhEPO (recombinant human erythropoietin).

Erythropoietin: the molecule



The hormone erythropoietin (EPO) is used in the treatment of chronic anemia. A glycoprotein, it bears several carbohydrate side chains. These slow down the breakdown of the molecule in the body.

Thanks to its numerous uses, rhEPO is one of the top-selling drugs worldwide. The Roche Group markets rhEPO under the proprietary names NeoRecormon and Epogin (Chugai).

**A new innovative drug:
CERA**

CERA (continuous erythropoietin receptor activator) is a chemically modified protein under investigation for therapeutic use in patients with anemia associated with chronic kidney disease and chemotherapy. Chemically synthesised, it binds to the erythropoietin receptor differently than EPO and is also broken down more slowly. The result is that EPO receptors on the surface of erythrocyte precursor cells remain permanently active, thus maintaining the production of new red blood cells. This means that patients require fewer intravenous or subcutaneous injections. In renal clinical trials untreated anemic patients can experience a correction of their anemia with one injection twice a month. Patients who are in maintenance can be managed with a single monthly injection whether they have reached end stage renal disease (chronic kidney disease stage 5) or not (typically chronic kidney disease stages 3 and 4). Less frequent administrations reduce the oscillation in hemoglobin levels outside the optimal range of hemoglobin as defined by best practice guidelines, which is often seen with existing short-acting compounds (epoetin, darbepoetin). Such excursions are associated with adverse events and considered to contribute to further deterioration of cardiac and renal functions. It is believed that less frequent administrations represent a significant gain in quality of life for patients but also allow overworked nephrologists and nurses to concentrate on the other serious medical conditions affecting many of these patients such as hypertension, diabetes, chronic heart failure and obesity. CERA is being developed for the treatment of anemia in patients with chronic kidney disease and for the treatment of anemia associated with cancer.

**The principle of
pegylation: Pegasys**

Improved efficacy of proteins can be achieved with the help of specific modifications. One method for inducing modifications in proteins is known as pegylation. PEG (polyethylene glycol) is a very large family of molecular entities with a common building block. These molecules vary in size and in shape (branching versus linear). It is essential to select the proper moiety that will confer

A pegylated protein: Pegasys



The molecular structure of interferon with protective PEG shell (red). Pegylation with polyethylene glycol (PEG) slows the breakdown of the drug in the body, thus permitting longer intervals between injections and greatly enhancing the drug's efficacy.

to the active protein the desired properties. The choice of linker is also very important as its rigidity (or lack thereof) will influence the ultimate properties of the new medicine. Roche has successfully applied this principle to develop a drug for the treatment of hepatitis C and B. In this method the drug is enveloped in one or two highly branched molecules of polyethylene glycol. These PEG barriers protect the molecule from the protein breakdown machinery in the body's cells, thus prolonging the drug's activity.

Pegasys is a modified interferon-alfa-2a molecule. This natural chemical messenger

inhibits hepatitis C virus replication. It has been used for decades for treating hepatitis C, a widespread infection which causes inflammation of the liver. To date no treatment exists that is able to eradicate the hepatitis C virus from the body.

The standard treatment requires at least three interferon injections per week. As a result, drug levels in the patients' bloodstream undergo significant fluctuations in a two-day rhythm, giving rise to side effects and limit efficacy. It is also considered that fluctuation is instrumental promoting the appearance of resistant viruses.

Thanks to a carefully selected pegylation with the appropriate bond with the protein, Pegasys is broken down much more slowly than simple interferon and therefore remains active in the body longer. This has several advantages for patients: Firstly, Pegasys only has to be given once weekly. Secondly, the dose does not have to be adjusted gradually – at least not to the same degree – according to the patient's age, hepatic status and renal function, a time-consuming process. Thirdly, interferon levels in the bloodstream are subject to less fluctuation, making the side effects more tolerable and improving patient compliance.

And fourthly, the efficacy of the drug is increased thanks to the molecular modifications and the relative constancy of drug levels in the blood. A much larger percentage of patients benefit from a long-lasting effect.

First approved in 2002, Pegasys quickly became the international market leader in the hepatitis C sector. The drug was also the first pegylated therapeutic protein in the world to be approved for the treatment of chronic hepatitis B.

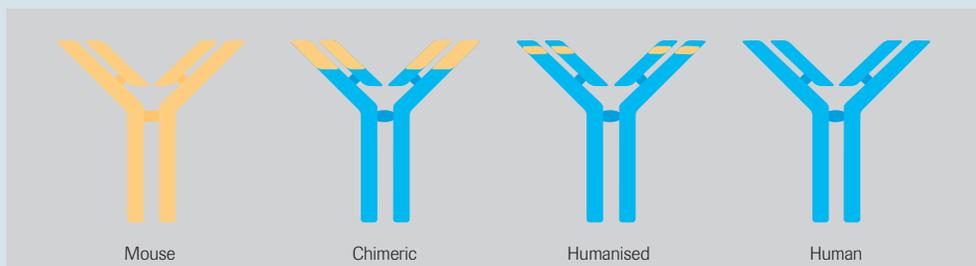
A new drug class: therapeutic antibodies

Therapeutic antibodies form a relatively new drug class that was only made possible by modern biotechnology. Antibodies are components of the immune system. They recognise foreign structures in the body, e.g. molecules on the surface of body cells, bacteria or viruses, and mark them out for elimination by the immune system. They belong to a class of proteins known as immunoglobulins (Ig). Several classes of antibodies exist, each of which has a different function. IgG antibodies are the most abundant. These Y-shaped proteins bear on their two short arms two identical regions that recognise a specific foreign structure. The long stem of the molecule interacts with other components of the immune system, which then initiate destruction of the intruders.

In 1972 César Milstein and Georges Köhler, who later received the Nobel Prize, found a way to produce copies of identical antibody molecules in unlimited amounts. Within a few years these so-called monoclonal antibodies had revolutionised biological research, allowing any desired molecule to be reliably identified and marked. However, it took more than 20 years for monoclonal antibodies to find widespread use in therapy. Not until the late 1990s did researchers succeed in exploiting the specificity of monoclonal antibodies for therapeutic purposes. For example, monoclonal antibodies can be designed to bind to specific molecules and block their disease-causing effects.

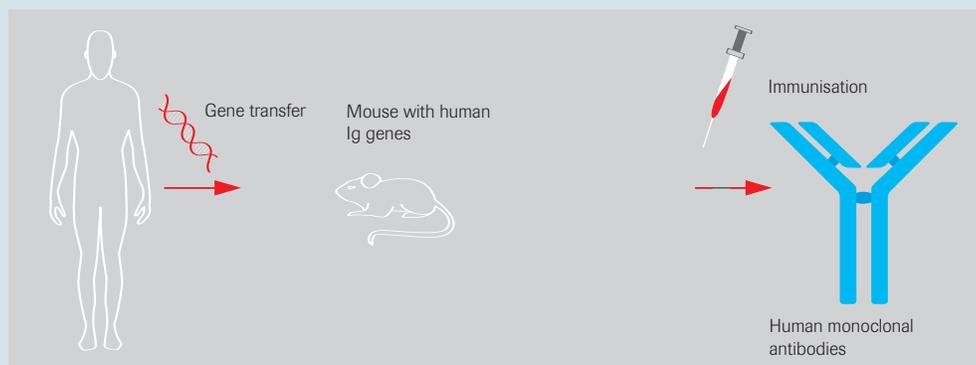
However, drug developers were unable to use antibodies obtained from standard mammalian (usually mouse) cells. Because the molecules differ in structure from one species to the next, mouse antibodies proved to be of very limited benefit in humans. In addition, dangerous side effects occurred. Researchers therefore turned their attention to what are known as chimeric and humanised antibodies, where only the recognition regions are based on mouse genes. It is now possible to insert all the human genes required to produce antibodies into laboratory

A new drug class: therapeutic antibodies



Each antibody bears on its two short arms identical regions that recognise a specific foreign structure, to which they bind. This principle is exploited in therapeutic antibodies in order to recognise pathogenic and other substances and

render them harmless. Whereas early therapeutic antibodies were still partly derived from mouse genes (yellow segments), therapeutic antibodies of the latest generation are indistinguishable from their human counterparts.



Fully human therapeutic antibodies are obtained by infecting a transgenic mouse that carries human genes for the

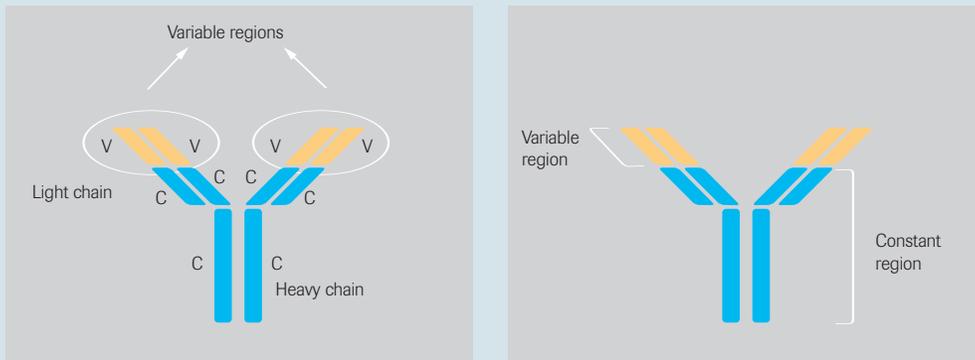
production of immunoglobulins (Ig) with the target for the antibodies that one wishes to produce.

animals. As a result, medical science now has at its disposal an arsenal of therapeutic antibodies that are structurally identical to their natural counterparts in the human body.

Example MabThera: hope for patients with lymphoma

A good example of a highly effective chimeric antibody is the Roche product MabThera/Rituxan (rituximab). MabThera/Rituxan is the world's first monoclonal antibody for the treatment of indolent and aggressive forms of non-Hodgkin's lymphoma (NHL). NHL is a type of malignant lymphoma, i.e. a cancer of the lymphoid tissues. The drug rituximab was developed to bind

A chimeric monoclonal anti-CD20 antibody



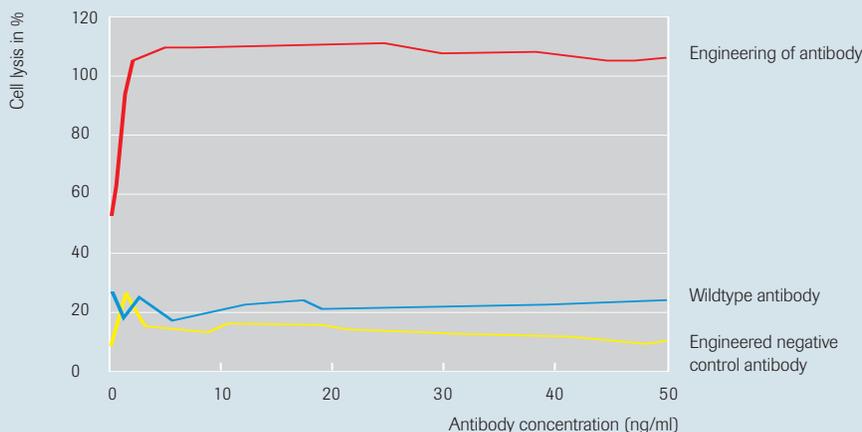
The constant region (C) of a human antibody is combined with the variable regions (V) of a mouse antibody.

specifically to the surface of lymphoma cells. The target protein of this therapeutic antibody is a receptor located on the surface of B lymphocytes (white blood cells), which in lymphomas grow uncontrollably. The antibodies bind to the cancer cells, marking them out for destruction by the body's immune system. At the same time rituximab makes the cells more susceptible to certain forms of chemotherapy, thus improving the survival chances of patients who previously had no further therapeutic options following unsuccessful chemotherapy.

A turbocharger for the immune system

Therapeutic antibodies such as rituximab help the patient's immune system to home in on diseased target cells. To achieve this, biotechnology has exploited a natural function of antibodies: the long segment of antibodies (the FC region, 'c' standing for 'constant') interacts with other components of the immune system to initiate a specific immune response against the recognised foreign substance. This immunological effect can be boosted by skilfully modifying the molecule, e.g. by adding further sugar molecules to the FC region of a therapeutic antibody (see box p. 48).

Enhanced immune response: modified therapeutic antibodies



Specifically modified therapeutic antibodies can induce a five to eight times stronger immune response (e.g. lysis of tumor cells) than natural antibodies.

The next drug generation: small molecule conjugates

The next step was to link therapeutic antibodies with small molecules to form what are known as small molecule conjugates. Antibodies have a disadvantage that they share with other therapeutic proteins: they are too bulky to penetrate into the interior of cells. Potential targets are therefore limited to molecules located outside of or on the surface of the body's cells. By contrast, many conventional, chemically synthesised small molecule drugs can readily pass through the cell membrane to targets within the cell or even the cell nucleus.

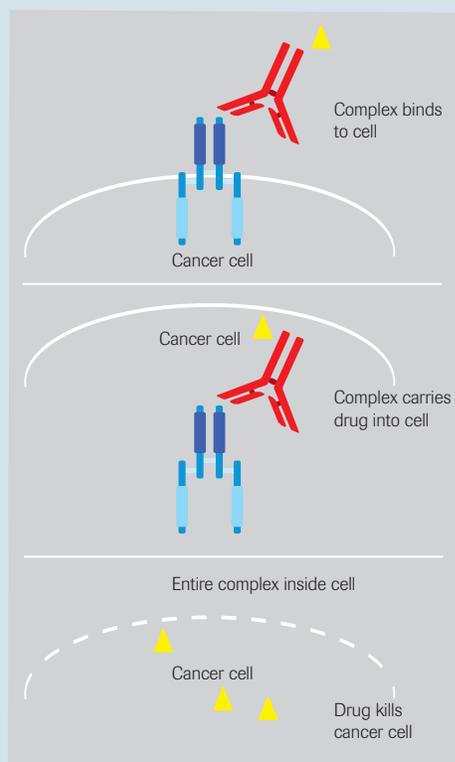
Small molecule conjugates combine the specificity of therapeutic proteins – especially antibodies – with the broad target range of small molecules. To produce them, researchers have developed complexes, or conjugates, consisting of therapeutic antibodies coupled to low-molecular-weight drugs. In such conjugates the antibody's role is to ferry the actual drug directly to its target in the body.

An important area for the use of conjugated antibodies is cancer therapy. Drugs commonly used to destroy cancer cells also attack healthy cells in the body. This results in numerous side effects, some merely unpleasant (e.g. the typical side effect of hair loss) and some life-threatening (e.g. when the drugs attack vital cells such as the precursors of red and white blood cells).

Among other things, Roche is working on conjugated antibodies that will bind specifically to structures (e.g. a receptor) on the surface of cancer cells. Once this occurs, the entire conjugate is internalised in the cell. In cancer cells the antibody is digested and releases the small molecule, which then destroys the diseased cell. In this way cancer cells can be specifically targeted and adverse effects on healthy cells can be minimised.

Small molecule conjugates represent a new generation of biopharmaceuticals. If the findings from tests are borne out, the latest generation of these drugs could signal a breakthrough not only in cancer therapy but in many other therapeutic areas where medical science has hitherto had to contend with severe side effects caused by the unspecific actions of conventional drugs.

Linking of antibodies to small drug molecules (small molecule conjugates)



Conjugated antibodies combine the specificity of therapeutic proteins with the broad target range of small molecules. The antibodies target a specific structure on the surface of cancer cells. Once the antibody has located its target and bound to it, the conjugated small molecule drug is released, penetrates the cancer cell and kills it.

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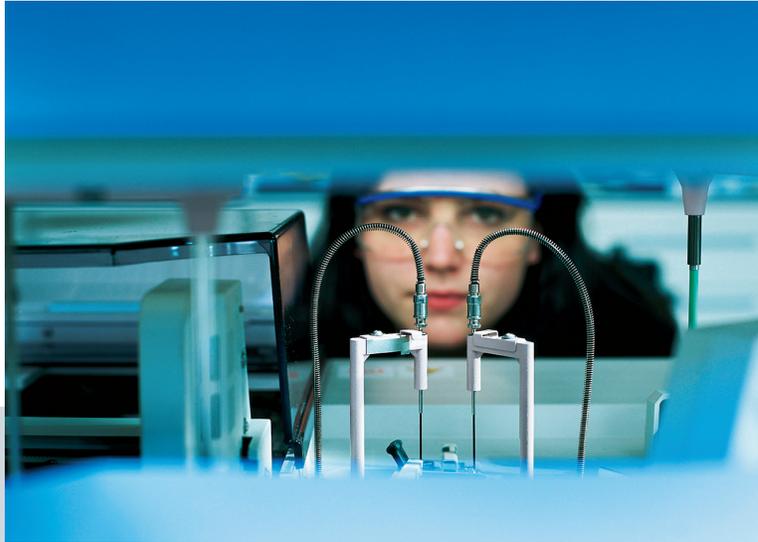
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Treatment begins with diagnosis



Molecular diagnosis provides modern medicine with an entirely new tool. As well as the therapeutic possibilities it offers, modern biotechnology can lead to novel ways of combating diseases such as diabetes, cancer and rheumatic diseases. For example, early and specific diagnosis, and also tests that can monitor treatment and the course of an illness, can result in more effective treatment of patients.

The biotechnology market grows apace

Medical science can only be as good as its understanding of disease processes. The more doctors know about the causes of diseases, the more effectively they can deal with them. This realisation may sound simple, but translating it into practice remains difficult, because the critical part of treatment is often finding the right diagnosis. It is precisely in this area that biotechnology has made tremendous strides in recent decades.

Thus, for example, alleviating pain should not be the only goal when treating patients with chronic pain. It is only when the source of the pain has been identified that steps can be taken to counter it in the long term. Yet pain patients in particular often have to undergo veritable medical odysseys as a result of uncertain diagnoses, failed treatments and ever increasing pain. Despite having similar symptoms, painful rheumatic diseases can

be caused by very different disorders, each of which requires a distinct treatment. Whether a treatment is successful therefore ultimately depends on a rapid, precise diagnosis.

The picture is similar with cancer, where the sheer variety of causes requires a new diagnostic approach. A tumor can remain completely harmless or rapidly develop into aggressive malignancy, depending on the tissue of origin and genetic pattern of the cells as well as the immunological constitution and lifestyle of the patient. Which therapy is the right one for an individual case depends largely on these factors, and whether those factors

are identified in time can spell the difference between life and death. In this respect, biotechnology has devised new means for identifying the precise molecular causes of such disorders.

Terms

Biopharmaceuticals drugs manufactured using biotechnological methods.

Enzymes biocatalysts; proteins able to facilitate and accelerate chemical reactions.

Epigenomics the science concerned with the variable modifications of DNA and their effects.

Genome the (largely unalterable) complement of all genes of an organism.

Genomics the science concerned with the form, function and interaction of the genes of an organism.

Genotype the variants of a given gene possessed by an organism; as a rule a human can have no more than two variants of each gene – one from the father and the other from the mother.

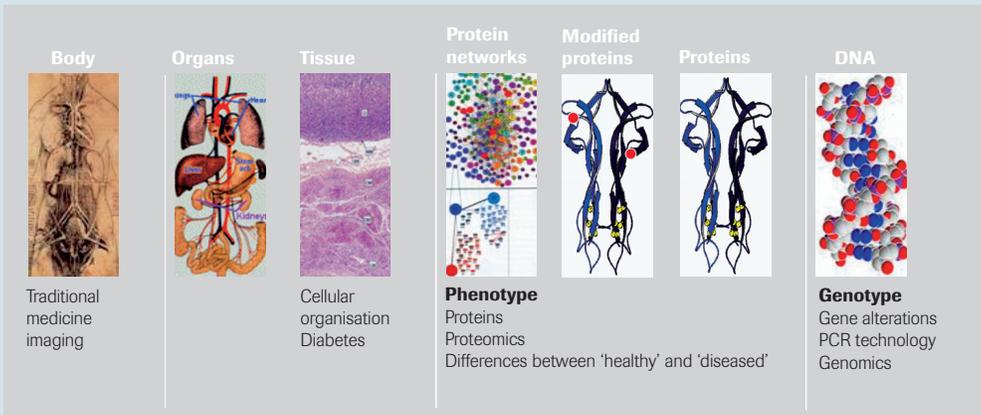
Phenotype the physical constitution of an organism with regard to a specific trait as determined by the interaction of its genotype and the environment.

Proteomics the science that deals with the form, function and interactions of the proteins of a biological system.

Recombinant proteins proteins obtained by recombining DNA, e.g. by inserting human genes into bacterial or mammalian cells.

Single nucleotide polymorphisms (SNPs) differences in individual building blocks (base pairs) randomly distributed in the genome which are inherited from generation to generation.

From body structure to the genome: the diagnostics playing field



Conventional diagnostics at the body level

In conventional medical diagnostics doctors mainly observe their patients' manifest signs and their excreta. For thousands of years experienced doctors have gathered crucial information about their patients' health from visible wounds, bone structure, posture and the colour of the skin, eyes, blood and excrement. Other methods of conventional diagnostics include palpation, for example for muscular indurations or masses, and an in-depth exchange of information between the doctor and patient. Modern medical science has supplemented this range of methods with imaging techniques, e.g. x-rays, computed tomography and magnetic resonance imaging. These routine methods still form the basis of every successful therapy – even if they often prove inadequate for the diagnosis of many diseases.

Diagnostics at the organ and tissue level

The next level of medical diagnostics concerns the internal structure of the body and focuses specifically on the functions and interactions of organs and tissues. In this area as well, modern diagnostic techniques such as sonography, computed tomography, intestinal endoscopy and arthroscopy have added to the arsenal of conventional examination methods. Nevertheless, tried-and-tested examination methods remain important.

Take liver biopsy tests, for example, which involve the removal of liver cells through a long needle inserted into the abdominal wall. Examining these cells closely under a microscope is still the most reliable way to identify diseases of the liver. However, in most cases biopsy is the final link in a diagnostic chain that starts with laboratory tests.

As early as 1970 Boehringer Mannheim (BM), which was taken over by Roche in 1998, developed the gamma-GT test, which is used to measure a metabolic enzyme whose levels are elevated in patients with inflammation of the liver. This sensitive, noninvasive technique is now an important laboratory test for the early detection of hepatic infections: Only in cases where the gamma-GT concentration is significantly elevated or slightly elevated over an extended period do doctors order further tests such as sonography or a liver biopsy.

Such tests became possible only with the advent of enzymes produced by biotechnological means. Thanks to such screening tests, which do not require surgical intervention and produce reliable results quickly and easily, doctors are now able to recognise and treat many more functional disorders of organs and tissues. An added benefit is that if screening test findings are negative, patients are spared an unnecessary and relatively risky intervention.

Diabetes: better quality of life, fewer complications

In the case of diabetes, the advantages of quick tests go even further: such tests are actually an integral part of diabetes therapy. Diabetes is due either to deficient insulin production by pancreatic cells or to an acquired insensitivity of certain body cells to insulin. In either case, the detection and treatment of the disease require regular monitoring of blood glucose levels with the help of enzymes produced by biotechnological methods. On the basis of these measurements, diabetics are able to determine when and how much insulin they should inject.

Until just a few decades ago diabetics had to visit their doctor for such tests, making it all but impossible to adapt insulin doses individually. Instead, diabetics had to adapt their diet and lifestyle to a standard therapy. Today, by contrast, modern diagnostic devices like Roche's Accu-Chek allow diabetics to check their blood glucose levels themselves at any time and thus adapt their treatment to their individual needs. This advance has not only enhanced the quality of life of diabetics but has also led to a

marked reduction in complications due to inadequate diabetes therapy.

The enzymes required for measuring such blood or urine parameters were produced as early as 1954 by Boehringer Mannheim using conventional biotechnological methods. To this end, microorganisms were cultivated in 50,000-liter batches. From the biomass thus produced enzymes such as glucose oxidase and cholesterol oxidase were obtained for measuring blood glucose and cholesterol levels, respectively.

Molecular diagnosis: What is a 'disease'?

Modern biotechnology has recently opened up whole new prospects in the field of diagnostics: the search for the molecular causes of diseases.

This line of enquiry is based mainly on the sciences of genomics, which deals with our hereditary material, and proteomics, which deals with its manifestations in individuals at the protein level. This has led in recent decades to many fresh insights, with the result that we now know far more about the development, progression and treatment of most diseases than was the case a generation ago.

In fact, these profound insights into molecular relationships within our bodies allowed the term 'disease' to be comprehensively defined for the first time as a state caused by an altered flow of information in a biological system.

Genotype and phenotype

This comprehensive definition of disease forms the basis for molecular diagnoses, which can be

divided into two groups:

- **Genotype:** DNA, which makes up our hereditary material, acts as the main store of information in biological systems. Genetic factors not only cause hereditary diseases but are also implicated in the development and progression of noninherited diseases. The genotype can make a person susceptible or resistant to certain disorders, endow him/her with a strong or weak immune system

What is a 'disease'?

The 'disease' state is the consequence of an altered flow of information in a biological system. Information carriers include proteins. Only if we know what proteins are present in a biological system and at what concentrations can we describe the balance between health and disease. Proteomics is a powerful tool for describing protein variety.

and determine how he/she responds to drugs. Researchers worldwide are searching for the genes and gene segments responsible for these phenomena with a view to developing tests that will enable doctors to detect such predispositions in their patients. Such tests would make it possible to delay or even prevent the onset of disease and to select the best treatment for a particular patient.

- **Phenotype:** It is not always possible to draw direct conclusions from the genome about how a genotype is expressed, i.e. the phenotype. Various and variable signals help determine whether, how and how frequently individual genes are actually read. Only at the level of gene products, namely proteins, can a patient's state of health be accurately determined.

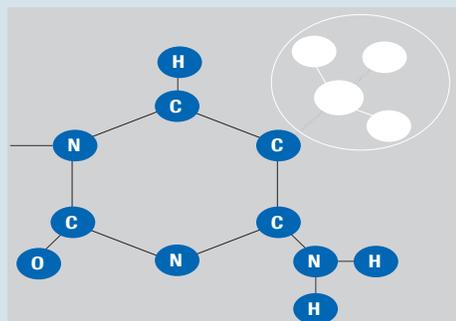
Genomics: diagnostic goal information store

Large and small differences in the genome make each of us a unique individual – not only in appearance and behaviour but also in terms of our health risks and response to treatments. Because the reasons for these differences were poorly understood, medical science was

unable to respond to them except to a very limited extent. Finding the right treatment for a given patient was therefore often a matter of trial and error. However, if the genetic basis of individuality in terms of disease and treatment is known, doctors will be better able to tailor therapies to patients' needs. But our genome is not as immutable as was believed for many decades: By modifying their DNA, cells are able to disable or activate specific genes or even alter the way they are read (see box).

A person's genotype is therefore not the immutable link in the information chain of biological systems that it was long thought to

Changeable hereditary material: methylation



Methylation is the most important long-term, but not irreversible, modification of the hereditary material in our body's cells. In this process, small organic molecules known as methyl groups are attached to the backbone of the DNA molecule. DNA sections marked in this way are no longer read and therefore are not expressed in the form of proteins. Although DNA methylation patterns are hereditary, they can be altered by environmental conditions – unlike the DNA itself. This enables cells to respond swiftly and on a long-term basis to changes in their environment.

be. This fact makes the hereditary material all the more important for the molecular diagnosis of diseases. If a person's life circumstances are reflected at the DNA level, this could offer key insights to help find the right treatment. Moreover, gene modifications of this nature may present suitable sites for drugs to act upon.

**Markers of individuality:
SNPs**

Molecular diagnostics is especially advanced in the field of single nucleotide polymorphisms, or SNPs for short (pronounced 'snips'). These are randomly distributed variations of individual building blocks of the genome, which can differ between individuals. Most SNPs have no effect on the phenotype, i.e. the physical constitution of an individual. However, if one building block is replaced with another within a gene, the consequences can be far-reaching. Often in such cases the corresponding gene product – usually a protein or a protein complex – is altered with the result that it acts faster or slower or reacts differently to external influences. In extreme cases, the exchange of a single building block can render the gene product useless, usually resulting in a severe hereditary disease.

That's why researchers, doctors, and the pharmaceutical industry have been paying greater attention to SNPs in recent years. Many polymorphisms are widespread in the population without causing any actual damage. The effects are not noticeable unless the affected gene products perform important functions, i.e. if they favour the development of certain diseases or are involved in the breakdown of toxins or drugs in the body. In such cases the choice of the right drug – and above all its dosage – can depend on the SNP variants a patient carries.

**New to medical science:
DNA chips**

The first diagnostic product that allows patients to benefit from these findings has been commercially available in Europe since 2004. Roche's AmpliChip CYP450 test rapidly identifies the most important variants of two genes involved in the breakdown of many drugs. If the drug breakdown process proceeds too quickly, it leads to a loss of drug efficacy; if it proceeds too slowly, it leads to an increased risk of side effects. Doctors can use the AmpliChip test to predict how their patients will react to a drug and adjust their therapy optimally.

**The first pharmacogenomic product:
AmpliChip CYP450**



The AmpliChip CYP450 test, which was launched in Europe in 2004, was the first commercially available pharmacogenomic product. (Pharmacogenomics describes how the effects of drugs depend on a patient's genome.) The AmpliChip can distinguish between the most important variants of two genes involved in the breakdown of many drugs, enabling doctors to determine the right dosage of a drug for a given patient even before the treatment is started.

The AmpliChip CYP450 test is an example of a new group of tools in modern diagnostics, the DNA chip. Most are thumbnail-size silicon wafers on which short fragments of DNA are deposited. If a solution containing longer DNA strings, for example one obtained by PCR amplification of DNA extracted from a patient's blood cells, is applied to a DNA chip, the fragments bind to complementary, i.e. mirror-image, sections of the longer DNA strings. In this way specific genes, gene regions and even SNPs can be detected.

**A tool for genetic
diagnostics: the PCR**

Another technique used for detecting DNA segments is the polymerase chain reaction, or PCR.

The PCR is used to make any desired number of copies of specific DNA segments. This is also an important prerequisite for DNA analysis on the AmpliChip CYP 450. Since its invention in 1982, the PCR has been a major factor in the rise of biotechnology. No genetics laboratory could do without it, and genome sequencing projects, of which there are many, would be inconceivable without it. It has even revolutionised forensic science with the introduction of genetic fingerprinting, which is based on the PCR. In medical science the technique forms the basis of nearly all genetic investigations:

- Scientists engaged in *research* into the molecular basis of diseases depend on the PCR. DNA, as a ubiquitous quantity in the information system of all life forms, can only be analysed with its help. Many pioneering findings are based, for example, on the Human Genome Project, in the course of which the human genome was sequenced. A number of

follow-on projects are now looking for genetic variation relevant to the development and treatment of diseases.

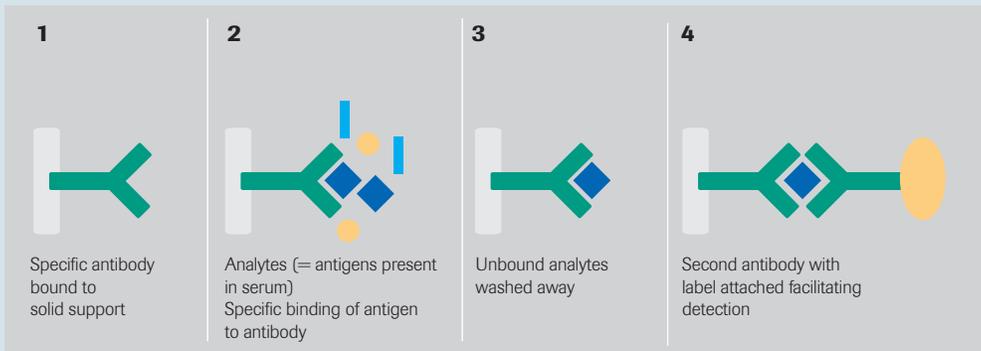
- The PCR is also important in drug *development*. Every biotech drug has to undergo several development phases in which the PCR is required. To manufacture a therapeutic protein, for example, it is first necessary to identify the corresponding gene in the human genome – an extremely difficult task without the PCR. The gene is then transferred to a special cell line, and this step too requires DNA ‘amplification’ with the PCR to determine if the gene was successfully inserted.
- In most *genetic testing* the PCR is needed to make copies of a patient’s DNA so that enough of it is available to be analysed by other methods. In this way, patients can be tested for susceptibility to a certain hereditary disease, for example. Prenatal and preimplantation diagnostic tests also make use of the same process. And finally, the PCR can be used to quickly and accurately detect SNPs and other medically relevant genetic variations. Molecular diagnostic tests at the DNA level will continue to rely on the PCR as an essential tool for the foreseeable future.

Proteins – information carriers par excellence

As the most important group of biological substances, proteins (gene products) are key targets of molecular diagnostics.

- Various *metabolic proteins* serve as the targets of diagnostic tests, because their activity may indicate the presence of certain diseases. One example is the previously mentioned gamma-GT test to detect liver damage.
- *Restriction enzymes* (used for accurately cutting DNA strings into shorter lengths) and *proteases* (which cut proteins at specific sites) are basic tools used by molecular biology institutes everywhere. Molecular diagnostics uses these tools, among others, to identify genes and proteins associated with diseases.
- *Antibodies* are another powerful tool used in modern biology. They form the basis of the ELISA, the most important method for identifying biomarkers in solutions and body fluids (see box, p. 60).
- As *active substances*, proteins are attracting increasing attention. In comparison to most conventional drugs, therapeutic proteins can be used to target the molecular causes of many

One principal method of measuring biomarkers in peripheral fluid: ELISA
Enzyme-Linked Immunosorbent Assay (simplified)



ELISA (enzyme-linked immunosorbent assay) is one of the foremost tools in the search for biomarkers. In this method antibodies against the specific protein being sought are attached to a carrier (1). Any biomarker molecules present in the test solution bind to the antibody (2). The surplus so-

lution is washed away (3) and a solution with labelled antibodies, which also bind to the biomarker, is added (4). By observing the labels it can be determined whether and how much biomarker the sample contains.

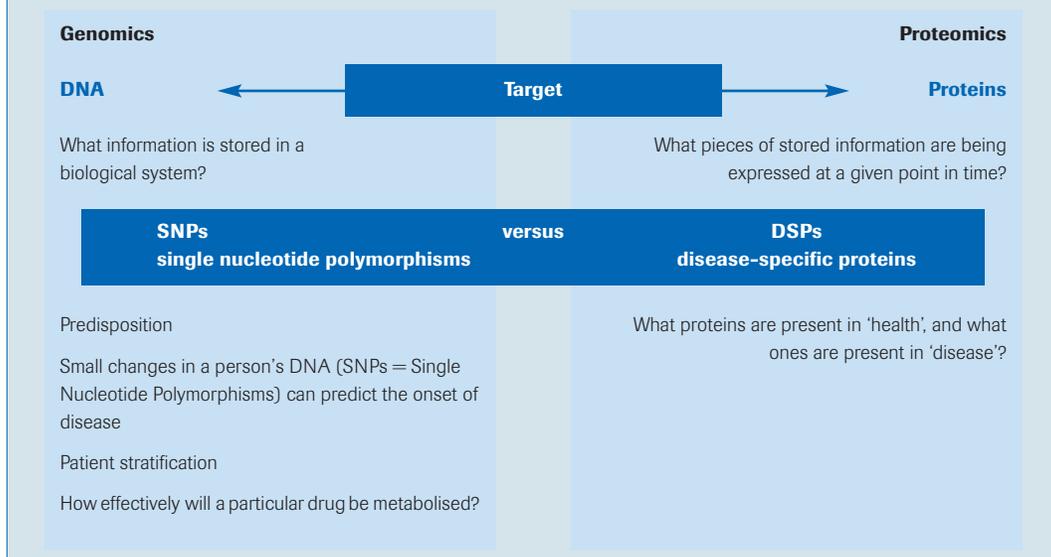
diseases with great specificity, making a precise diagnosis of the underlying disorder all the more important. Particularly in the field of biotechnology, treatment and diagnosis go together hand in glove.

Proteins as biomarkers

A protein that is suitable for detecting altered information flow in a biological system is called a biomarker. The aim of diagnostic research is to find such disease-specific proteins (DSPs) and develop tests to detect them in patients' body fluids, e.g. blood, urine or saliva (see box above for a description of the ELISA principle).

The main areas of research are the major prevalent diseases for which only unsatisfactory diagnostic tests and therefore treatment options are available – mainly malignant diseases such as intestinal, lung or breast cancer, and systemic diseases such as rheumatic diseases and diseases affecting the central nervous system, e.g. Alzheimer's disease. What all these disorders have in common is that they lack a clearly defined cause. Rather, they are caused by an unfortunate chain of multiple genetic and environmental factors. It is therefore all the more important to recognise the early phases of these diseases and break the chain

Molecular diagnosis: new prospects opened up by genomics and proteomics



through targeted treatment. If the disease does develop, early and specific treatment is often life-saving, and this, in turn, depends on finding the right diagnosis. Biomarkers can therefore bring about progress at four levels:

- **Screening markers** can help even in the asymptomatic phase to detect the start of the disruption of information flow that is responsible for disease. For this purpose entire population groups are examined, e.g. everyone above a certain age with a familial predisposition or carriers of other broadly defined risk factors. To ensure that as many people as possible benefit from such preventive examinations, the procedures should be as painless, simple and safe as possible. This category includes various cancer screening tests.
- **Prognostic markers** indicate how fast a disease is progressing in an individual. Forms of the same disease that differ in their virulence often require entirely different therapies. For example, early rheumatic symptoms are usually treated by conservative methods such as physiotherapy or the use of anti-inflammatory ointments and drugs. In especially rapidly progressing cases, aggressive therapeutic intervention may be indicated, even in early stages, despite an increased likelihood of side effects.

- *Stratification markers* enable doctors to predict whether and how well a patient responds to a certain type of drug. This depends largely on genetic variations of drug metabolising enzymes, which in most cases can be detected at the gene level using modern techniques, Roche's AmpliChip CYP450 test being a prime example.
- *Efficacy markers*, finally, describe how well a drug is working in an individual patient. Here again it is often not enough to rely on the improvement of symptoms. Only at the molecular level can the effects of many drugs really be assessed. For example, the success of HIV therapy must be continuously monitored in order to be able to transfer a patient to other drugs if the virus starts replicating again rapidly because it has developed resistance to the drugs being used.

Example of cancer prevention: early intestinal cancer detection

The fight against cancer is one of the greatest challenges facing modern medicine. According to an estimate by the International Agency for Research on Cancer, part of the World Health Organization, over 1.7 million people died from cancer in Europe in 2004, mostly lung cancer, followed by intestinal and breast cancer. Intestinal cancer is one of the most underestimated forms. Although screening programs are in place in most industrialised countries, people do not avail themselves of them to the necessary extent. Yet up to 90 percent of all fatal cases of intestinal cancer, says the German Felix Burda Foundation, could be prevented in the space of ten years by instituting a program of regular endoscopic checks. The major misgiving is that although intestinal endoscopy is effective, it is also unpleasant and, being invasive, not without its risks. To date there is no screening method that is able to identify high-risk patients simply and safely. The early detection of intestinal cancer still relies for the most part on the results of an occult blood test, which detects hidden ('occult') blood in the stool. Depending on the study concerned, however, this test fails to identify up to half of positive cases. In addition, one in five patients proves to be healthy after subsequent endoscopy. Given the large number of patients with intestinal cancer, medical researchers are therefore working intensively on alternatives to the occult blood test. Strong hopes are pinned on biotechnology to find an answer. Suitable screening tests based on protein biomarkers could become available within just a few years.

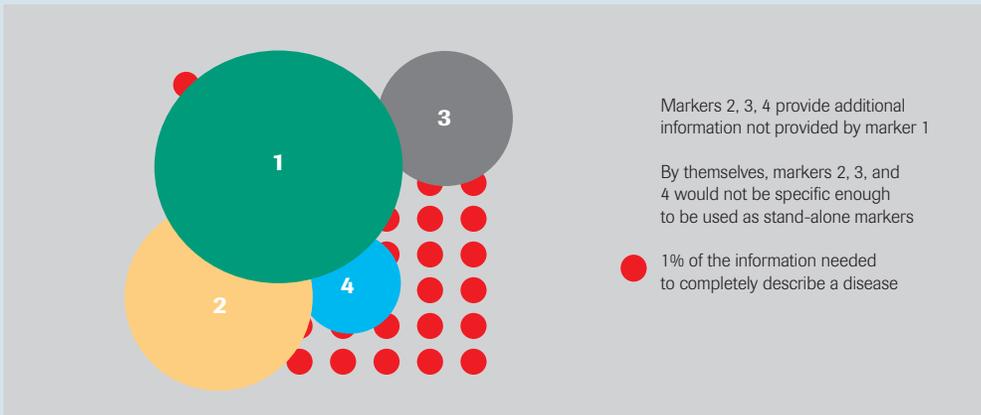
Example of a complex disease: rheumatoid arthritis

Different causes, similar symptoms: Arriving at an accurate diagnosis is especially difficult in the case of complex diseases, which include rheumatic diseases. It is now known that over 100 different

disorders – some degenerative, some inflammatory – are subsumed under the umbrella term ‘rheumatism’. That alone shows to what extent doctors have to depend on modern diagnostic testing, especially since the right treatment often depends on the actual cause of the pain symptoms.

The most common inflammatory form of rheumatic disease is rheumatoid arthritis (RA). RA is thought to be due to an autoimmune reaction, where the immune system attacks tissues of the body itself, often causing destruction of the joints. Women are affected more often than men. Patients usually have to contend with severe pain and considerable impairment of movement. The causes of the disease are still unknown, but it appears certain that genetic predisposition, previous diseases and probably also lifestyle are all factors.

Marker selection = Search for additional information



Often one biomarker is not enough to detect a disorder with certainty, particularly in the case of complex diseases such as rheumatoid arthritis (circle 1). For this reason researchers look for an optimum combination of markers which together describe as many disease factors as possible (circles 2 to 4). This approach is based on a mathematical model known as Regularised Discriminant Analysis (RDA).

RDA is less concerned with testing the suitability of individual markers than with determining how much additional information each provides. The best marker combinations therefore do not necessarily contain the best individual markers.

The fact that diverse factors contribute to the development and progression of rheumatoid arthritis is also reflected in the search for suitable biomarkers. Not a single protein is known which can be used to diagnose a disease with absolute reliability – a fact that has become increasingly clear in recent years. All the molecular candidates so far tested either do not occur in all patients or occur also in other inflammatory diseases. Biologists have therefore teamed up with mathematicians to develop a model to help in the search for an optimum combination of multiple markers (see box, p. 63).

Prospects: diagnostics and treatment evolve together

Biotechnology has made key contributions not only to therapy but also to diagnostics. Armed with molecular diagnostic tests at the gene and protein levels, doctors can already search much more effectively for the causes of a patient's illness and adapt the treatment accordingly, and not just in the early phases. Biomarker analyses can also be used to monitor the success of a treatment. Diagnostics, treatment and treatment monitoring are evolving together, and research in this area is being intensively pursued.

An example that illustrates this development is the detection and treatment of HIV-positive individuals. The behavior of the HI virus is highly variable: Without treatment some people infected with the virus develop AIDS symptoms within just a few months, while others remain healthy for decades. The reasons for this are varied, ranging from differences in the immune response between individuals to significant variations in the genome of the virus.

Biotechnology therefore figures prominently in the diagnosis and treatment of AIDS. Thus, the HI virus is routinely detected indirectly with the help of specific antibodies which are usually present in sufficient quantities for testing some six to twelve weeks after infection. The antibodies therefore serve as biomarkers for the infection.

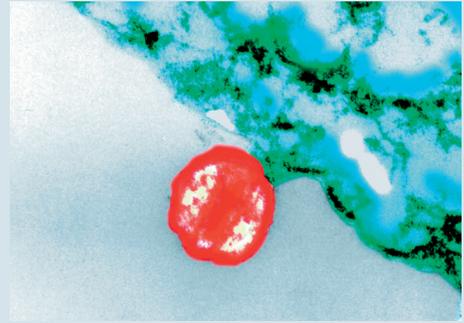
However, as early as 1996 Roche introduced a far more sensitive diagnostic test. At the time, the Amplicor HIV-1 Monitor test was the first diagnostic PCR test, and it is still used today for detecting viral RNA directly. PCR has two advantages: First, HIV RNA can be measured with reliable and consistent quantitation down to 50 copies/mL. This sensitivity enables physicians to accurately quantitate and track HIV viral load levels – even at ex-

tremely low levels. This enables physicians to confidently initiate proper therapeutic regimens that will deliver more effective viral load suppression, and transition to alternate therapies if and when viral outbreak should occur. Secondly, PCR tests e.g., the Amplicor HIV-1 Monitor test Version 1.5, provide quantitation of a variety of HIV-1 subtypes. Since the HIV-1 virus is comprised of multiple subtypes, accurate quantitation of HIV-1, regardless of subtype or genetic diversity, is critical to ensuring effective patient management.

Because HI viruses change very rapidly and become resistant to the drugs used,

HIV therapy must be continuously adapted to the individual patient's needs. HIV-positive patients usually take a cocktail of three or four different drugs to keep the viruses in their body under control. Modern molecular diagnostic methods are therefore needed not only at the start of the therapeutic process but throughout treatment. The treatment regimen must be continuously adapted to the patient's current viral status, and in the case of HIV that means for the rest of the patient's life.

Diagnosis and treatment evolve together: example of HIV



The HI virus specifically attacks those immune cells that are meant to prevent such attacks, and victims usually die as a result of an acquired immunodeficiency syndrome, hence the acronym AIDS. Thanks to molecular diagnostic methods and new biopharmaceuticals, the pathogens can now be held in check for decades.

To ensure that HIV-positive people in poor countries, where HIV is rampant, can also benefit from this medical advance, Roche decided in 2003 to sell its drugs in such countries at a 'nonprofit price'.

Treatment begins with diagnosis

As in the case of HIV, the diagnosis and treatment of other diseases are also merging together. The more specifically a drug is directed against the cause of a disease, the more important it is for doctors to identify the cause accurately. For pharmaceutical companies that are active in both areas, this development has opened up a unique opportunity: Now diagnosis and therapy can be considered together to help patients individually.

Progress in the treatment of complex diseases in particular shows that molecular diagnostics holds new promises for med-

ical science. In this area, biotech drugs and diagnostic agents are not competing with conventional therapies but in many cases permit specific therapy for the first time where before the aim of treatment was merely to relieve unspecific symptoms – a real blessing for patients.

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