



Targeted transport of active ingredients with antibodies

Double whammy

In the fight against cancer, one of the weapons in the doctor's arsenal is chemotherapy. But chemotherapeutic drugs attack healthy tissue too, and sometimes have very debilitating effects on the patient. Bayer HealthCare researchers are working on a new therapeutic approach which gets the cytotoxic drugs right into the cancer cells. An immunoconjugate or "antibody-drug conjugate", CA9-ADC, is currently being administered for the first time in Phase I clinical trials.

Scene of the crime – a tumor. The reason why cells should suddenly get out of control often remains unexplained to doctors. This much is clear: the genetic material of cancer patients is so radically altered in a number of places that the cells can no longer fulfill their original function and go completely haywire. They demand space where none is to be had, or move off through the human body and deposit themselves in organs or bones as metastases. At first, they often lie dormant and merely disrupt the processes taking place in the human body – and then threaten the patient's life. The faster they are detected and destroyed, the greater the patient's chance of a cure.

However, the degenerated tissue cannot always be removed by surgery. Doctors also use radiation treatment (see also "Fighting tumors with alpha

radiation," page 10) or attack the tumor with a wide range of cytotoxic agents. The problem is that chemotherapy of this kind often is a rather imprecise weapon, causing significant collateral damage also on healthy cells.

Tumor markers: targets for Y-molecules and cytotoxins

This leads to side effects such as hair loss, vomiting, nausea and a weakened immune system. For this reason, patients can usually tolerate only a very low dose of the drug, which may not be enough to defeat the tumor. So doctors are looking for new ways of getting cancer drugs even more precisely to the site where they are needed.

A promising way they have found of doing this is to use antibodies: the "arms" of these Y-shaped protein

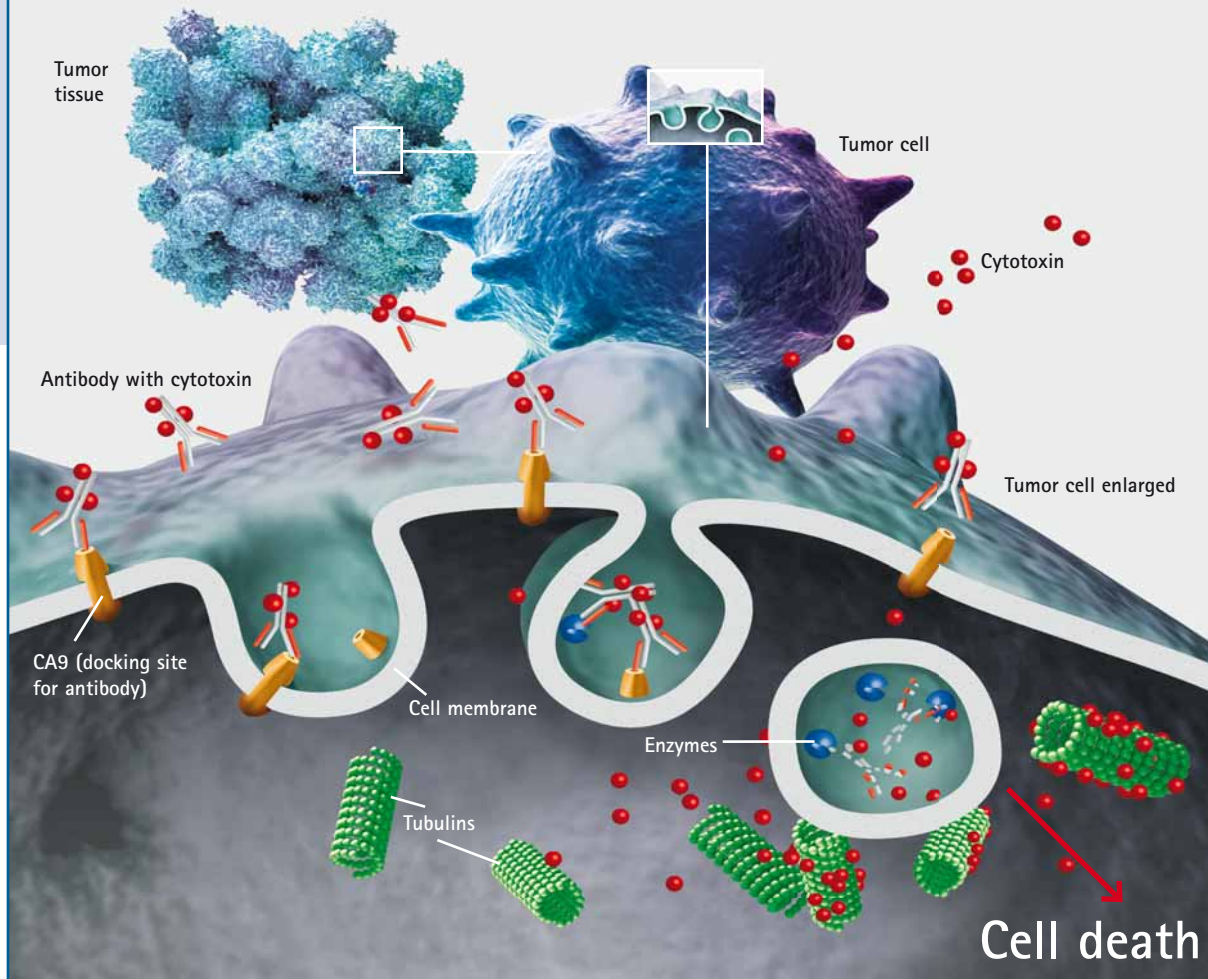
molecules bind only to very specific structures – for instance, to the surface molecules which distinguish tumor cells from healthy ones, known as tumor markers. On their own, antibodies are only effective against the mutant cells in suitable circumstances. It is only in combination with a highly effective cytotoxic agent that they create a powerful combined agent to combat cancer. "An antibody tracks down the cancer cells and gets the cytotoxic directly to the place where it can act, inside the tumor cell," explains Dr. Hans-Georg Lerchen, a researcher in Medical Chemistry at Bayer HealthCare in Wuppertal. Because antibodies are also an important part of the immune system, the dual-action molecule is called an immunoconjugate or antibody-drug conjugate – ADC for short.

Fight against cancer: Bayer HealthCare is constantly on the lookout for new ways to target cancer cells, for example in the lungs (large photo), more selectively with drugs. Scientists Dr. Berthold Boedeker (left) and Dr. Hans-Georg Lerchen (small photo) are working on a solution with antibodies that transport an active ingredient directly to the cancer cells and thus selectively target the tumor.



The Trojan horse in the cell

The Y-shaped antibodies coupled with the cytotoxin bind to the tumor marker (CA9) on the surface of the cancer cell. The cell membrane bulges inwards, transporting the antibody and cytotoxin into the interior, where specific enzymes break down the link between the antibody and the cytotoxin and release the active ingredient. The cytotoxin attaches itself to the tubulins (protein molecules), blocking them and thus preventing cell division. This causes the cancer cell to self-destruct. The cytotoxin can also migrate through the membrane to neighboring tumor cells and exert its action there as well (the so-called "bystander effect").



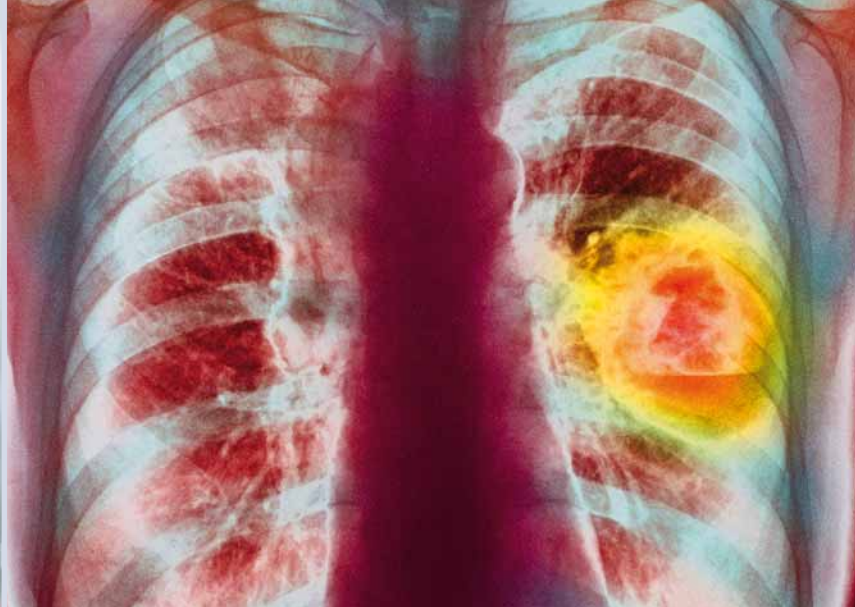
In a current research project, scientists at Bayer HealthCare are working on the cell toxin monomethyl auristatin E, or MMAE for short. MMAE is used under a license granted by the U.S. company Seattle Genetics, which is working on the project as a collaboration partner of Bayer HealthCare. MMAE is a chemical which launches a highly effective suicide program in the cancer cells. "In a cell culture, the toxic agent is fatal to the cells in only two hours," says Lerchen, describing the researchers' observations. Powerful cytotoxic agents like these can help to defeat tumors – provided that they attack

only the mutant cells. However, on their own, cytotoxic agents are blind and cannot distinguish between good and bad cells. For this reason, MMAE must be transported directly to where it is needed, or it would prove too aggressive for the patient. Indeed, by linking the chemical to the antibody, the Bayer researchers have created the perfect combination for a potential treatment. "We give our patients a prodrug of the actual drug itself. The cytotoxic agent only becomes active when it has arrived at the cancer cell," says Dr. Bernhard Fritz-Zieroth, Global Project Leader at Bayer HealthCare.

MMAE acts on cells which are in the process of dividing. "High cell division activity is a notable feature of tumor cells and one involved in the action of many other cytotoxic agents," says Dr. Berthold Boedeker of Biotechnology Development, Bayer HealthCare, Wuppertal. Boedeker, a biochemist, works together closely with Lerchen.

Active ingredient blocks tubulins: tumor cell division halted

The Bayer researchers used special fluorescent dye markings to prove that the cytotoxic, aided by the antibody,



Cancer medicine: Dr. Bodo Brocks (photo, left) checks the fermentation process for the production of recombinant antibody fragments in the laboratories of the biotech company MorphoSys. The finished immunoconjugate is designed to destroy tumor tissue in the body and help people with lung cancer (right), for example.

is really getting through to the cells. Once there, it attaches itself to molecules called tubulins which are essential for cell division. If the tubulins are blocked, the tumor cells cannot divide. When certain molecules within the cell register this error, they activate the crucial self-destruction program: the cells shrink and break down into a large number of individual fragments, which are eventually destroyed by the body's own phagocytes.

This newly developed type of drug transport is highly specific. "The principle only works if the antibody reaches its target on the cancer cell and actually succeeds in binding to it," says Lerchen. Choosing the appropriate places to attack is crucial. The more specific the target, as the molecule is also called, the better the prospects for treatment. Targets are tested according to various criteria during the process of drug development. "As far as possible, they should be present only in tumor cells, not in healthy cells – and should then transport the bound antibodies, with their payload, inside the cells," explains Boedeker.

CA9: the gateway to the inside of the cancer cell

The docking sites on the antibodies are molecules which are primarily located on the surface of a tumor. In this case,

the molecule is known as carboanhydrase 9 (CA9). This molecule is the gateway to the inside of the cancer cell. "It frequently undergoes endocytosis, i.e. it is transported from the cell surface inside the cell, and then back to the cell surface," explains Lerchen. The real function of carboanhydrase 9 is not yet fully understood, but this protein molecule appears to be present more frequently in the external cell membrane when the tissue is poorly supplied with blood, and thus with oxygen, which is often the case with a rapidly growing tumor.

The more of this substance which can be detected on the cell surface of a tumor, the more malignant the tumor appears to be – and the worse the prospects of survival for the patient. The researchers have found the molecule in various types of tumor. In theory, therefore, it is a possible target for various cancer therapies, e.g. for lung, stomach, colon or kidney cancer. Bayer researchers are currently investigating the therapeutic potential of their new antibody-drug combination in Phase I clinical trials in patients with various types of tumor. In future trials, CA9-ADC is due to be administered in combination with other types of chemotherapy which have proved effective against the type of tumor concerned. The latter will further disrupt cell division

and increase the effectiveness of the immunoconjugate.

Nevertheless, there are still some obstacles to overcome. "In individual cases, we do not know whether all cancer cells actually secrete CA9 on their surface," says Lerchen. One advantage here is that MMAE which has been released in a tumor cell can enter neighboring cells and destroy them, even if they have not produced their own CA9.

The molecular giant: 20,000 atoms per antibody

It has also not been reliably established whether the antibodies can penetrate the dense tumor tissue and gain direct access to the center of every tumor, or whether they need to work their way in gradually from the outside. Antibodies, with over 20,000 atoms, are also comparatively huge molecules for use in therapy. Compare them with acetylsalicylic acid – the active ingredient of Aspirin® – with less than 40 atoms, or insulin with 788 atoms.

A "magic bullet" of that size was probably not what the German Nobel Prize winner Paul Ehrlich had in mind when he dreamed, back in the late 19th century, of identifying pathogens and tumors by their unique characteristics and destroying them – without harming healthy cells in the body. In the 1960s and 1970s, scientists worked out the



Antibody production: in the special laboratory for highly effective active ingredients, Dr. Joachim Krüger (photo, left) from Chemical Development at Bayer HealthCare manufactures the cytotoxin MMAE. It is then combined with the antibody in Krüger's laboratory. The glovebox (right) is used to process substances in an environment that is sealed off from external influences.

function of antibodies in the human immune system and recognized their therapeutic potential: then, in 1975, the Argentinian César Milstein and Georges Köhler from Germany developed a mass-production technique for specific antibodies, for which they received the Nobel Prize for medicine in 1984.

In the meantime, however, the manufacturing process has been changed: originally, scientists derived the first instructions for building elements of an antibody indirectly from mouse cells, but nowadays the gene sequence for modern "monoclonal antibodies" is constructed entirely from human DNA. However, the production process still relies on mammalian cells. "We could not synthesize a large, complex protein like this consistently in the laboratory," says Boedeker.

Genetic engineering for high-purity therapeutic antibodies

Using a process licensed by the biotech firm MorphoSys, the Bayer researchers identified the basic structure of a suitable antibody and tailored it for the target molecule CA9. They incorporated the relevant gene sequence into the genetic material of hamster cells which, using this detailed blueprint, are able to produce monoclonal antibodies in large quantities. The cells are grown in a nutrient medium in large biore-

actors with a capacity of around 200 liters. The cells gradually release the protein molecules into the surrounding culture medium. In a number of stages of purification, the desired antibodies are eventually separated off from other extraneous components and processed into an ultra-pure product by Boedeker's team, for subsequent use in cancer treatment.

The production of the actual cytotoxic agent, MMAE, is the responsibility of a research group under Dr. Joachim Krüger of Chemical Development, Bayer HealthCare, Wuppertal. To produce the cytotoxic agent, they go through an extremely laborious chemical synthesis process comprising around 30 steps. It is in Krüger's laboratory, too, that the two components of the conjugate, the cytotoxic MMAE and the antibody, are finally linked together. In a chemical reduction process, up to eight sulfur-hydrogen binding sites are freed up for the cytotoxic to attach itself. Statistical distribution determines whether the antibody carries the comparatively tiny but deadly messengers at two, four, six or eight sites. "We have designed the synthesis process to ensure that four toxic agent molecules are bound, on average," explains Krüger. "Although, naturally, we are aiming for a product which is as uniform as possible, we are not starting from a pure substance, as is the case with many other synthetic

drugs. We are dealing here with a combination, and one which we have to produce consistently and reproducibly with an identical composition," says the chemist. This development is unique in his field of work. Many tests had to be redesigned and adapted to the requirements of the antibody. Boedeker and Krüger have succeeded in their "double whammy": in 2009, they and their teams were awarded the internal Global Drug Discovery research prize for their innovative work.

A Trojan horse taken into the heart of the cancer cell

Just as these two areas of development are intertwined, the antibody and the drug must remain linked at all times. The cytotoxic must not separate from its carrier molecule while the antibody is circulating in the blood – and the molecules sometimes remain in the body for up to four weeks before they are completely broken down by the liver. "It is not until the antibody has docked with a tumor cell and been internalized that the drug should separate off, as smoothly as possible," explains Lerchen. The company Seattle Genetics has found a suitably sophisticated solution: special enzymes called cathepsins, already present in the cell, take over this task, cleaving the link between the antibody and the

An invention of the immune system

Although the production process for immunoconjugates may seem very artificial, its main component is entirely natural: antibodies are a very important part of the human immune system. The body expends a great deal of energy on the production of these Y-shaped protein molecules. They bind their target molecule, called the antigen, on their two short arms. And they are very selective: often it is a tiny aspect – an individual turn or charge in the molecule – which, after the initial contact, means that it does not fit into the narrow binding site after all. However, when a molecule does finally fit into the arms of the antibody, it becomes firmly attached and is difficult to detach. The exact shape of the binding site varies from antibody to antibody. Certain cells in the human immune system, the B lymphocytes, combine some 170 genes at random on various chromosomes. This jigsaw puzzle, in combination with other modifications to the molecule, produces up to 10^{11} different antibody frameworks – an almost infinite number of structures which bind promptly to and identify external invaders in the body, such as bacteria or viruses – a kind of health police.



Active ingredient carrier: the computer animation shows the molecular structure of the antibody. Coupled to it, the active ingredient is transported directly to the cancer cell without being recognized by the immune system.

drug, which is called the "linker." The toxic agent is then free to act within the cell. In the same way as the Greek soldiers concealed in the Trojan horse outwitted their opponents, the cytotoxic first infiltrates the enemy ranks unseen, linked to its antibody, and then embarks on its work of destruction in the tumor cell. The Bayer researchers have obtained a license for the linker design from Seattle Genetics.

Personalized medicine in the battle against cancer

During the production process, the immunoconjugate comes into contact with 130 chemicals and reagents. At the end, a freeze-dried white powder is produced which, after some six months of production time and numerous quality and toxicity tests, is finally distributed to the hospitals taking part in the current Phase I trials. In these trials, doctors in the United States and the Netherlands are testing the CA9 immunoconjugate on patients for the first time. They dissolve the drug in half a liter of saline solution and adminis-

ter it intravenously. "The optimal dose depends on the amount of toxic agent required to fight the tumor and how much of the drug the body can actually tolerate," explains Fritz-Zieroth.


The actual dose which may be required to treat a particular type of tumor will be established later. Not all cancers are alike: the treatment must always be adapted to the cellular changes associated with a particular type of tumor. Diagnostics will therefore become increasingly important in the medicine of the future: cell biologists are searching for the specific features of each type of tumor tissue. For example, CA9 immunoconjugate will only be used in patients whose tumor cells secrete carboanhydrase 9 on their surface. "With this personalized treatment approach, we hope to give patients the treatment which is most appropriate for them and spare them unnecessary side effects," explains Krüger.

In order to select the right group of patients for the new treatments, says Fritz-Zieroth, an appropriate diagnostic procedure is chosen at the very earliest stages of the research. In this area,

Bayer HealthCare enters into external collaboration agreements and is constantly expanding its diagnostic platform for cancer treatments by bringing in the latest technologies, including a cooperation project with the specialized pharmaceutical and diagnostics company Prometheus Laboratories Inc. in San Diego, USA.

New analysis methods help in drug development

Specific analysis methods for the identification of certain molecules in tissue or blood samples from cancer patients should not only help with cancer therapy itself, but should also reduce development times for new drugs. The work of the forensics specialists at the "scene of the crime," the cancer cell, brings many challenges – and hope for many patients.

 www.research.bayer.com/immunoconjugates
Links to further materials and more information on this topic