

Sagopilone shows promise in combating tumors

A vice-like grip on the cytoskeleton





Taxanes are among the most effective anticancer medications known. They block the breakdown of the cytoskeleton and prevent tumor proliferation. Tumor cells have pumps with which they can expel taxanes, however. Bayer HealthCare is now conducting clinical tests on sagopilone, a new and promising active ingredient from the epothilone group of substances, which has a cytoskeleton-stabilizing action but is not recognized and expelled by the pumps.

The tumor cell moves forward like a tiny, tremulous worm. Blistery offshoots form in several places. Suddenly the cell inflates into a ball, which is then instantaneously surrounded by a dark, spherical sheath. A few seconds later, the ball pops apart to form two smaller cells. The round sheaths disappear and two little worms crawl off. This amazing spectacle – a time-lapse video recording of an event which actually took several hours – is a scene that plays out millions of times over each day in the world's laboratories. It is the method used by scientists to analyze the behavior of tumor cells, especially in cancer research such as that conducted in the oncology laboratories of the Bayer Schering Pharma Division in Berlin-Wedding. Following administration of sagopilone to the cells, however, this spectacle plays out very differently. The cells in the Petri dish never get beyond the spherical stage. While normal cells spend only about an hour in the spherical stage, these cells take a solid ten hours to divide. And instead of splitting into two equal halves, they simply explode at the end of the process.

Under the guidance of Dr. Ulrich Klar, intensive structural modifications were performed to overcome the limitations associated with epothilones, a novel class of anticancer substances. During this optimization process, more than 350 biologically active epothilones were synthesized, from which

sagopilone was selected for clinical development because of its outstanding preclinical properties. Sagopilone exhibits a high level of in vitro and in vivo activity against a broad range of different human tumor models, including those that are particularly sensitive to taxanes but also those that are resistant to taxanes or other commonly used chemotherapeutic agents and therefore no longer respond to these compounds.

Numerous tumor types targeted by cancer researchers

The substance is currently being evaluated in a comprehensive proof of concept program covering many types of tumor where there is still significant unmet therapeutic need. "In addition to the standard, well-known applications for the taxanes," says Dr. Klaus Bosslet, Head of the Oncology Therapeutic Research Group, "sagopilone appears to be effective in combating other tumors as well. Sagopilone could, for example, represent a major medical advance in the treatment of brain tumors."

As clearly illustrated in the video recording of real cancer cells, epothilones inhibit cell division. Researchers have long focused on cell division, as the majority of tumors become dangerous only as a result of their unchecked growth. The first drug products introduced were the cytostatics, which typically

Cancer researcher Guido Piechowiak (large photo) visually examines crystals in Bayer Schering Pharma's research laboratory in Berlin, where Bayer's active substance sagopilone was developed primarily by (from left) Dr. Jens Hoffmann, Dr. Ulrich Klar, Dr. Bernd Buchmann, Professor Werner Skuballa, Dr. Wolfgang Schwede and Dr. Johannes Platzek. The scientists are standing in front of a projected image of the active substance molecule and its target.



damage cells by interfering with metabolic activity during cell division. Epothilones target a very different area. They attach themselves like a vice to the network of protein threads that crisscrosses the inside of the cell, the part of the cytoskeleton that both stabilizes the cell and makes it flexible.

Point of action: protein threads inside the cancer cell

The individual threads in the cytoskeleton are simple in structure. Two types of the framework molecule together form sub-units which polymerize in long strands. Several slightly interwoven strands combine to form a pipe-like skeletal thread.

The term "skeleton" is somewhat misleading, however, because unlike the human skeleton made of bones it is continuously built up and destroyed again. This has an especially dramatic impact on cell division, as skeleton build-up and breakdown are particularly active during this phase and take place in accordance with set rules. As soon as the chromosomes have been bound in the typical X-shaped spindle and reached the center of the cell, the two halves of the X are separated and pulled into the two sides of the cell by the fine threads of the cytoskeleton. Cell division is complete when the two sides of the cell are distinct from one another and the

chromosomes each grow a new second half. If the cytoskeleton could not break down, the chromosome threads would remain in the center of the cell and nothing at all would happen for a time. "At some point, the cell's own molecular control functions report that a problem of possible relevance to cell security exists and it destroys itself," says Klar, explaining the outcome.

The taxanes, already in successful use for many years now, prove that the cytoskeleton provides a suitable target for cancer medication. Originally harvested from the bark of Pacific yew trees, they are now synthesized from substances found in the needles of the European yew. Like the epothilones, the taxanes inhibit the breakdown of the cytoskeleton.

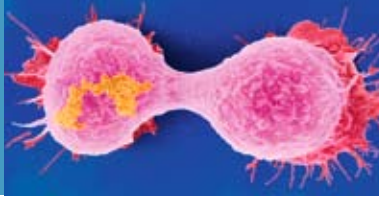
But why all the effort to develop the epothilones as an alternative to the taxanes when they both target the same site? As potent as the taxanes are, they have drawbacks: tumor cells are often able to expel the taxanes via pumps before they can attack the cancer cells. Due to this tendency to develop resistance, other agents are very welcome. In addition, unlike the epothilones, the taxanes are not water soluble and must therefore be administered in an oily solution that can induce allergies.



Preparatory work: Frank Kuczynski sets up distillation equipment at the Bayer Schering Pharma research laboratory in Berlin – one of many steps in the synthesis of the new active ingredient sagopilone for test purposes.



Cancer researcher Dr. Klaus Bosslet believes that sagopilone may also represent a major medical advance in the treatment of brain tumors.



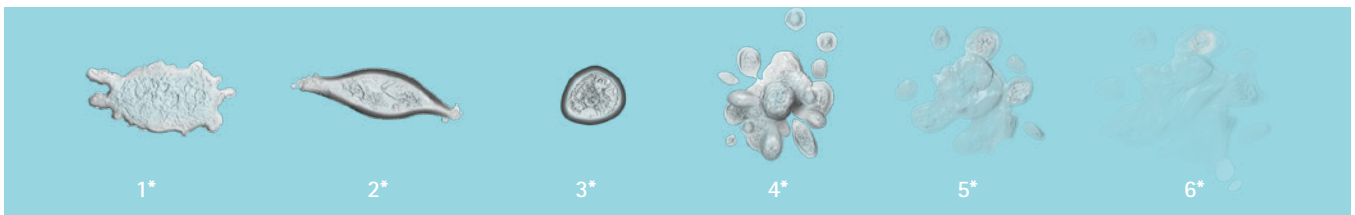
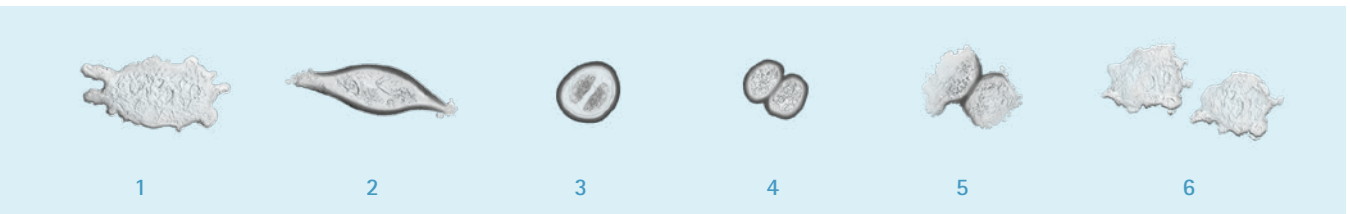
"The process of epothilone development at Bayer Schering Pharma was a long and winding one," says Ulrich Klar. One day, an external researcher offered the company a substance he had discovered and which he considered to be a possible anticancer medication. Schering was focusing on other areas at the time, and so the offer was rejected. By the time interest developed in the new cancer drug, it was too late – a rival company had already secured the rights to the substance.

River bank bacteria as the inspiration for the new active substance

This company concentrated on the natural product and was in fact able to culture the hard-to-raise river bank bacteria

and derive the agent from it. As is so often the case, however, the missed opportunity proved to be a new chance. The Bayer researchers synthesized their own epothilone and Bayer Schering Pharma now owns an epothilone with a structure somewhat different to the naturally occurring one, giving it especially desirable characteristics.

Reaching this point wasn't easy, however. Klar and his team first tried to reconstruct the natural substance by chemical means. As a result of its complex structure, however, synthesizing the molecule proved extremely difficult. Chemical ingenuity was once again needed to modify the structures in a way that preserved their destructive effect on tumors while simultaneously minimizing side effects. The task, as Klar explains it, was thus to further open the "thera-



How sagopilone works

A look through the microscope reveals how Bayer's new substance sagopilone destroys tumor cells.

The stages of a normal cell division process are pictured in the top row. The tumor cell (1), floating in a nutrient solution, first surrounds itself with a solid cell sheath (2). Inside, the chromosomes form X-shaped threads and move to the center of the cell (3). Each half of the X-shaped spindles contains a complete set of chromosomes. The cytoskeleton now pulls the two equal halves apart so that each half has a copy of the complete set of chromosomes (4). After the division (5), the solid cell sheath dissolves. The set of chromosomes has now been repli-

cated in both daughter cells, the cell division process is complete (6). As seen in the bottom row, cell division begins as usual in the presence of the new Bayer active ingredient (1*, 2*). The cell makes the necessary preparations, but then becomes stuck in the spherical phase (3*). This is because the accumulation of sagopilone causes the cytoskeleton to harden to the extent that it can no longer pull the chromosomes apart. The cell's own control functions recognize that a serious error has occurred and trigger the self-destruction mechanism. In the end, the cell explodes (4*) and its components are broken down in the body (5*, 6*).

peutic window". Approximately half of the 450 compounds that differed only slightly from the prototype epothilone were still very promising. The cultured cells quickly developed resistance to some, while others proved too toxic. After innumerable in vitro efficacy tests in close cooperation between chemical synthesis and the laboratory for in vivo tumor pharmacology headed by Dr. Jens Hoffmann, five to six candidates with favorable therapeutic windows were selected. Ultimately, sagopilone was declared the favorite because it received top marks in all categories and could also be synthesized relatively well.

The synthesis process was complex, however. Starting with three purchasable initial materials and more than 100 additional ingredients, Klar's team required a total of 39 individual steps to produce three initial sub-units which they then used to construct the finished molecule. But this impressive chemical accomplishment discouraged rather than delighted the company management. How could such a complex process ever be implemented cost-effectively on a large scale? "After all, the key question for a pharmaceutical company is ulti-

mately: can synthesis be carried out in such a manner that it yields sufficient quantities of the test substance and later of the marketable product?" explains Klar.

Persistence and persuasion brought research success

Even colleagues were skeptical at first, recalls the cancer researcher. The Head of Chemical Development, Dr. Harribert Neh, believed that large-scale synthesis was possible, however, and provided sustained support for its realization under the guidance of his department head, Dr. Orlin Petrov. Feasibility wasn't the only problem: there was also the issue of time to consider. Converting one step in the chemical process from the laboratory to production-scale implementation, a procedure known as upscaling, normally takes a month. This meant that 39 steps would take more than three years. Klar didn't want to wait that long, nor could he afford to. In order to obtain a sufficient amount of material for the upcoming preclinical trials, he therefore quickly assembled a team of



Hopeful candidate: the Bayer researchers analyzed approximately 450 different epothilone compounds until sagopilone – 5.5 mg of which is contained in this little vial – emerged as the favorite.



Purification of active substances: Bodo Röhr fills a chromatographic column used for separating substance mixtures and purifying reaction products.



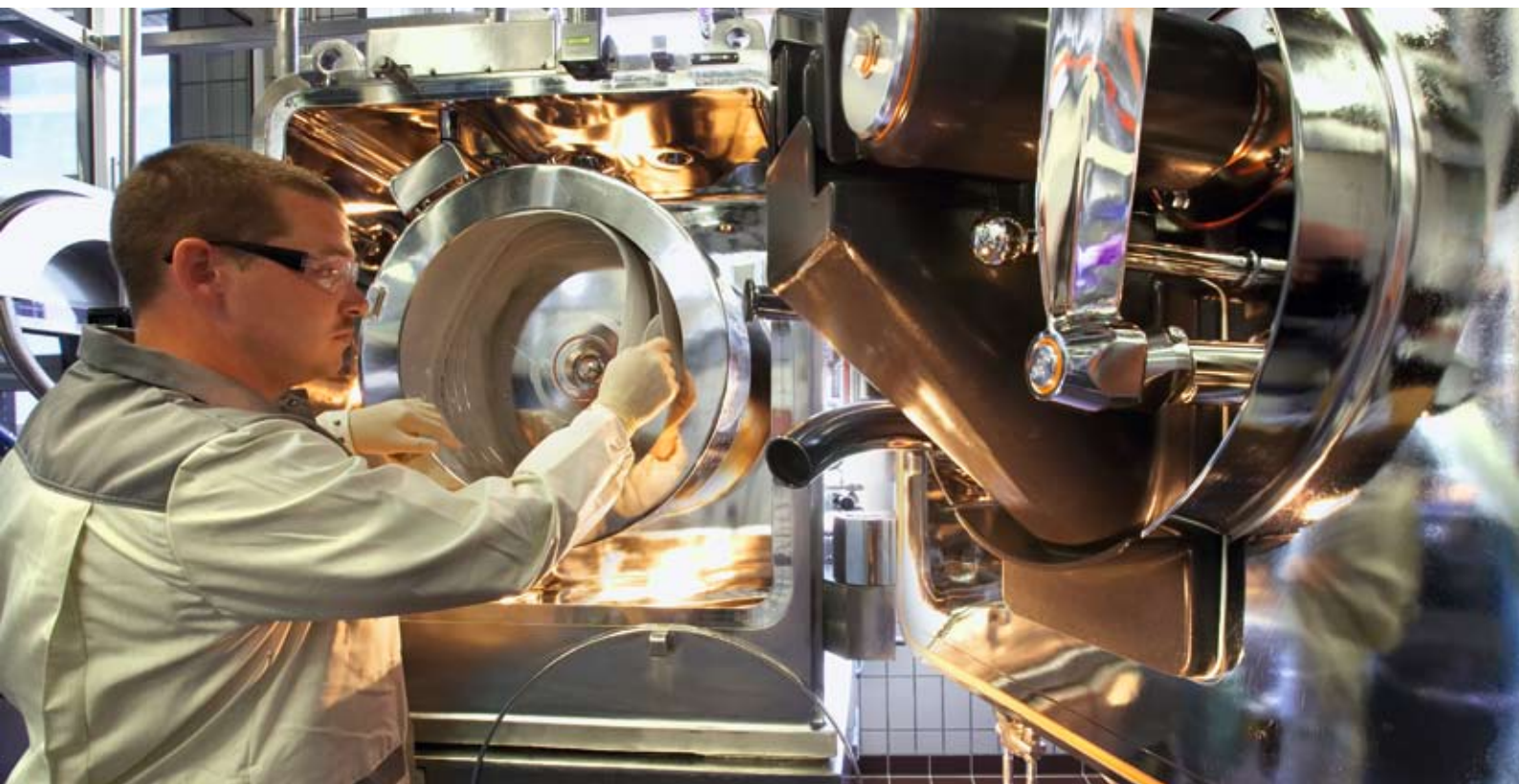
chemists and laboratory technicians. After a little over half a year, they had produced 36 grams of the substance, an amount theoretically sufficient for the treatment of approximately 200 patients. At the same time, colleagues under the direction of Dr. Johannes Platzek of Chemical Development were working on upscaling. In an impressive success, they were able to reduce the chemical development time from the estimated three years to a little over twelve months. These efforts have been well rewarded: their output is now five times that of Klar's laboratory. Recognition from external researchers also helped move the project forward. Bayer is now the only company with a fully synthetic epothilone, sagopilone, in advanced clinical development.

Clinical experts must now prove that sagopilone is really as promising as previous studies indicate. If the results of the following clinical studies are good, cancer researcher Ulrich Klar predicts that sagopilone, either alone or together with other medications, will play an important role in getting a vice-like grip on cancer of various origins and in all of its stages.

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The University of Bonn website features a spectacular photograph of the cytoskeleton of a tumor cell. To find it, simply enter the word "Zellskelett" [cytoskeleton] in the search field on the website.



The search for a cure: in the chemical pilot plant, Dennis Albrecht prepares a centrifuge to isolate an intermediate stage of the finished active substance sagopilone.