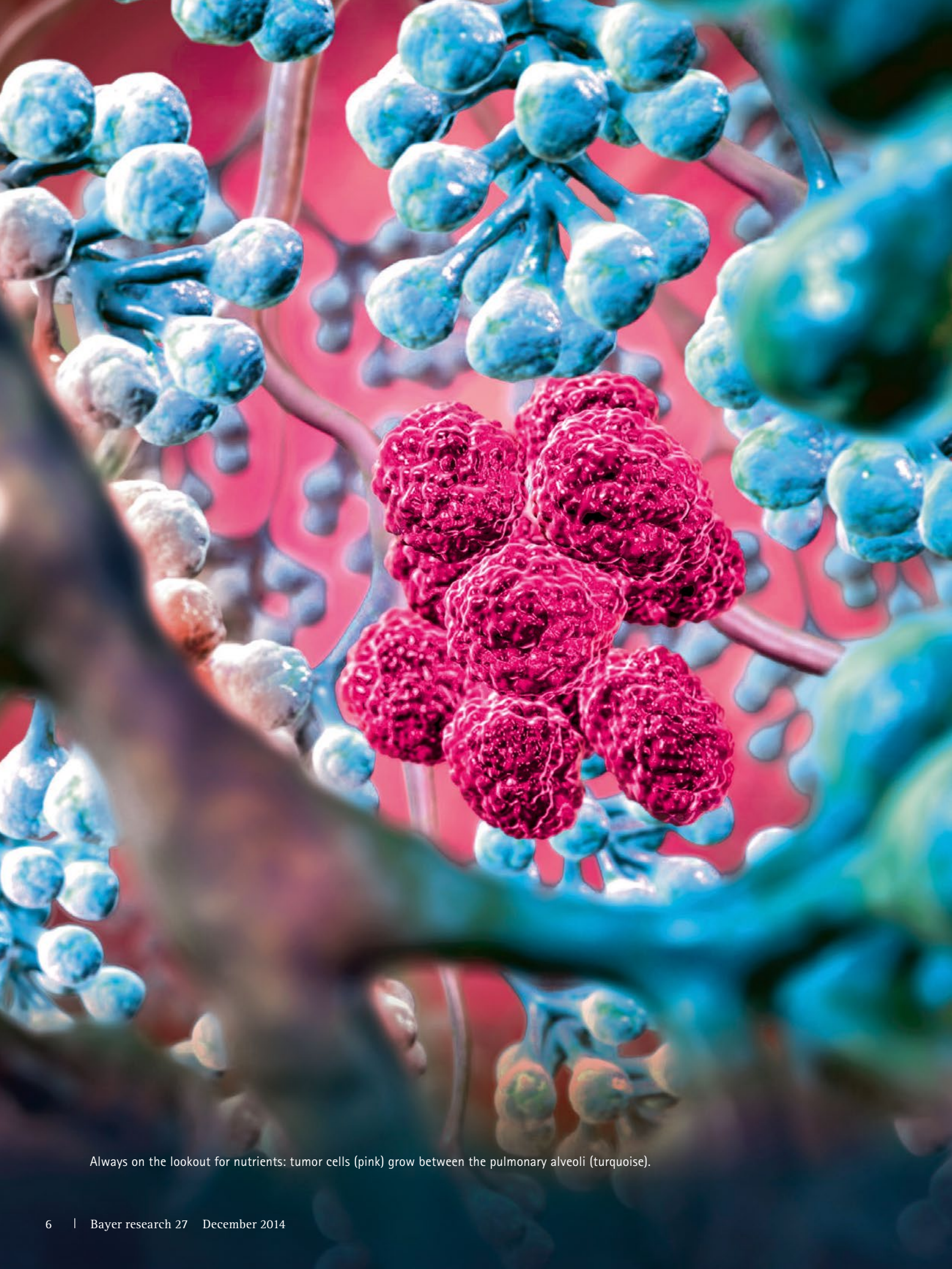


Always on the lookout for nutrients: tumor cells (pink) grow between the pulmonary alveoli (turquoise).

BAYER RESEARCHERS USE INNOVATIVE METHODS TO TRACK DOWN EFFECTIVE DRUGS FOR FIGHTING CANCER

Screening in 3D

The purpose of chemotherapy is to destroy all cancer cells in the body – and heal the patient. Using a process known as 3D tumor screening, Bayer researchers are heading in a new direction in the hunt for active substances that may be able to supplement conventional therapies and make them more effective.

Tumors are extremely voracious. To grow – and for their cells to keep dividing – they need vital substances from a patient's blood. New blood vessels therefore form and grow into the tumor to supply the cancer cells with oxygen and nutrients. Frequently, however, a tumor grows so fast that the blood supply cannot keep up. In consequence, the cells inside a tumor are undernourished. They respond to this scarcity of supply with a trick: they feign sleep, cease to divide and wait for replenished supplies. This mechanism is a cause for concern among cancer researchers and physicians everywhere, because many cancer drugs, known as cytostatics, mainly target the well-nourished cells multiplying in the peripheral region of a tumor.

Dormant cancer cells can wake up again at any time

They attack these sites directly, put a stop to cell division and shrink the diseased tissue. Frequently, however, a tiny core of the tumor remains behind after chemotherapy: the dormant cells inside the tumor. It is these cells that can reawaken all too quickly in some cases and continue to grow when the supply of nutrients from the blood resumes after treatment has ended.

"This is one of the main reasons why a cancer can reoccur even after apparently successful treatment," explains Dr. Patrick Steigemann, a biologist who works in Lead Discovery at Bayer HealthCare. To prevent such an outcome, he and his colleagues in oncology research are studying how to also effectively eliminate the dormant cells inside tumors in the course of chemotherapy. Their long-term goal is to develop a new cancer drug that joins forces with standard cytostatics to attack these sleeping cancer cells as well and thus minimize the risk of recurrence. "There's still a gap here in cancer treatment," Steigemann says.

3D tumor spheroids on the screen: Dr. Steigemann and his colleagues have essentially taught the computer how to see. This technology is very useful in the search for suitable candidates among approximately 500,000 substances.

Searching for a completely new active ingredient

Searching for an active substance with these characteristics is like searching for the proverbial needle in the haystack. "The known substances obviously don't help us at all; we need an entirely new active substance," Steigemann says. He and his colleagues therefore started off by consulting Bayer's in-house substance library encompassing some three-and-a-half million compounds, and together with the experts from Medicinal Chemistry initially selected 500,000 substances that were as diverse as possible. "An interesting trail could potentially lead to a whole range of related substances that

500,000

substances screened

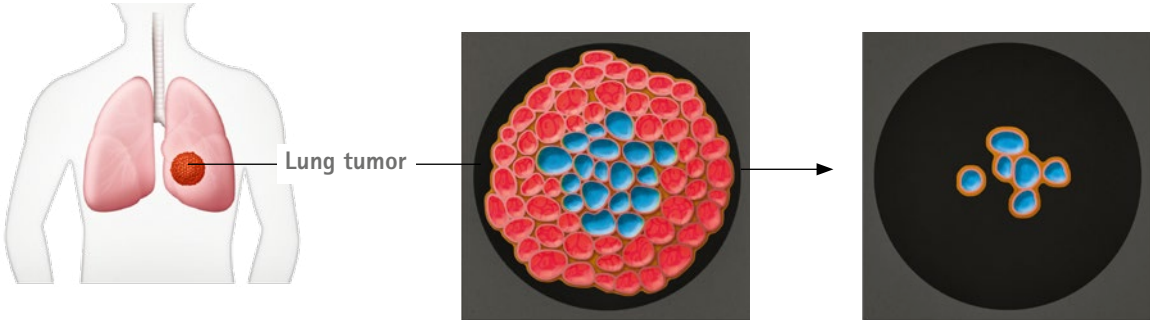
Steigemann and his team are screening 500,000 substances to find potential hits.

Source: Bayer

Photos: Corbis Creative (1), Matthias Lindner/Bayer AG (1), Bayer AG (2), Privat (1)

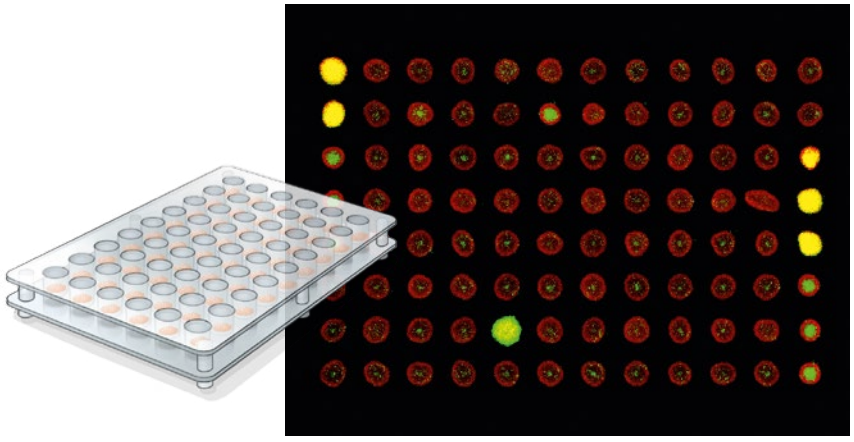
Inside the tumor

For cancer treatment to be successful, every last tumor cell must be eliminated. If any remain, new tumors can quickly develop again, leading to a recurrence of the disease. Bayer scientists are therefore looking for a substance that primarily targets the cells inside the tumor and, combined with conventional cytostatics, has the potential to completely eradicate the cancer cells.



With **conventional chemotherapy,** *the risk remains that* **dormant cells** *are left intact.*

The **outer cell layer** *of a lung tumor divides very quickly. The* **cells on the inside** *however are under-supplied with nutrients. They therefore switch into energy-saving mode: they stop dividing, feign sleep and wait for the supply to recommence.*

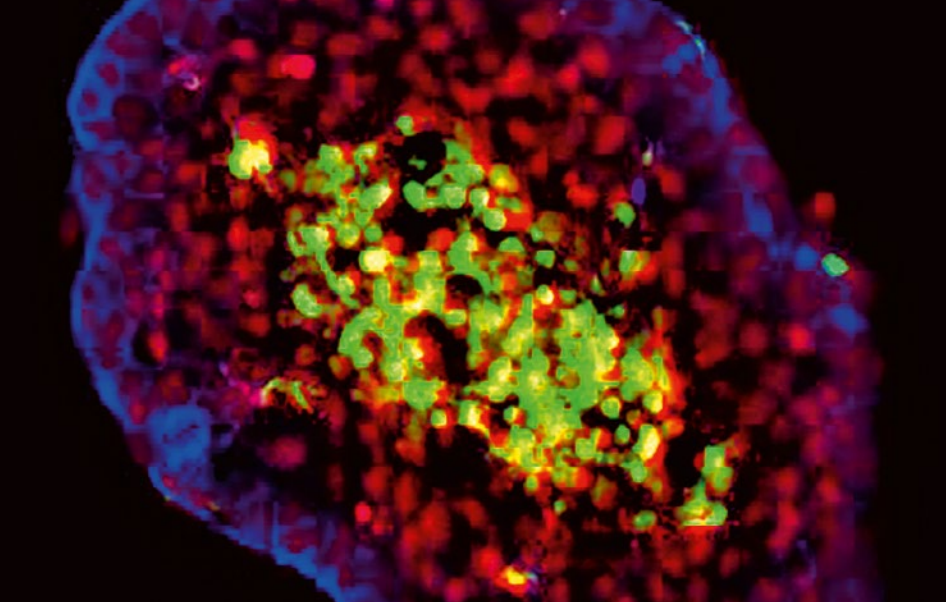


This plate with 96 wells is filled with spheroids treated with substance candidates. After 72 hours in the incubator, the results are visible: in the middle of the second row from the top, one tumor spheroid shows the desired outcome of green on the inside (dead cells) and red on the outside (living cells). In the center below is a test substance that has destroyed all the cells, appearing as a green patch. This active ingredient is useless. The columns on the left and right contain reference substances such as respiratory chain toxins and cytotoxic substances.

could include new drug candidates," Steigemann explains.

"We can't just test these compounds on individual cancer cells in a Petri dish, however," says Steigemann. Since he and his colleagues are searching for substances that specifically attack dormant cells in cancerous tissue, they need access to spatial cell structures that are as similar as possible to real tumors in patients, because cancer cells behave differently in the body than individual cells in a laboratory. "In a tumor, cancer cells react to one another. And every single cell reacts in turn with its extracellular environment," Steigemann says.

To recreate the situation inside the human body as realistically as possible in the laboratory and make possible sequential testing by means of automated screening, he and his colleagues breed micro-tumors – microscopically small cancer structures that act like real tumors in the body. They first fill a nutrient liquid and individual cancer cells into the wells of microtiter plates. Over a period of four days, the cells inside the wells in these plastic trays form small, round cancer cell clusters, also referred to as tumor spheroids. The researchers then add a test substance to each well and place them in incubators heated to body temperature for three days to give them enough time to exhibit their effects on the tumor spheroids. Some 320 substances per plate can be screened



Bayer scientists use dyes to make the individual parts of the tumor spheroid appear in different colors. The green elements in the image above are dead cells, the cell nuclei are red and the cytoskeleton, which is important for mechanical stability, is blue.

in this way using special automated robot systems.

The subsequent analysis process has also been automated by the researchers. An automated microscope camera shoots two photos of the spheroids in each well, marked beforehand with a fluorescent stain: living cells glow red, dead cells green. The computer superimposes the two images to produce a two-color photo, which clearly separates the living cells from the dead ones, thereby showing if the substance has had the hoped-for effect.

Understanding how cells behave in their natural environment

"We've essentially taught the computer how to see," Steigemann says. "High-content analysis" is the term that he and his colleagues use for this method. A total of some 2,000 plates are analyzed in each screening cycle. Thanks to the new automation technology, the Bayer researchers needed only three months to complete their analysis instead of several years like before. "Testing the tumor spheroids helps us to understand how cells behave in their natural, three-dimensional environment, and we can then use this knowledge for our therapeutic approaches," says Steigemann. The images are evaluated to detect the precise phenotype, or visual result, that they are looking for: a spheroid with as many green, dead cells inside as possible and a red, living outer cell layer. The researchers are not overjoyed about every dead cell they come across in their trials, however. As Steigemann says, "Our approach aims to specifically target only the undernourished, dormant cells inside the tumor." If an active substance simply kills off all cells, without making a distinction, that usually also includes the healthy cells in an organism. "We need a therapeutic window that makes healing possible," the scientist explains.

With their high-tech approach, the active substance detectives at Bayer have already found promising substance classes among the investigated compounds. Their task now is to investigate how exactly the substances work and whether the results in the lab can be transferred to humans. Only after the researchers have tested these aspects sufficiently can they send their active substances on the long road through clinical development. It will take at least ten years before these discoveries in the laboratory can be turned into a drug, but Steigemann is optimistic. "The dream of every cancer researcher is for his work to be able to help seriously ill people."

Guido Krömer

"Cancer develops if the immune system fails"

research *talked to Dr. Guido Krömer from the French research institution INSERM and Paris Descartes University in France about current and future developments in cancer research.*

What has cancer research accomplished in the last years?

Nowadays, it is possible to understand the particular molecular makeup of each tumor. However, the most important thing was understanding that cancer is not just the disease of cells that have adopted a selfish behavior. Rather, cancer is a systemic disease that can only develop and spread if immunosurveillance – that is the immune system – fails.

What challenges does it meet today?

We have to understand the complexity of the tumor – at the level of the cancer cells themselves. The tumor is a system composed of different body cells. We need to understand the relationships among these cells because they ultimately drive or suppress the development of cancer and tumor progression.

What are the most promising approaches?

We are all hoping that the improved understanding of each tumor on the molecular level will ultimately lead to a "personalized" therapy. I believe that there will be spectacular advances in the field of immunotherapy. We have the opportunity to stimulate anticancer immune responses by specific and hence "personalized" interventions.

www.research.bayer.com/3d-tumorscreening
More information on this subject