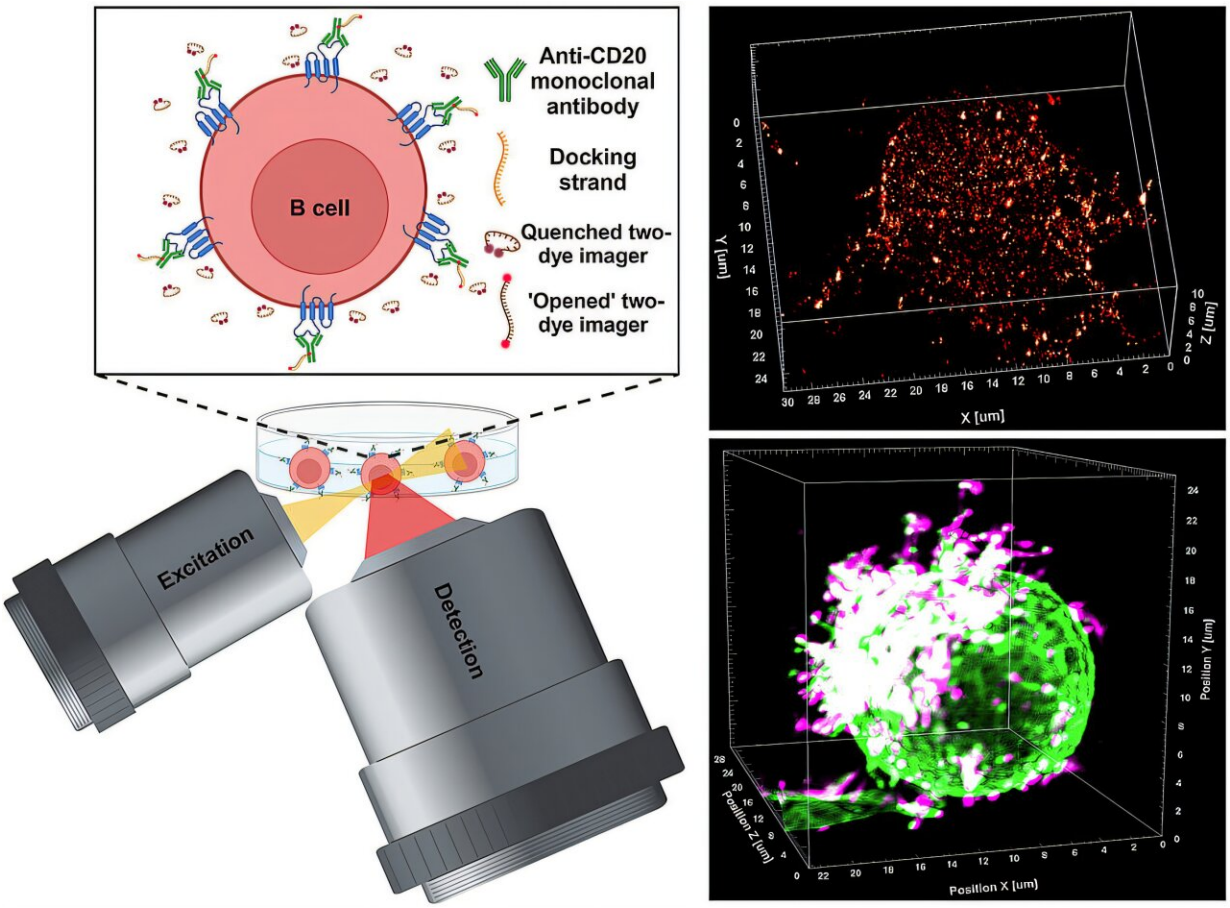
Super-resolution microscopy shows how therapeutic antibodies work against cancer cells

January 10 2025



Mode of action of the new microscopy method LLS-TDI-DNA-PAINT. On the top right, the RTX antibody was visualised on a Raij-B cell: it is easy to see how it links the CD20 molecules in the membrane. Bottom right: the hedgehog-shaped appearance of a living Raji B cell after the antibody has bound. The surface protein CD45, which is homogeneously distributed on the cell surface, is

also labelled in green. Credit: Arindam Ghosh / Universität Würzburg

In blood cancers such as chronic lymphocytic leukemia, B cells of the immune system multiply uncontrollably. One form of therapy involves labeling the CD20 protein on the surface of the B cells with customized antibodies. This triggers a chain of immunological reactions and ultimately leads to the destruction of the cancer cells.

Such immunotherapeutic antibodies have been used against tumor diseases for 30 years. "Although it is crucial for the success of the therapy, we still know very few details about how the antibodies bind to CD20 and how the subsequent reactions take place," says Professor Markus Sauer from the Biocentre of Julius-Maximilians-Universität (JMU) Würzburg in Bavaria, Germany.

Tracking down the effectiveness of the antibodies

This is now likely to change. A team led by the JMU biophysicist has developed a new super-resolution microscopic method. It makes it possible for the first time to investigate the interactions of therapeutic antibodies with target molecules on tumor cells in 3D with molecular resolution.

The research is published in the journal *Science*.

"We can now observe how effectively the antibodies work and thus contribute to the development of improved therapies," says Sauer.

The new microscopic method is termed LLS-TDI-DNA-PAINT. In the journal *Science*, first author Dr. Arindam Ghosh and a team from Sauer's chair describe how the newly developed technology works and what

findings have already been obtained with it. Dr. Thomas Nerreter and Professor Martin Kortüm from the Medical Clinic II at Würzburg University Hospital were also involved in the study.

B cells take on the shape of a hedgehog

The Würzburg researchers carried out their studies on fixed and living Raji B cells using the new microscopy method. This cell line originates from a patient's Burkitt's lymphoma and is often used in cancer research. The researchers brought the cells into contact with one of the four therapeutic antibodies, RTX, OFA, OBZ and 2H7.

All four antibodies crosslink the CD20 molecules in the cell membrane, resulting in strong localized accumulations of the antibodies. This activates the so-called complement system and initiates the killing of the cells by the immune system.

In contrast to the current classification of therapeutic antibodies, the results show that the concatenation of the CD20 molecules occurs independently of whether the antibodies belong to type I or II.

The experiments also show that all four antibodies crosslink CD20 molecules that are located at specific sites on the membrane—on micrometer-long protrusions of the membrane called "microvilli."

At the same time, the binding of the therapeutic antibodies polarizes the B cell and the outstretched microvilli are stabilized. As a result, the B cells take on a kind of hedgehog shape because the membrane protrusions are only located on one side of the cell.

The next steps in research

What happens next? "The previous classification of therapeutic antibodies into types I and II can no longer be maintained," says Dr. Arindam Ghosh. Until now, research has assumed that therapeutic antibodies of type I have a different mechanism of action than those of type II. However, the Würzburg studies disprove this.

"The hedgehog shape makes the B cells appear as if they want to form an immunological synapse with another cell," says the JMU researcher.

It is conceivable that the treated B cells activate macrophages and natural killer cells of the immune system in this way. The research team will now clarify whether this assumption is correct in further studies.

More information: Arindam Ghosh et al, Decoding the molecular interplay of CD20 and therapeutic antibodies with fast volumetric nanoscopy, *Science* (2025). DOI: 10.1126/science.adq4510

Provided by Julius-Maximilians-Universität Würzburg