Beating Bladder Cancer: Novel Treatment Combinations with CDK4/6 Inhibitors

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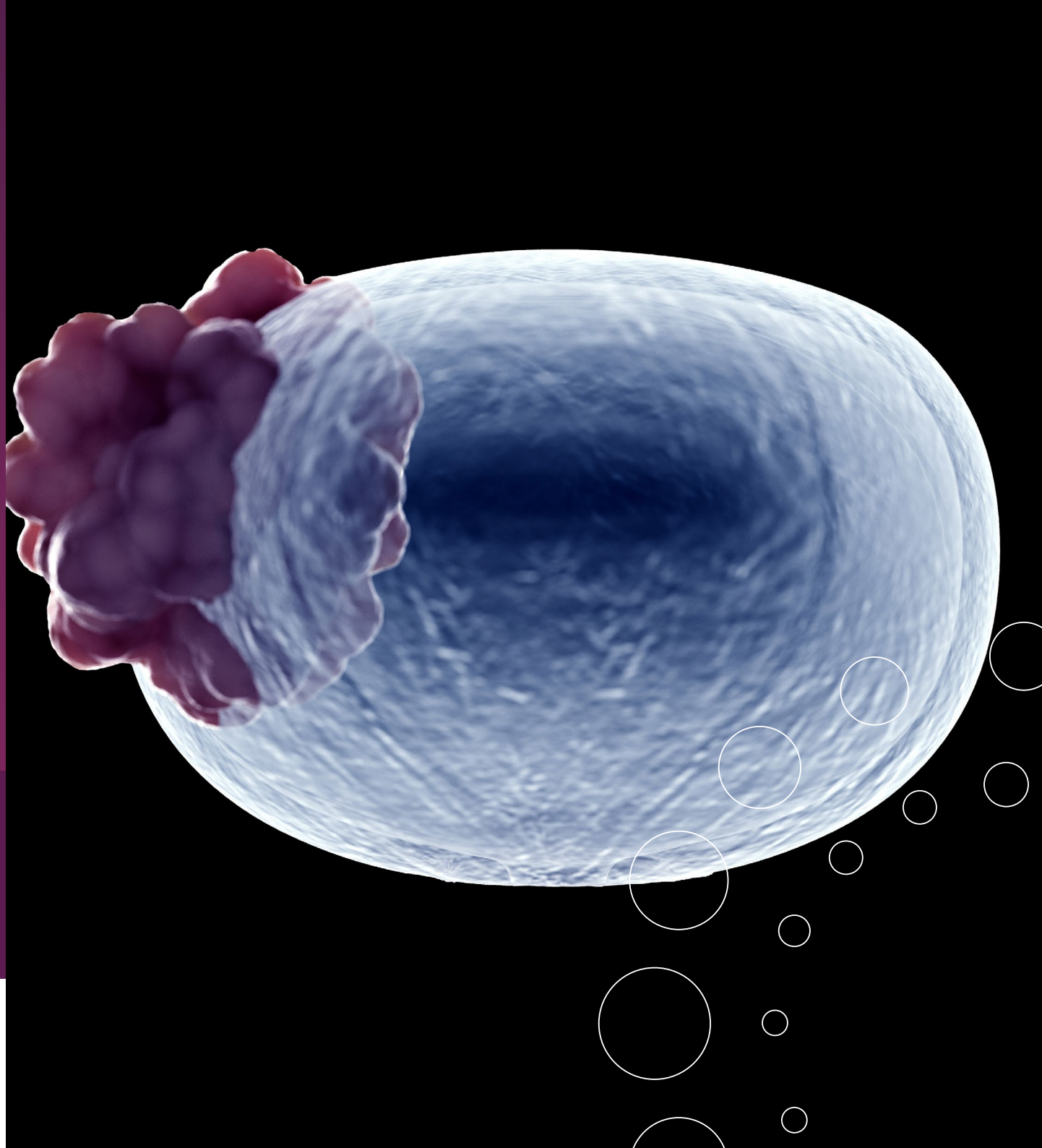

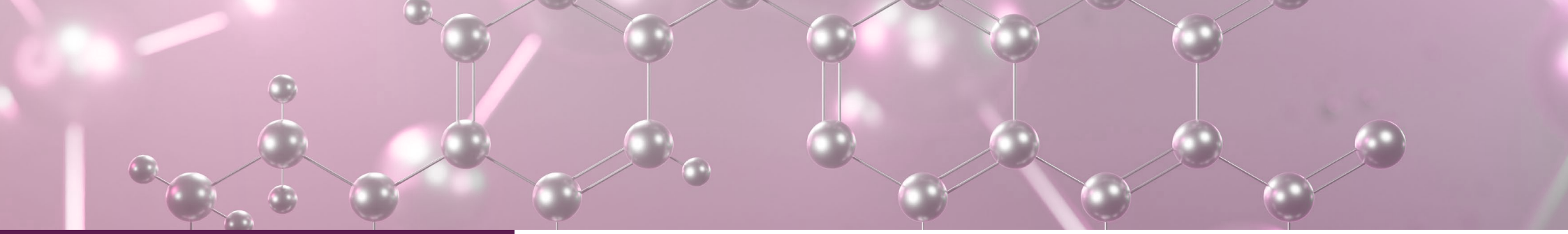

Beating Bladder Cancer: Novel Treatment Combinations with CDK4/6 Inhibitors

Cancer is one of the leading causes of death around the world. Research into this disease is vital to the development of new treatments, bringing fresh hopes to those affected by this potentially devastating diagnosis. Dr Roman Nawroth and Dr Ting Hong carry out their ground-breaking research at the Technical University of Munich in Germany. They focus their efforts on novel approaches to fight bladder cancer, exploring the use of CDK4/6 inhibitors.

Making the Top Ten

Bladder cancer features in the top ten of the most commonly occurring types of cancer, according to the World Health Organization. Globally, in 2022, over 600,000 people were diagnosed with this form of cancer, and it accounted for over 200,000 deaths. Although it is highly treatable when caught early, bladder cancer remains one of the most expensive and challenging cancers to diagnose and treat, since detection relies predominantly on an expensive and invasive procedure called cystoscopy, which involves looking inside the bladder with a camera.

Additionally, mortality and incidence rates have not changed significantly over the past decades despite advances in diagnosis and treatment. However, novel therapeutic approaches are currently being investigated. Dr Roman Nawroth and Dr Ting Hong from the Department of Urology at the Klinikum rechts der Isar of the Technical University of Munich carry out their vital research into a relatively new class of small molecules called CDK4/6 inhibitors. They work tirelessly to develop effective and personalised therapeutic combinations to beat bladder cancer.

Promising New Treatment?

Dr Nawroth and Dr Hong explain that CDK4/6 inhibitors have recently been approved by the US Food and Administration (FDA) as a standard treatment for certain types of breast cancer in combination with hormone therapy. These medicines disrupt a process which cells use to divide and multiply, targeting and blocking the action of specific proteins called cyclin-dependent kinases 4 and 6 (CDK4/6). CDK4/6 strictly regulates the cell cycle in normal cells. However, in certain types of cancer cells, it can become overactive, causing the tumour cells to uncontrolled proliferation. The colleagues add that a significant proportion of bladder cancers show mutations in the genes which regulate the growth and multiplication of the cells. Moreover, preclinical studies have shown that CDK4/6 inhibitors are promising in treating bladder cancer.

Unfortunately, a phase II study using the CDK4/6 inhibitor called palbociclib was not so successful. Additionally, other trials found that CDK4/6 inhibitors failed to provide long-term anti-tumour effects, in particular when administered as single agent therapies. The colleagues say that CDK4/6 inhibitors do offer a promising treatment option. However, their effectiveness is impacted by the molecular background of a tumour, which influences both initial response and development of resistance throughout therapy. Understanding how and why this occurs is critical to making CDK4/6 a viable option for treating bladder tumours.

Unravelling the Resistance

Functional genomics is an area of molecular biology which focuses on understanding the interactions and functions of genes and, in turn, the production of proteins. The team carried out a functional genomics study to find out how the resistance to CDK4/6 could be occurring and if anything could be done to stop or minimise it. They used a genome-scale CRISPR-dCas9 activation screen, which allows the enhanced activation or expression of certain genes to be studied. A screen for resistance to the CDK4/6 inhibitor, palbociclib, was conducted in lab-grown bladder cancer cells, and data was also gathered using a 3-dimensional model in the chicken egg, which enables the study of cancer-like structures, impact of genes and treatment methods.

Screening results, which identify signalling pathways, chain reactions of events after gene expression, and clinically relevant changes to molecules, were then compared using four methods/databases: DAVID, Reactome, DGIdb, and cBioPortal.

The colleagues reported that their screening found 1024 sgRNAs, a particular form of genetic material, which represented 995 genes showing signs of markers linked with resistance to palbociclib. They were able to identify eight sgRNAs linked to specific genes and, thus, certain cell signalling pathways, specifically, members of receptor-tyrosine kinases, PI3K-Akt, Ras/MAPK, JAK/STAT and Wnt signalling pathways. If these pathways could be blocked with a suitable inhibitor, then the development of CDK4/6 resistance could be prevented. The team proceeded to combine palbociclib with inhibitors against these signalling pathways, which did indeed reveal beneficial effects.

New Targets Identified

Having successfully identified the resistance mechanisms to allow suitable combination therapies to be achieved, using the CRISPR-dCas9 screening approach, Dr Nawroth and Dr Hong continued their investigations. This time, they focused on analyzing molecules that are involved in the cell responses to CDK4/6 inhibitors treatment. They explain that identifying these molecules would contribute to the development of new combination therapies. Matching the most suitable inhibitor to the CDK4/6 inhibitor could enhance the targeting of bladder cancer cells more effectively by minimising the development of resistance.

The team used the data from their previous investigation along with information gathered from an analysis of published studies to gain a full picture of the genes involved with CDK4/6 inhibitor (specifically palbociclib) response and resistance.

They then compared the genes that were down-regulated ('switched off') when treatment was started with genes that were known to lead to resistance when they were up-regulated ('switched on'). Five relevant genes were identified, and the team proceeded to focus on the KIF and MCM protein families, specifically MCM6 and KIFC1. They combined inhibitors against both MCM6 and KIFC1 with the palbociclib CDK 4/6 inhibitor, discovering that this resulted in synergistic inhibition of cancer cell growth.

Novel Approaches with RNR Inhibition

Next, Dr Nawroth and Dr Hong started investigating other potential inhibitors that could be used in combination therapy. The colleagues highlight that CDK4/6 inhibitors work by halting cell growth and division, but after the 'sensitive stage', the cancer cells have some kind of compensatory mechanism causing resistance to the treatment. The team identified another culprit responsible for this resistance. The ribonucleotide reductase regulatory subunit M2 (RRM2), which is a crucial component of the ribonucleotide reductase (RNR) complex, was identified as a key mediator in the acquisition of resistance.

The colleagues also investigated whether palbociclib activates the breakdown of the RRM2 proteins using a particular process called the ubiquitin-proteasome system during the sensitive stage, also exploring whether RRM2 is controlled by a particular molecule called E2F transcription factor 3 (E2F3), once resistance occurs. After conducting their detailed investigations, Dr Nawroth and Dr Hong concluded that RNR inhibition in combination with CDK4/6 inhibition offers a promising new therapeutic strategy for patients with bladder cancer, in particular with forms resistant to chemotherapy.

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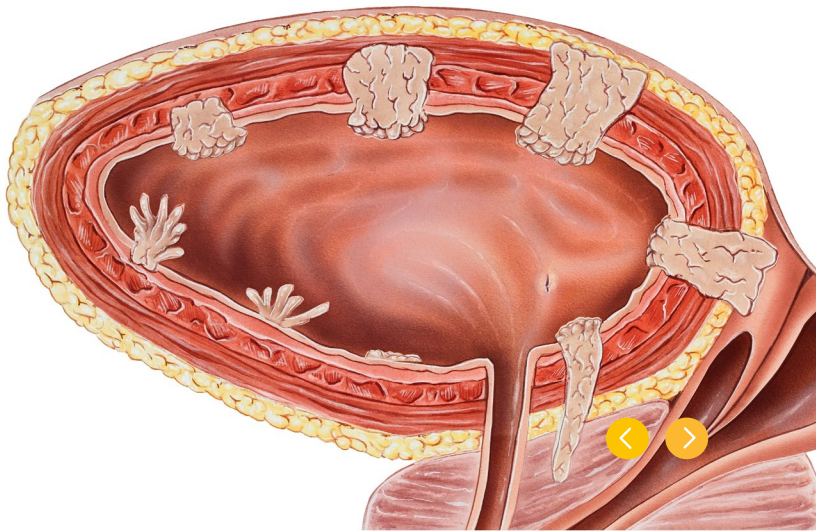
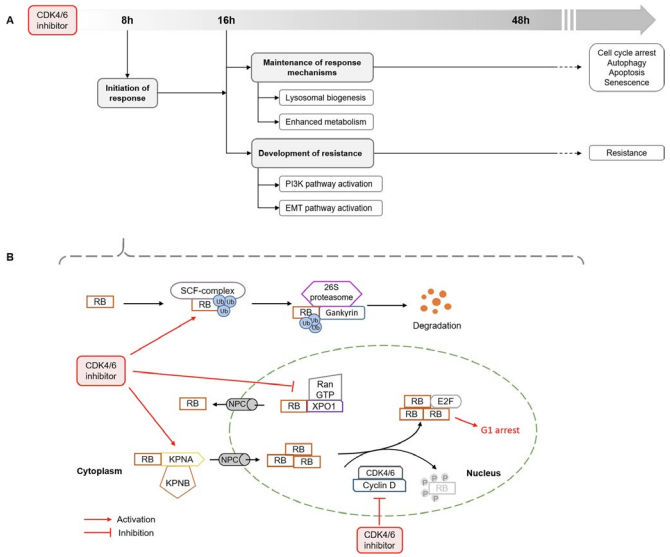


A working model for the molecular response to CDK4/6 inhibition



A The therapy response to CDK4/6 inhibitors can be divided into distinct steps: Initiation followed by a plasticity between maintenance of response or development of resistance and finally the separation into a long-term response and resistance. **B** The molecular mechanisms of the initiation. CDK4/6 inhibitor triggers RB proteolysis in the cytosol and nuclear translocation. The accumulation of dephosphorylated RB leads to cell cycle arrest.

Initiation of Cell Cycle Arrest

In their most recent study, Dr Nawroth and Dr Hong identified and explained the step-by-step process by which CDK4/6 inhibition initiates cell cycle arrest, stopping the growth and division of bladder cancer cells. Together with the data from their previous investigations, a better understanding of the underlying molecular mechanisms could help further improvement of therapy design, as resistance can develop at different stages of treatment triggered by various pathways.

Over a 48-hour time course, the team analysed the response of bladder cancer cells to palbociclib, using a process called RNA sequencing, and discovered a multi-step response mechanism to this therapy. They then translated these results to the molecular mechanisms occurring in the cancer cells. They found that the first steps involved the movement of a molecule called RB protein into the nucleus of the cell by a specific mechanism involving the activation of specific transport proteins called importin α/β. At the same time, the RB protein left in the cytoplasm of the cell is degraded, which is a process regulated by the so-called proteasome complex that involves the activation of a multi-protein complex SCF and gankyrin. The colleagues highlight that only RB protein, which is hypophosphorylated (meaning that it has low numbers of phosphate groups attached to it, altering its biochemistry), accumulates in the cell nucleus. This is an essential step for a successful therapeutic response, initiating cell cycle arrest, thereby preventing its growth and division.

CDK4/6 Inhibition: The Future of Bladder Cancer Therapies

Dr Nawroth and Dr Hong explain that patients who are RB protein negative or have certain mutations relating to it tend to have a poor response to treatments with CDK4/6 inhibitors. Their understanding of the cell cycle arrest process and the role of the RB protein could account for this problem. The team also reported an increased expression of the MiT/TFE protein family in the later stages of treatment, which is an essential step to maintain a further response to the treatment. They add that, in the final stages, they observed involved cancer cell senescence (a process where they stop growing and dividing) or the development of resistance to CDK4/6 inhibition.

Dr Nawroth and Dr Hong are paving the way for novel bladder cancer treatments, and opening the doors for personalised cancer therapies. Being able to tailor treatment to the individual based on the genetic makeup of the cancer cells allows for more effective therapy. The team's ground-breaking research exploring the molecular mechanisms involved in the resistance process and novel treatment combinations brings new hope to those impacted by bladder cancer.

MEET THE RESEARCHERS

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Dr Roman Nawroth received his PhD in Molecular and Cellular Biology from the University of Muenster, Germany, in 2002, having studied the control of endothelial cell junctions during the transmigration of leukocytes at the Max-Planck-Institute. From 2003 to 2006 he trained in cancer biology whilst pursuing his postdoctoral studies at the University of California in San Francisco, USA. Currently, he holds the position of Assistant Professor in the Department of Urology at the Technical University of Munich. Since starting here in 2007, his research has focused on three main areas. He is developing an understanding of the mode of action of targeted therapies, which could result in personalised cancer treatments. His group is also working on oncolytic adenovirus-based therapy, having developed a combination therapy that is due to enter phase 1 clinical trials. Additionally, he is involved in the development of methods to monitor therapy success and maintenance based on patient-derived liquid biopsy samples.

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Dr Ting Hong is an MD student at the Technical University of Munich, Germany, who has been a member of Dr Nawroth's research group since 2020. His work focuses on exploring the molecular response mechanisms of CDK4/6 inhibitors, and he has made significant steps towards understanding the multistep molecular responses involved, particularly at the initiation of the response. Additionally, he has demonstrated novel approaches to design combination therapies which enhance the efficacy of the CDK4/6 inhibitors, or help to overcome drug resistance. He is also researching oncolytic adenovirus-based therapies, with a focus on understanding the adenovirus replication process, and discovering new methods to make these viral treatments more precise and effective for targeting bladder cancer.

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FURTHER READING

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