New Approaches to Fighting Cancer: Annexin Proteins and Carbon Nanotubes

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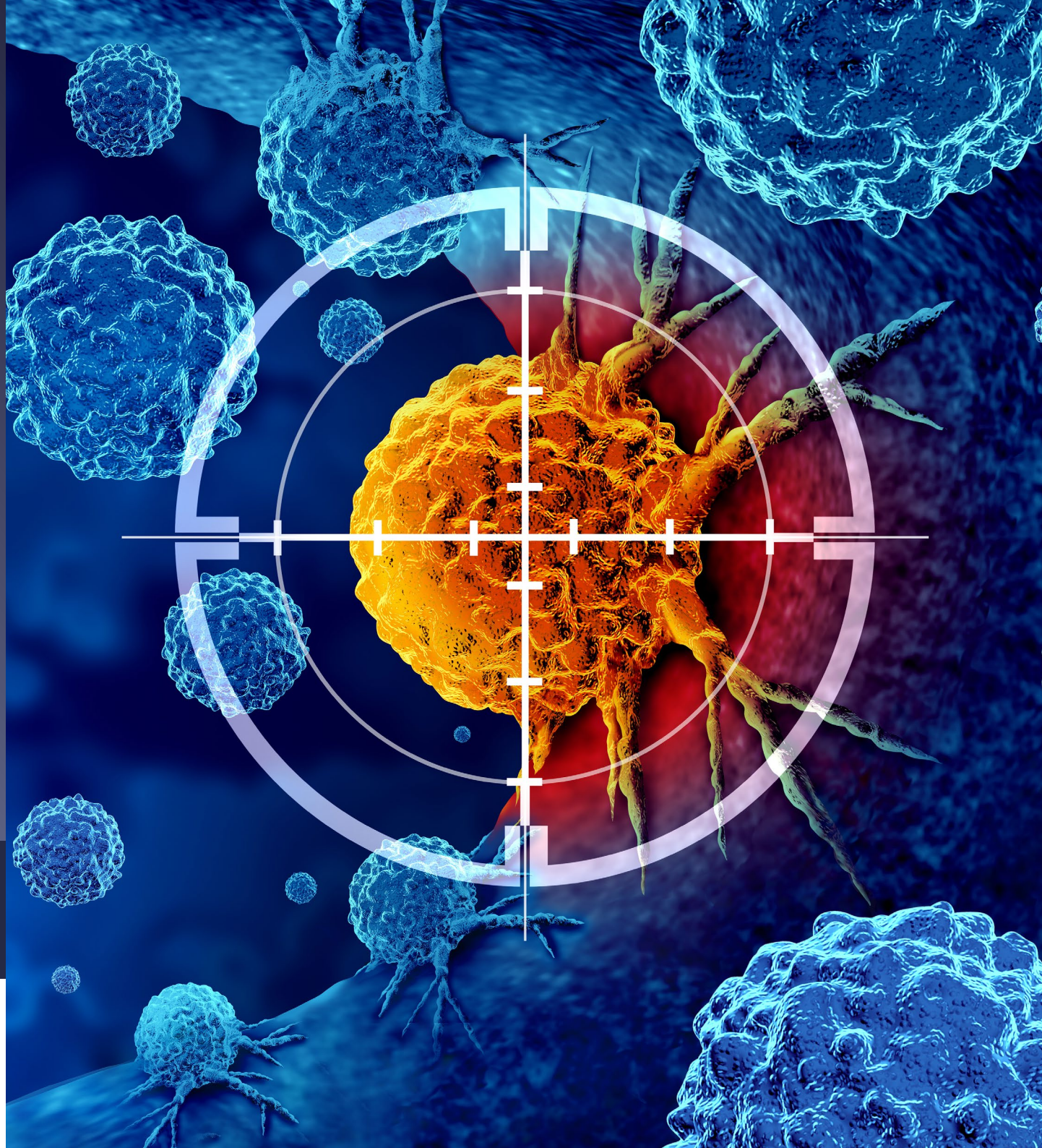

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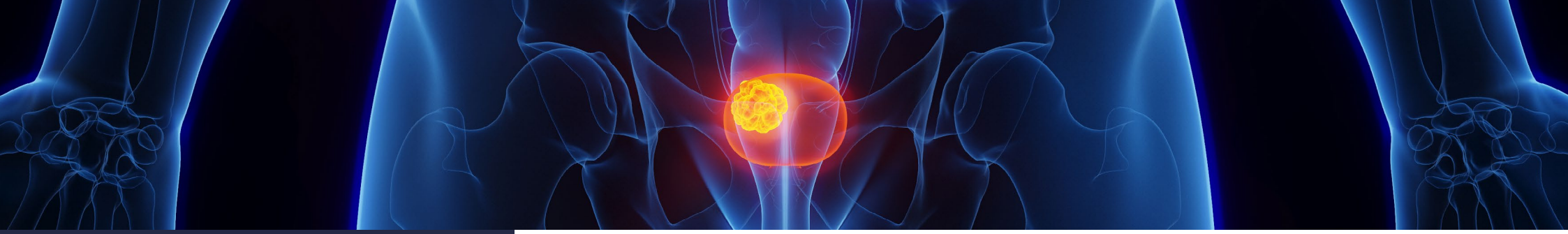


New Approaches to Fighting Cancer: Annexin Proteins and Carbon Nanotubes

Cancer is a leading cause of death worldwide, and efforts to provide new and more effective treatments are critical. Professor Roger Harrison from the University of Oklahoma has developed new methods to target cancer using annexin proteins and carbon nanotubes. The annexin proteins allow tumour cells to be selectively targeted while healthy cells are spared. Combining these novel molecules with established options, such as immunotherapy, is proving very effective.

Photothermal Therapy to Treat Cancer

According to the World Health Organization, cancer accounts for almost one in every six deaths around the world. Fortunately, the ongoing development of new treatments offers hope that, eventually, the devastating grip that cancer holds on human health can be weakened.

An approach known as photothermal therapy uses light at a specific wavelength alongside a carbon nanoparticle coupled to a protein that binds selectively to the surface of cancer cells to target these cells. After the nanoparticles s are administered, they are activated with particular electromagnetic frequencies. When this happens, the nanoparticles get very hot, heating the cancer cells to 42–60°C or above in a short time frame, causing irreversible damage to the cancer cells in a process called thermal ablation. This therapy can be administered using light at a certain frequency to tumours on the skin or on the surface of certain organs; alternatively, the light can be delivered inside tumours anywhere in the body with an image-guided optical fibre.

Professor Roger Harrison from the University of Oklahoma in the USA explores the use of carbon nanotubes as photosensitising agents for photothermal therapy. Carbon nanotubes are cylinder-shaped molecules made up of a single-layered sheet of carbon atoms. When exposed to certain wavelengths of light, they heat up quickly. However, it is vital that the carbon nanotubes only attach to the cancerous cells, ignoring any healthy cells. With this in mind, Professor Harrison is developing methods to target only the unwanted cells using annexin proteins – a group of molecules found naturally in the body.

Targeting Bladder Tumours

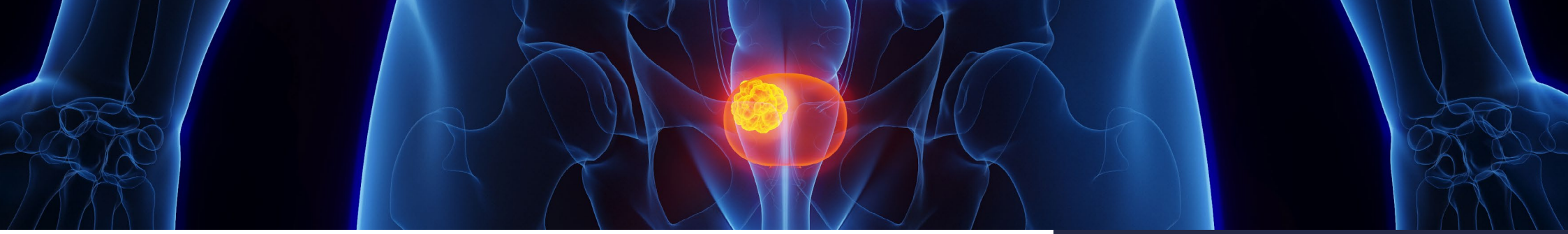
Bladder cancer has a recurrence rate of up to 70% after some types of surgery, usually due to a few cancerous cells being left behind. Professor Harrison and colleagues conducted a study using carbon nanotube-based photothermal therapy to target bladder tumours with the aim of reducing the incidence of tumour recurrence. Bladder tumour-specific single-walled carbon nanotubes (SWCNTs) were delivered into the bladders of mice with cancer at a very low dose. One day later, the mice received a short treatment of near-infrared light which heated the nanotubes, resulting in ablation of the cancerous tissues.

Professor Harrison explains that the nanotubes were specifically designed to only target and bind to the bladder tumour cells. The protein annexin A5 (ANXA5) was joined to the nanotubes, creating the SWCNT-ANXA5 conjugate. Annexin A5 binds strongly to a fatty molecule called phosphatidylserine which is exposed on the outer surfaces of cancer cells but only found inside the cells of normal healthy tissues.

Promisingly, no tumours were visible on the wall of the bladders 24 hours after the light treatment. In addition, the healthy parts of the bladder remained undamaged, confirming that the specific targeting of tumour cells had been successful. To look at long-term survival, the team monitored the mice for a total of 116 days, achieving a cure rate of 50%. Importantly, there were no signs of toxicity or spread from the nanotubes to other organs.

Joining Forces with Checkpoint Inhibitors

The immune system uses checkpoint proteins to help identify foreign or unwanted cells, such as pathogens and cancerous cells. These proteins are like switches that must be turned on or



off (depending on the particular protein) to initiate a response from the immune system. Cancer cells can sometimes use these checkpoint proteins to avoid being spotted by the immune system. Checkpoint inhibitors are a type of immunotherapy that influences how checkpoint proteins respond. While they do not directly kill cancer cells, they help the immune system find them and, thus, destroy them.

Professor Harrison and his team carried out a ground-breaking study combining photothermal therapy with their targeted SWCNTs and a checkpoint inhibitor to treat metastatic breast cancer in an animal model. They used the mammalian protein annexin A5 (ANXA5) bound to SWCNTs to create an SWCNT-ANXA5 conjugate. This was injected into the mice, where it accumulated in the tumour, and light treatment was later administered. One group of mice also received immunotherapy with anti-CTLA-4-based checkpoint inhibition, and another group only received the checkpoint inhibition. An increased survival rate was apparent in the group that received both treatments (55% after 100 days) compared to the other groups – far higher rates than either of the two treatments alone.

Promising Results for Treating Metastatic Breast Cancer

Professor Harrison explains that globally, there is a high prevalence of breast cancer but a lack of safe and effective treatments for the advanced stages of the disease. The positive results from combining carbon nanotube photothermal therapy with immunotherapy encouraged the researchers to explore the benefits further. Again, annexin A5 protein was joined to the nanotubes, as it binds so effectively to phosphatidylserine found on the outside of cancer cells and tumour blood vessels but remains hidden to healthy cells.

This time, the SWCNT-ANXA5 conjugate was injected directly into the breast tumours of the mice before undergoing photothermal therapy. This enhanced the therapeutic effects of SWCNT-ANXA5 conjugate, particularly when the mice were also given checkpoint inhibition immunotherapy at the same time. The survival rate of the mice receiving this treatment combination was high – an impressive 80% after 100 days, despite having a very aggressive form of breast cancer. Clearly, combining the newly developed nanotube treatment with stimulation of the immune system using checkpoint inhibition offers a promising step forward.

Safety First

Findings also supported earlier work on the safety and toxicity Findings also supported earlier work on the safety and toxicity of carbon nanotubes, confirming that the single-walled carbon nanotubes were low in toxicity. Professor Harrison emphasises that administering SWCNT-ANAX5 conjugate directly into the tumour also helped to prevent the build-up of nanotubes in the organs which work to clear substances from the body, including the kidneys and liver.

While carbon nanotubes show extremely promising results as photothermal agents, there have been challenges over the years in developing them into safe forms for use in the clinical environment. Obtaining data about the safety of nanotubes (for example, regarding how they are removed from the body) is vital if they are to be used in practice. Professor Harrison adds that nanoparticles can behave in unpredictable ways compared to traditional chemotherapy drugs. The length, diameter, and stiffness of nanotubes can all affect the level of toxicity of these molecules in living organisms. However, the work of Professor Harrison and his colleagues has confirmed safe nanotube size and structure, as well as the best route of administration to minimise

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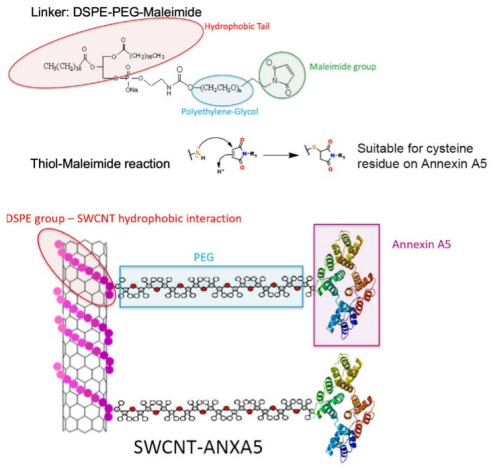
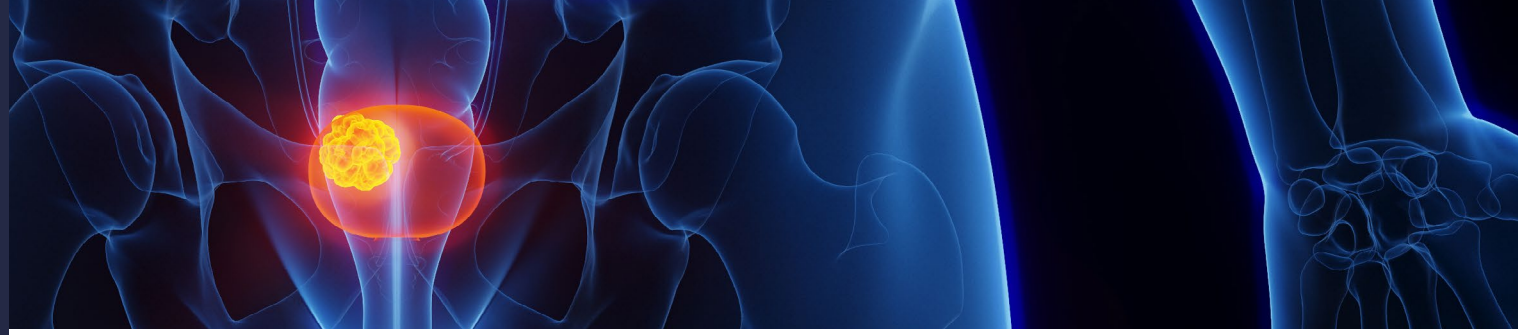


Conjugation of a single-walled carbon nanotube (SWCNT, left) to the protein annexin A5 (ANXA5, right) connected by a DSPE-malemide-PEG linker. Annexin A5 binds selectively to phosphatidylserine (a fat molecule) on the surface of cancer cells.



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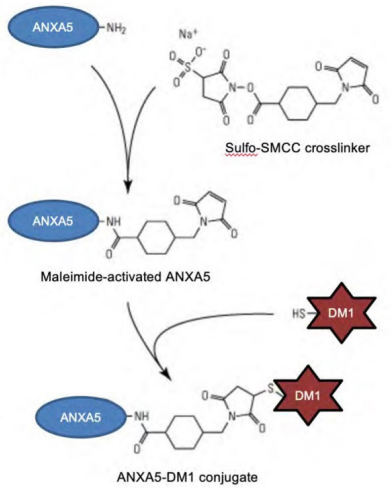


The DM1 anticancer drug is linked to ANXA5 in a two-step reaction. First, the lysine residues on ANXA5 are activated with sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (Sulfo-SMCC). Second, DM1 is introduced and the maleimide on ANXA5-sulfo-SMCC reacts with the lone sulfhydryl group on DM1.

build-up in the organs. Although these results were obtained from animal models, the findings open the doors to new approaches for treatments in humans.

A New Annexin A5 Conjugate

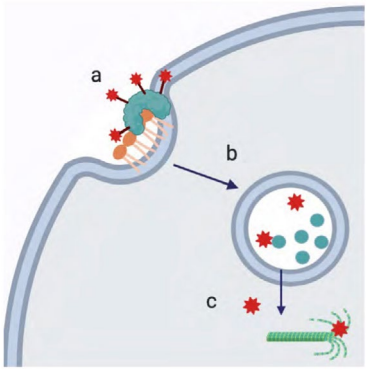
Professor Harrison and his colleagues decided to explore other ways to use the annexin A5 protein. The most aggressive type of breast cancer is triple-negative breast cancer (TNBC), characterised by high levels of phosphatidylserine on the tumour surface. The team built a new annexin A5 conjugate by linking this protein to the potent anticancer drug emtansine (DM1), creating the ANXA5-DM1 conjugate. As the annexin A5 binds to the phosphatidylserine on the tumour cell surfaces, the DM1 is delivered directly to them, resulting in cell death.

The validity of this approach was confirmed in *in vitro* lab testing, where the ANXA5-DM1 conjugate was found to be specifically toxic to the TNBC cells and capable of quickly initiating cell death. This toxicity was enhanced compared to just using the DM1 alone. Additionally, the researchers found that the conjugate did not show toxic effects against healthy breast cells. Spurred by these excellent results, Professor Harrison now plans to investigate the ANXA5-DM1 conjugate in animal models to determine optimal dosing as well as the effects of combining the treatment with immunotherapy.

The Next Steps

Professor Harrison highlights that the number of cancer cases around the world is growing, and that cancer is the single most critical barrier to improving life expectancy. Worldwide, 1 in 20 women are affected by breast cancer, with 10 to 20% of those being diagnosed with the aggressive form known as TNBC, which has a very poor prognosis. Currently, there is no cure for metastatic breast cancer, and treatment options are limited, compounded by a myriad of associated unpleasant side effects. There is a desperate need to find new treatments.

Professor Harrison and his team are making huge strides forward in developing novel therapies. They are now in the next phase of testing SWCNT-ANXA5 conjugate photothermal therapy, having received funding to investigate its use in combination with immune system stimulation for the treatment of breast cancer. At the same time, the ANXA5-DM1 conjugate offers another promising treatment for aggressive metastatic breast cancer as it moves into the next phase of *in vivo* testing. Together, these key advances bring fresh hopes for cancer patients worldwide..



ANXA5-DM1 mechanism of action. a) ANXA5-DM1 binds to phosphatidylserine (PS) expressing cancer cells. b) ANXA5-DM1 is internalized, and ANXA5 is broken down in the lysosome. c) Free DM1 diffuses out of the lysosome and causes mitotic catastrophe.

MEET THE RESEARCHER

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Professor Roger Harrison obtained his BS in Chemical Engineering from the University of Oklahoma in 1967. In 1968, he attended the University of Wisconsin-Madison, where he received his MS and, later, PhD in Chemical Engineering. He worked as a research engineer at three companies and, in 1988, was appointed Associate Professor of Chemical Engineering at the University of Oklahoma. Over his well-published career, he has held the role of Visiting Professor at Blaise Pascal University, the University Institute of Technology at the University of Limoges in France, and the School of Pharmacy at the University of Cairo, Egypt. In 2014, he became the Interim Director for the newly established School of Biomedical Engineering at the University of Oklahoma. He remains at the University of Oklahoma in the position of Professor of Chemical Engineering. In 2017, he was inducted into the College of Fellows of the American Institute of Medical and Biological Engineering, a select group of the top 2% of medical and biological engineering professionals, and in 2023, he was awarded the David Ross Boyd Professorship, one of the University's highest honours.

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FURTHER READING

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