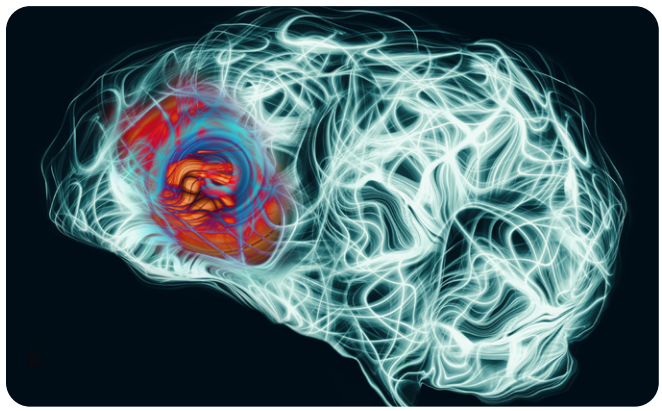
EXPERT INTERVIEW

Assessing nanomedicine delivery across the blood-brain barrier using pre-clinical *in vivo* imaging



The blood-brain barrier (BBB) presents a formidable obstacle for the effective delivery of therapeutics to the brain and consequently impedes effective treatment of brain cancers. In recent years, various novel techniques and approaches have been adopted to address this challenge, including the development and use of nanomedicines. However, interrogating the ability of nanomedicines to cross the BBB and retain in the tumor has proved difficult due to a lack of suitable models for study and limited understanding of the tumor-brain physiology during disease progression.

In a recent study,[1] a team of researchers based at the Centre for Advanced Imaging at the University of Queensland in Australia utilized various pre-clinical *in vivo* small animal imaging techniques to explore the uptake of custom-designed nanomedicines at different stages of brain cancer (Figure 1). By evaluating the retention of these nanomedicines and establishing a relationship between structure, tissue accumulation, and BBB leakiness, their work holds great potential for informing on the optimal timing of nanomedicine administration in a clinical setting in relapsed patients.

In this article, we hear from three of the study's authors-Professor Kris Thurecht, Dr. Zachary H. Houston, and Dr. Nick Fletcher – who discuss the rationale behind their approach and the personalized therapeutic potential of this methodology for patients with brain tumors in the future.

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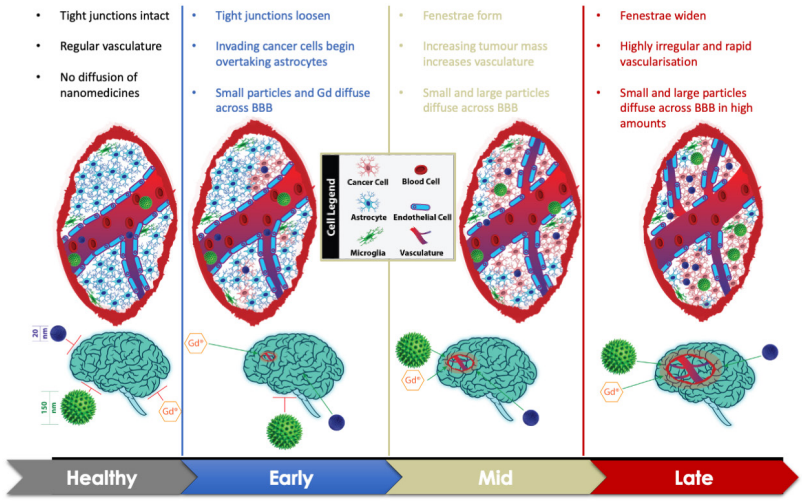

Figure 1: A schematic representation of brain tumor development utilizing an endogenous mouse model and illustration of the disease progression in the context of nanomedicine and BBB permeability, as well as cellular microenvironment. Blue particles are small nanoparticles (sNPs), and green particles are the big nanoparticles (bNP). Figure provided by Houston Z, et al.

The brain tumor challenge

Brain cancer presents unique treatment challenges for clinicians and remains one of the most difficult malignancies to treat. Prognosis is often poor for patients and for those diagnosed with glioblastoma, which is the most common and aggressive form; median survival is just 15 months. Treatment often includes surgical resection of the tumor mass, followed by radiotherapy and chemotherapy. However, around 90% of patients do not survive beyond 3-5 years.

One of the major obstacles limiting the efficacy of treatments for brain cancers is the BBB, though several novel drug delivery methods for treatment of primary brain tumors are being actively studied (Figure 2). The BBB is a highly selective and semi-permeable structure primarily formed of endothelial cells, and it serves to protect neural tissues in the brain by preventing diffusion of certain compounds from the blood, including potential therapeutics. In patients with brain cancer, the permeability of the BBB changes as the tumor progresses, allowing larger and previously excluded therapeutics to cross the barrier. This 'leakiness' is triggered by not only physical disruptions of a growing mass, but also alterations in various signaling pathways, which cause the normally tight junctions between cells to expand. Most efforts in this area of research have focused on exploiting the leaky BBB, with the aim to enable more accurate brain tumor diagnosis and develop personalized, effective treatments dependent on these progression markers.

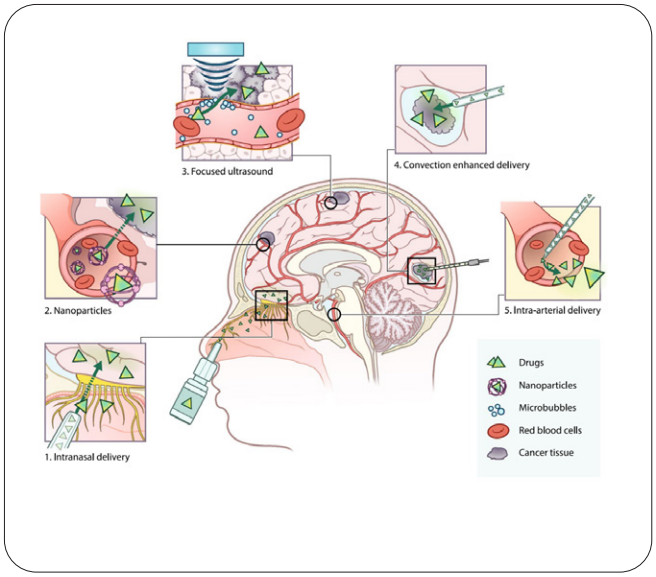

Figure 2: Representative overview of recent drug delivery methods for targeting primary tumors, some of which relies on the disruption/weakening of the selectivity of the BBB. Taken from Figure 1 of Overview of Current Drug Delivery Methods Across the Blood-Brain Barrier for the Treatment of Primary Brain Tumors[2]; additional figure details can be found in the original figure legend of https://doi.org/10.1007/s40263-020-00766-w.

Nanomedicines offer promise for targeted drug delivery

The field of nanomedicine has rapidly been gaining attention for its use in diagnosis, prevention, and treatment of various cancers. What makes nanomedicines attractive to many researchers is their ability to be engineered to target specific receptors and labeled with dyes or radiopharmaceuticals for imaging and quantitative assessment of drug delivery and tumor progression (Figure 3). "One of the great things about nanomedicines is the flexibility in their design," enthused Dr. Houston, who dedicates much of his time to fine tuning novel nanomaterials for enhanced therapeutic efficacy. "You can pick and choose what you want to put on them to modulate their properties and behavior." The ability of nanoparticles to cross the BBB also makes them an attractive approach for targeted delivery of drugs to brain tumors. However, ensuring the medicine not only crosses the BBB but also penetrates through the breadth of the tumor tissue can be challenging.

To streamline their design and development, screening, and initial applicability of their nanomedicines, their lab employs optical imaging of fluorescently labeled materials to initially qualify the effectiveness and biodistribution of their candidate molecules and their subtle iterations. This provides a rapid route for design-optimization, matching the physicochemical properties of the nanomaterial to their behavior *in vivo*. Once their potential therapeutic or imaging moiety has reached a desired performance, which could be leveraged to assess a possible biomarker, imaging modalities such as PET and MRI imaging come into play where the nanomaterial behaviors can be interrogated more rigorously in complex animal models.

Prof. Thurecht's team used pre-clinical *in vivo* imaging to assess the effects of custom-designed nanomedicines at different stages of brain cancer in a spontaneous murine model of glioblastoma. "We want to understand the tumor biology and how the materials that we were making interacted with different sub environments of the tumor," explained Dr. Houston, adding that it was previously unclear whether nanomaterial accumulation was influenced by the volume of the tumor alone or the leakiness of the tumor environment.

The genetically engineered mouse model used for this study was designed to form spontaneous tumors, allowing the team to study the BBB and brain tumors at various stages during the progression of the disease. "With our collaborators at the Queensland Brain Institute, we designed the models specifically to be more like the human or clinical disease so we could answer questions about the physicochemical

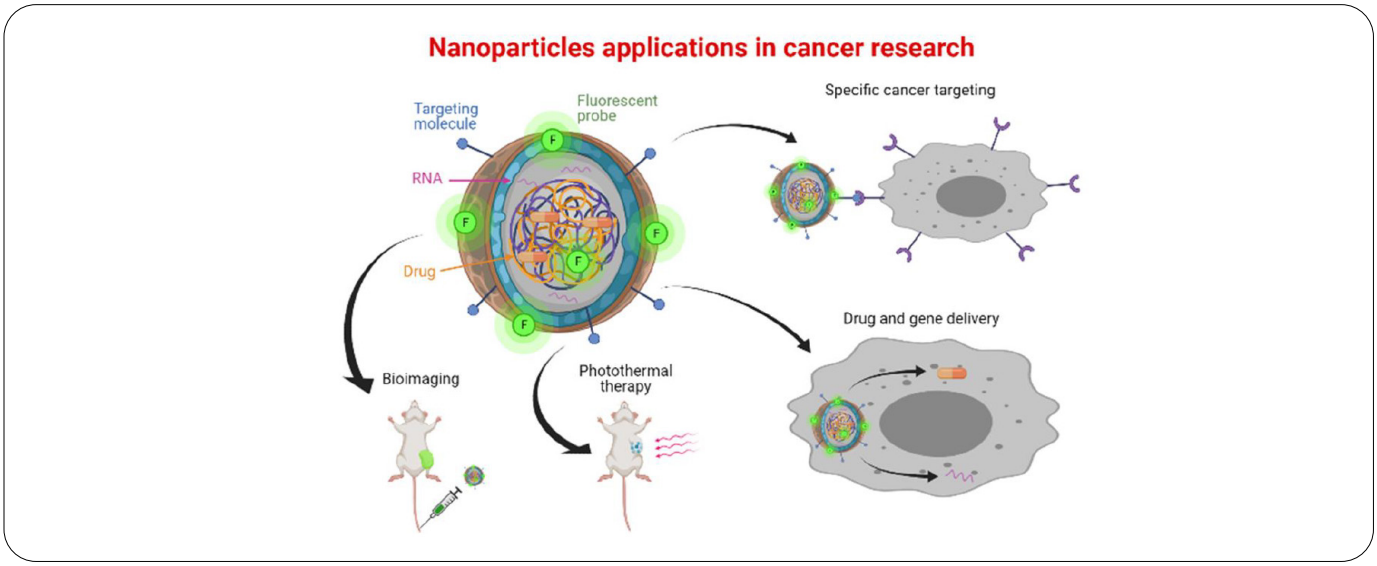

Figure 3: Overview of nanoparticles (NPs), broad selection of functionalization, and their applications in cancer research. Taken from Figure 4 of Smart nanoparticles in biomedicine: an overview of recent developments and applications[3]; additional figure details can be found in the original figure legend of https://doi.org/10.20944/preprints202102.0619.v1 (pre-print, ahead of review).

qualities of our potential medicines," explained Prof. Thurecht. "Our model allows the tumors to develop without us artificially interfering with the BBB, offering unique insight into how the evolving biology within the brain during tumor growth affects our treatment efficacy."

The mice were scanned with MRI before and over the course of brain tumor development to identify the tumor and measure its volume at different stages of tumor maturation. The degree of tumor-associated leakiness was also determined by monitoring the dynamic uptake of Gadovist, a small molecule gadolinium chelate that does not cross a healthy BBB, into the tumor tissue using T1-weighted imaging. The team used quantitative PET imaging of small (20 nm) and large (100 nm) nanoparticles to probe the effect of nanomaterial size on their ability to cross the BBB and accumulate within the tumor. Both nanomedicines were labeled with a radioisotope (64Cu) tag for visualization and conjugated with a bispecific antibody (BsAb) with dual affinity for ephrin type A receptor-2 (EphA2), a protein which is highly expressed in most human and murine glioblastomas, as well as polyethylene glycol (PEG) to decorate the surface of the low-fouling PEG based materials and generate a targeted construct.

Commenting on their approach, Dr. Houston said: "The standard way to look at a tumor in the clinic is to measure its largest diameter in any plane, but that doesn't provide any indication of what is going on in terms of tumor pathophysiology. We therefore also looked at the leakiness of the tumor using PET with and without targeting vectors to gain more insight into the potential influence of the tumor physiology."

The 'Leakiness' factor

One of the key findings of the study was that the accumulation of nanomedicines in brain tumor tissue was better correlated with the leakiness of the BBB than actual tumor volume (Figure 4). "We initially thought that by looking at volume, the bigger the tumor, the leakier it is, and the better it would be for our nanomedicine," explained Prof. Thurecht. "Instead, we found that volume was a poor measure of predicting how a nanomedicine might accumulate in tumors." This surprising finding suggested to the researchers that a smaller tumor with a higher degree of leakiness might show a higher uptake of nanomedicines than a larger one.

The researchers then used a ranking system for tumors based on their leakiness to examine the influence of nanomedicine size on tumor accumulation. "As you might expect, we observed that larger and smaller particles had different time windows relating to the development of the tumor and when they get across," said Dr. Houston. Specifically, the team observed that smaller nanomedicines typically crossed the BBB and accumulated at early stages of tumor development, while larger particles accumulated much later. "The major output from this finding is that these nanomaterials could potentially be used for treatment at a very particular time window," noted Dr. Houston. As the imaging methodology used in their pre-clinical study can be applied to clinical routines, the researchers say their work holds potential to inform on the optimal timing of nanomedicine administration in a clinical setting in relapse treatment in a personalized manner.

The challenge of spontaneity

The current study presented several challenges for Prof. Thurecht's team, including the spontaneous and erratic nature of the murine model. "The fact that they in many ways mimic the clinic is good because it has translatability and allows us to answer complex questions about our delivery system, but awful because they are just as spontaneous and

irreproducible," said Dr. Fletcher. "They also grow at different rates – some take 20 days while others take 120; some might only grow a little and then just stop. That was a massive limitation for us to get enough power in the statistics to draw meaningful conclusions from some aspects of the study."

Following the success of their work, the researchers have moved into comparative studies on canines, which, like humans, develop brain tumors spontaneously. Prof. Thurecht explained that they were able to effectively use, test, and evaluate their nanomedicines on these models as there are no side effects for the dogs. "Moving from mice to canines is the middle step before humans," he said. "However, not only are there similarities between the two models but there are also differences. When we studied the canines, we observed certain behaviors that were not the same as in the mice, and that is all part of understanding the process." Interestingly, the team found that the biodistribution of the nanomedicines was far more favorable in the dog than it was with the exact same material in the mouse model. "We're still trying to understand that, but it makes us more confident and happier moving to human clinical evaluation," said Prof. Thurecht.

The team are now in the process of preparing for a human clinical trial which they hope will start early 2022 in glioma patients. "This is the stage where we are able to evaluate and really understand how these materials behave in real patients in a clinical setting and infer what the benefits to the patients are going to be."

Access to technologies and instrumentation

Prof. Thurecht noted that they were lucky enough to have access to instrumentation that lends itself well to investigating diseases of the brain. "Having the infrastructure and technologies allowed us to investigate these questions around compromised conditions of the BBB," he said. "We have always pushed for imaging and imaging technologies, particularly because of the amount of information it can give us in experiments. In my opinion, running complementary imaging and diagnostics along with therapeutic studies is now key." The team also leverages significant collaborative engagement with both academics and industry. Their research forms part of a major national consortium under the umbrella of the Australian Research Council Centre of Excellence in Convergent BioNano Science and Technology, bringing together scientists across the globe to address key challenges in bio-nano science. Training of new scientists in the area of imaging is also enabled by the Australian Research Council Training Centre for Innovation in Biomedical Imaging Technologies, where industry-led training programs drive innovation in pharmaceutical science. These two initiatives provide a unique pipeline of both capability and capacity to translate lab-scale discoveries through to pre-clinical and clinical assessment of new therapeutics.

References

1. Houston Z, Bunt J, Chen K, Puttick S, Howard C, Fletcher N et al. Understanding the Uptake of Nanomedicines at Different Stages of Brain Cancer Using a Modular Nanocarrier Platform and Precision Bispecific Antibodies. *ACS Central Science*. 2020;6(5):727-738.

2. Haumann R, Videira JC, Kaspers GJL, van Vuurden DG, Hulleman E. Overview of Current Drug Delivery Methods Across the Blood-Brain Barrier for the Treatment of Primary Brain Tumors. *CNS Drugs*. 2020;34(11):1121-1131.

3. Campora, S.; Ghersi, G. Smart Nanoparticles in Biomedicine: An Overview of Recent Developments and Applications. Preprints 2021, 2021020619. (viewed on Nov 22, 2021).

About the researchers...

Professor Kristofer Thurecht

Prof. Thurecht is Deputy Director and a senior group leader within the Centre for Advanced Imaging (CAI) and a senior group leader at the Australian Institute for Bioengineering and Nanotechnology (AIBN) at the University of Queensland where he currently holds a National Health and Medical Research Council fellowship. His research focuses on developing improved understanding of the nano-bio interface, particularly using molecular imaging tools to address some of the complex questions in this field. His team works across the boundaries of chemistry and materials, biology, and imaging science to probe how nanomaterial properties affect their function in living animals. He is a CI in the ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, and theme leader in the ARC Training Centre for Innovation in Biomedical Imaging and Technology.

Dr. Zachary H. Houston

Dr. Houston is a Postdoctoral Fellow at the Centre for Advanced Imaging (CAI) at the University of Queensland (UQ). Dr. Houston is highly experienced in organic chemistry, nanomaterial design, radiochemistry, and *in vivo* molecular imaging (fluorescence, luminescence, PET-CT, MRI, and PET-MRI) in small animal tumor models. In pioneering work, Dr. Houston established the ability to track nanomedicine efficiency in permeating the blood-brain barrier in a spontaneous murine tumor model through the use of PET-MRI that has resulted in significant progress towards clinical translation. Dr. Houston was the lead coordinator for a first-in-the-world Phase 0 clinical study in a comparative oncology program where a personalized treatment using nanocarriers and custom bespoke targeting technology was used to treat canines with prostate cancer. Additionally, Dr. Houston will lead a Phase 1 clinical trial in humans in 2022 to assess if the applicability of nanomedicines for the treatment of brain cancer.

Dr. Nick Fletcher

Dr. Fletcher is a Postdoctoral Research Fellow in the Thurecht Group working across the Centre for Advanced Imaging and the Australian Institute for Bioengineering and Nanotechnology at the University of Queensland. His work ranges from the design and functionalization of nanomaterials as probes or delivery vehicles, through to the study of material behaviors in complex biological environments. This work is particularly focused on both understanding bio-nano interactions at previously unexplored scales, as well as developing approaches to modulate and control these interactions to improve nanomedicines pharmacological behaviors and outcomes. He has spearheaded the development of pre-clinical molecular imaging approaches for polymeric nanomedicines, as well as developed complementary research programs in pharmacological approaches to modulate their behaviors. He is now focusing his work on transitioning this research program towards novel radiotherapeutic nanomedicines in the newly established ACRF Facility for Targeted Radiometals in Cancer.

The University of Queensland

The University of Queensland (UQ) is one of Australia's leading research and teaching institutions, striving for excellence through the creation, preservation, transfer, and application of knowledge. Within UQ, the Centre for Advanced Imaging (CAI) reflects UQ's vision to lead technological innovations in biotechnology and biomedical research requiring spectroscopic and imaging research capabilities. It brings together the skills of a critical mass of researchers and 'state-of-the-art' research instruments to address health challenges of the future.

Imaging and spectroscopic techniques are key platform research technologies for studying the structure and function of living organisms in health and disease and facilitating drug discovery and validation. Together they speed translation of scientific discoveries to clinical realization, enabling the goal of personalized medicine by better characterizing disease and response to treatment in the individual patient. The University of Queensland's Australian Institute for Bioengineering and Nanotechnology (AIBN) is an integrated multi-disciplinary research institute bringing together the skills of world-class researchers in the areas of bioengineering and nanotechnology. AIBN seeks to deliver innovative solutions to society's problems through sustainable materials, healthy living, and translational success.

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