From Lab to Life: Nanobodies as Biomarkers for Cancer

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*In the ongoing fight against cancer, one truth stands paramount: monitoring the progression of the disease is essential for patients and healthcare providers alike. The ongoing **FABXS** project opens up the opportunity to revolutionise cancer diagnostics through cost-effective and ethical methods.*

Led by Prof. Therese Hunter as the principal investigator, the FABXS team is unmasking hidden clues about cancer cells through proteins. The FABXS team is composed of a UM team that includes Prof. Gary J. Hunter, postdoctoral researcher Dr Brandon Charles Seychell, and the Protein Lab team from the Department of Physiology and Biochemistry in partnership with the SAMOC team led by Dr Nick Refalo. The researchers' primary focus lies in producing and characterising the structure and function of recombinant proteins. This work allows them to follow a protein's path and function in our body.

The researchers' deep understanding of how mutations affect protein function is crucial, especially in the context of cancer. Mutations can lead to significant changes in disease progression, altering how proteins behave within the body. This knowledge is pivotal in bridging the gap between basic protein research and practical applications in cancer diagnostics. This expertise forms the foundation for their ambitious project: developing a cutting-edge diagnostic kit for cancer biomarkers.

WHAT ARE BIOMARKERS?

Playing a central role in the researchers' work, biomarkers are measurable indicators of biological processes that offer invaluable insights into a patient's response to cancer or treatment. When cancer develops, it can cause certain proteins to be produced in higher or lower quantities than normal. These proteins can be used as biomarkers that indicate the presence of cancer cells. In oncology, these biomarkers can serve multiple vital roles: predicting disease progression, indicating remission, or even signalling potential chemoresistance – when a patient becomes unresponsive to chemotherapy.

By measuring these protein levels in tissue samples or blood, doctors can gain valuable insights into the presence, progression, or response to cancer treatment. Since protein levels can be measured, T. Hunter and Seychell have set out to produce and use nanobodies to detect four biomarkers. Nanobodies are protein molecules that act similarly to antibodies, whereby they recognise and specifically bind to the biomarker proteins and thus function as sensors for these biomarkers.

Effective biomarker monitoring is pivotal; it empowers healthcare professionals to make informed decisions about patient care. However, the cost and limitations of existing diagnostic kits can

Chromatography machine used for protein purification
Photo courtesy of the FABXS Team

hinder access, especially for those in underprivileged communities. By leveraging advances in molecular biology and ethical production methods, the team aims to provide healthcare professionals with powerful tools for monitoring disease progression effectively.

A CRUELTY-FREE ALTERNATIVE: NANOBODIES

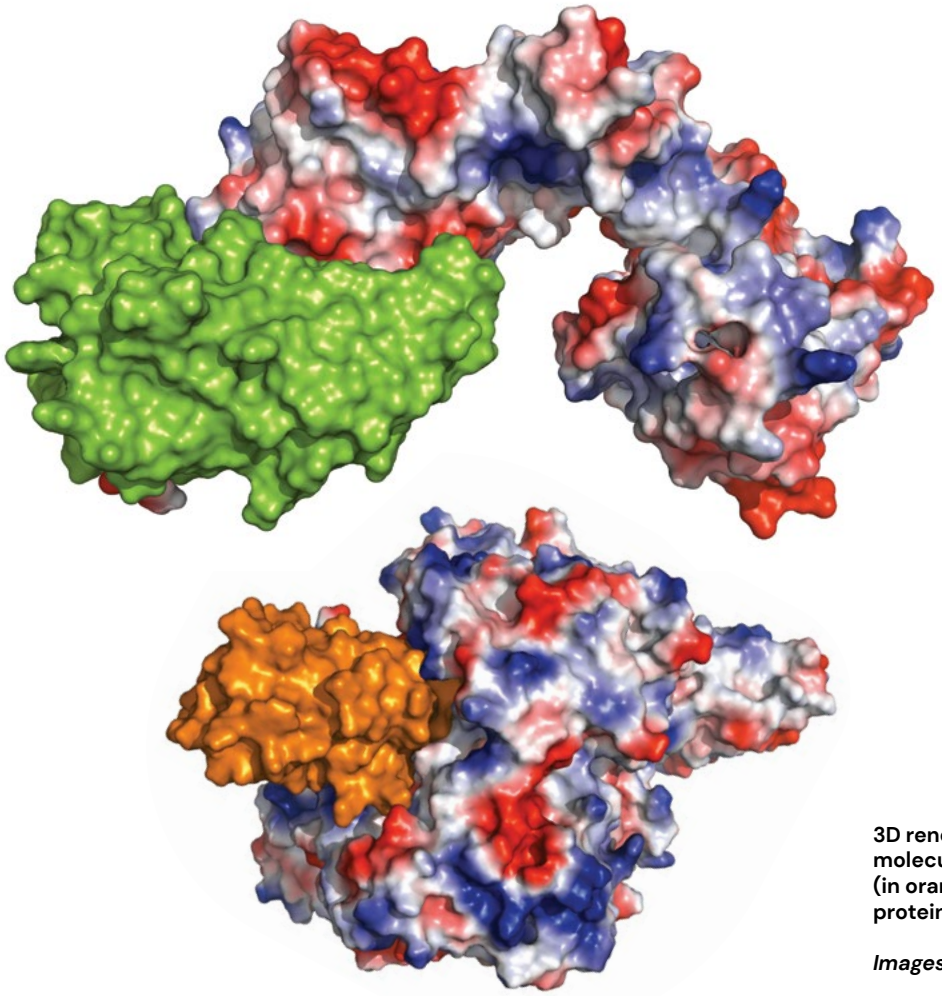
Traditionally, antibodies used in diagnostics are produced by injecting an antigen into animals like mice or rabbits. Antigens are molecules or structures that the immune system recognises as foreign or potentially harmful. By injecting an animal with an antigen, its immune system is triggered to produce antibodies against the antigen, which are then harvested from their serum. However, this method has several drawbacks: animal-derived antibodies can be unstable and costly. Moreover, since animals are euthanised after antibody harvesting, new antibodies would need to be harvested from a new animal, resulting in batch-to-batch differences. As T. Hunter explains, every time new antibodies are needed, the entire process must be repeated, leading to variability in results.

The FABXS project seeks to address these issues by utilising 'recombinant antibodies', specifically nanobodies – small single-domain proteins that can bind to specific antigens or target proteins with high affinity. The researchers have prioritised ethical considerations by ensuring that the process employed to produce these nanobodies does not involve animals.

The benefits of using nanobodies extend beyond ethical concerns; they also offer improved reproducibility. A current issue of animal-derived antibodies is that while they can be reproduced, it does not mean that all the reproduced antibodies are identical. Think of it like different solutions for the same answer. The animal's immune system will produce an antibody to bind to the triggering antigen; however, they might bind in different positions. Alternatively, once you have the map to produce the right nanobody for the right antigen, this would allow you to produce identical clones. This is extremely important for ensuring consistency and reproducibility across experiments and is critical for large-scale production and reliable diagnostic results.

THE JOURNEY OF NANOBODY PRODUCTION

Protein biomarkers were carefully chosen based on their established roles in oncology. One protein



3D renderings showing in silico molecular models of nanobodies (in orange and green) bound to protein biomarkers

Images courtesy of the FABXS Team

has been recorded in over 60% of cancers, and its different forms in cancer cells are linked to the spread of cancer. The proteins chosen are not solely biomarkers for use in oncology but also act as markers for other conditions, such as high blood pressure, heart failure, liver disease, and chronic inflammation.

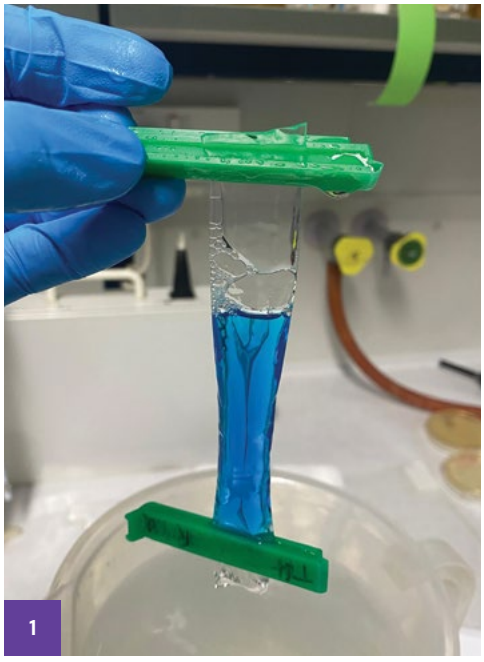
Building upon Seychell's Ph.D. research and other research conducted in UM's Protein Lab, the proteins were synthesised recombinantly in a bacterial host, purified and characterised, and sent to a partner company specialising in nanobody production. The large number of nanobodies generated in this manner then had to be analysed at the University of Malta. This step was very labour-intensive, as it involved the recombinant production of many nanobody proteins to assess which were viable by testing their solubility, stability, and degree of binding to the biomarker of interest. Seychell highlights that this method allows for reproducibility as long as consistent growth conditions are maintained – again highlighting the stark contrast to the variability seen with animal-derived antibodies.

WILL THE DETECTION SYSTEM WORK?

A significant challenge in developing a reliable detection system is ensuring that the binding of the protein actually occurs at the intended site on the antigen. To tackle this issue, the researchers needed to devise a method for labelling each nanobody with a fluorescent marker. Fluorescent markers function by attaching to the intended molecules (in this case, proteins) and emitting a fluorescent light when exposed to light of a particular wavelength. This fluorescence enables the detection and measurement of signal intensity, providing information about the amount of the biomarker of interest present in the sample.

To validate their detection system, Seychell explains that they employ a technique known as Fluorescent-Linked Immunosorbent Assay. By using known concentrations of nanobodies and biomarkers, they create a standard curve that helps determine the limits of detection.

With nanobody production underway and fluorescent tags in place, the final phase involves testing these components on biological samples – specifically patient



1. **Preliminary fluorescent protein used for detection**
2. **Microtitre plate reader used for fluorescence detection of the FABXS nanobodies**
3. **Microtitre plate prepared with a serial dilution setup**

Photos courtesy of the FABXS Team

blood samples – to assess their effectiveness in real-world scenarios.

REDEFINING THE FUTURE OF CANCER PATIENTS

Effective biomarker monitoring is pivotal in oncology; it empowers healthcare professionals to make informed decisions about patient care. Biomarkers serve multiple vital roles: predicting disease progression, indicating remission or relapse, and signalling potential chemoresistance. The FABXS project is the start of a journey not only toward sensitive and affordable diagnostic kits but also toward improved patient outcomes through timely interventions and tailored treatments based on accurate biomarker detection.

The potential applications of nanobodies extend beyond cancer diagnostics; they hold promise for various research fields requiring protein detection. For instance, traditional antibodies can be prohibitively expensive, costing hundreds of euros for just 100 micrograms, while laboratory-produced nanobodies can yield significantly larger quantities at a fraction of the cost. This cost-effective approach could democratise access to essential diagnostic tools, particularly benefiting underprivileged communities where access to healthcare resources may be limited.

T. Hunter and Seychell emphasise that this project represents an important first step. As T. Hunter says: 'I'd look at this project as the first stage for providing the tools that will validate the use of these nanobodies.' By ensuring that these innovative solutions are both effective and accessible, the team hopes to contribute toward enhancing the quality of life for cancer patients across Europe.

The collaborative effort between UM researchers and clinical partners at Sir Anthony Mamo Oncology Centre exemplifies how interdisciplinary approaches can lead to meaningful innovations in healthcare. With continued support and development, initiatives such as the FABXS project have the potential to not only transform cancer diagnostics but also pave the way for more equitable healthcare solutions worldwide.

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