Associate Professor Richard Tothill, Professor Sarah-Jane Dawson, and Professor Linda Mileshkin received a grant to develop a liquid biopsy test for CUP diagnosis. Photo by Wayne Taylor

On a mission to help oncologists diagnose a mysterious, aggressive cancer

Collaborators at the University of Melbourne and Peter MacCallum Cancer Centre are developing a liquid biopsy test for cancer of unknown primary

CANCERS ARE OFTEN NAMED for the organs in which they first appear. If cancer originates in the lungs, we call it lung cancer, even if it later spreads to other organs. But what if we detect cancer only after it has spread, and can't tell where it came from?

Cancer of unknown primary, or CUP, is diagnosed when cancer cells are discovered that are not typical of cancer cells usually found in that region of the body: They've spread from somewhere else. "There's nothing to associate them with," says Richard Tothill, an associate professor at the University of Melbourne and head of its Rare Disease Oncogenomics group. He explains that this general lack of awareness translates to less advocacy, funding, research, care, and support. "The patients fall through the gaps as well, in terms of medical care. The clinical problem is that we need to know where primary cancer arose in the body to direct an effective treatment." In other words, breast cancer patients respond better to breast cancer drugs, so it's important to resolve a cancer diagnosis.

Usually, when a cancer spreads from one organ or site to another, it retains many features particular to cancer cells from that site. However, CUP cancer cells seemingly don't point to any particular body part—or the evidence is contradictory and leads to uncertainty in diagnosis. Because of this, some researchers once considered CUP a separate kind of disease from cancer. Tothill's team thinks CUP is a collection of different cancer types that are atypical for their sites of origin—for example, a lung cancer with rare biomarkers—and this combination eludes diagnosis. "You could imagine that CUP is almost a human construct, as it's our failure to diagnose rather than being something unique," Tothill says.

Diagnosing CUP is difficult and time-consuming, and it moves fast. Because it's so aggressive, CUP patients have poor outcomes. In Australia from 2015 to 2019, just 20.6% of CUP patients were still living one year after receiving a diagnosis, according to the Australian Institute of Health and Welfare.[1]

Using genomics to solve the puzzle

Tothill and team are developing genomic technologies and tests to help clinicians identify where CUP's aggressive cells originally come from. Using

1. aihw.gov.au/reports/cancer/cancer-data-in-australia/contents/survival

whole-genome sequencing (WGS) in addition to imaging, pathology, and other approaches, Tothill believes they can resolve about two-thirds of these mysterious cases with comprehensive whole-genome sequencing. Impressively, they've been sequencing CUP patients using different genomic tests for an entire decade, in an Australian national cohort study called Solving Unknown Primary CancER (SUPER), which was originally founded at the Peter MacCallum Cancer Centre in Melbourne by longtime collaborator and medical oncologist Professor Linda Mileshkin.

Over the last 10 years, the SUPER study found that it takes about 57 days on average between when a patient has a scan suggesting they may have secondary cancer and when they are given a diagnosis of CUP because further investigations cannot find a primary cancer. After that, a physician may initiate genomic testing, which can take up to six more weeks to return results. "These patients have a really long, arduous diagnostic odyssey," Mileshkin says, "and that causes a lot of psychological distress for the patient and their family. The clinicians often have to make an educated guess about what treatment is best to prescribe."

The usual route is an empiric chemotherapy, which covers a range of possible tumor types, though many CUP patients don't get significant or long-lasting tumor shrinkage with it. Mileshkin points to evidence from a recent international clinical trial called CUPISCO[2] that she presented on behalf of the study team at the European Society for Medical Oncology (ESMO) Annual Meeting in 2023, showing that CUP patients fare better when they can access a targeted drug or immunotherapy, guided by the results of genomic sequencing of their tumor, as part of their first-line treatment.

One of the challenges of sequencing patient samples is the tissue itself. Sometimes there is no tissue available because prior tests have used it all up, and when there is tissue left over, pathologists have already treated it with a fixative called formalin to enhance their microscopic imaging.

Tothill and Mileshkin's current study, SUPER-NEXT, has been performing WGS using tissue biopsies since 2020 and is now developing a blood test in collaboration with clinician-scientist Professor Sarah-Jane Dawson at the Peter MacCallum Cancer Centre (Peter Mac),[3] who is a world expert in cell-free DNA.

Cell-free DNA is DNA that has been released from tumor cells into the blood. Fortunately, CUP patients typically have a higher percentage of this "cfDNA," which means doctors are more likely to get a result. Tothill estimates that 80% of CUP patients have a good amount of cfDNA.

In addition to cfDNA directing patients to a targeted therapy, it can also provide information about where the primary cancer came from. To find the origin, the team's approach is to take two kinds of information from the DNA. They look at changes in the code—the mutations—as well as a chemical modification called DNA methylation, both of which can help identify the cancer type or the primary cancer diagnosis.

If Tothill's team can combine or cross-reference the two types of information—the mutations and the methylation—machine learning could one day predict where the cancer came from. He explains: "Our overall goal is to develop a rapid liquid biopsy blood test for patients with CUP, and to try to apply that much earlier in their diagnostic journey to save them that arduous workup—to get a result quicker, and get patients onto the best trials for them. And of course, we'd probably be saving the health care system some money along the way."

Development of the cell-free DNA CUP test is supported by The Advanced Genomics Collaboration (TAGC), a partnership between the University of Melbourne and Illumina. Invest Victoria, Illumina, and the University of Melbourne established TAGC to advance biomedical research translation, expand access to state-of-the-art genomic testing and treatments, and create the necessary infrastructure and jobs. It is underpinned by three platforms in clinical genomics, bioinformatics, and health economics. (The unrelated Allied Genetics Conference, organized by the Genetics Society of America, also uses the abbreviation TAGC.)

The team is utilizing a significant number of patient samples collected over many years from SUPER studies. "It's an absolutely amazing resource," Tothill says. "Because we've already done DNA sequencing on the tissue for many of the patients, we can benchmark the cell-free DNA test against the genomics of the tissue for these patients."

They aim to sequence 300 more patient samples over the next two years, and by the end, they hope to have a test that is ready for initial testing in the clinic. They want to show clinical utility, speed up the diagnosis, get patients to treatments earlier, and improve outcomes.

All team members are making important contributions,

2. annalsofoncology.org/article/S0923-7534(23)04150-9/fulltext
3. petermac.org

since developing the technology and carrying out the study requires a variety of expertise. Tothill names scores of people in oncology, mathematics, and bioinformatics, including Hartwig Medical Foundation-Australia[4] managing director Peter Priestley, who developed a tool called CUPPA (CUP Prediction Algorithm) that they use in WGS. Priestley will help in developing it for liquid biopsy as well.

Tothill is excited about the winning combination of the team, the samples, the technology, and the support from TAGC.

In order to solve CUP, a variety of global organizations have come together—cancer centers, medical foundations, universities, and the World CUP Alliance,[5] which engages experts across the globe to bring together leading research. The group first organized World CUP Awareness Week in September 2021, but officially launched as an entity at ESMO 2023 and finally secured a dedicated CUP track for the upcoming ESMO 2024.

Barriers to treatment

In addition to solving CUP diagnoses through genomics, the SUPER-NEXT team is also tackling some of the practical aspects the disease entails for current patients and their families. "A lot of money goes into support and care for different cancer types, and nurses who will support particular patients with particular diseases," Tothill says. "But CUP education and patient support, again, often fall through the cracks."

In 2012, Mileshkin started Australia's first CUP Clinic to provide patients with the best possible workup, potential clinical trial enrollment, and other support services, such as support by nurses and psychologists. Patients who walk into the CUP Clinic at Peter Mac have access to all of this, and patients living some distance away can be seen by telehealth as well. The team is also working with collaborators at other hospitals to try to improve care for CUP patients nationwide.

Another issue CUP patients face is getting access to treatment. "There's a real drug access problem," Mileshkin says. Immunotherapy and some targeted therapies can work well for CUP patients if genomic sequencing suggests that a specific treatment be used, but Australia's public health care system doesn't always cover those drugs, as they are generally only funded for specific types of cancer, such as lung cancer. Furthermore, clinical trials are often cancer type specific, which leaves out CUP patients.

And while most Australians live in metropolitan areas, those living in rural areas, such as indigenous communities, have even less access to any kind of treatment or care. Generally, health services can be quite limited in remote regions, so the fairly specialized process of getting a tissue biopsy compounds in difficulty. This is another reason Tothill is excited about developing a test that requires only a simple blood draw—it can be done at remote sites.

Tothill and his team see all aspects of CUP. He feels fortunate to have been working on the disease for two decades, and to have seen the evolution of various technologies and approaches for it: "I feel like I'm in this spot right now where I know exactly what works and what doesn't. I can see the gaps in the clinical need and the practicalities of getting tests to patients and turning things around. I'm excited about the potential of the test to be able to effect change for these patients.

"There's a lot of hype around liquid biopsies at the moment, and it's great to be a part of that and to push the boundaries with new technologies," he continues. "I'm in the best place: to be collaborating with Peter Mac—the leading cancer hospital in the country—working with TAGC and the platforms with Illumina's support, and being involved with the World CUP Alliance. It's pretty amazing that this is all coming together." ◆

To read about how whole-genome sequencing is bringing hope to CUP patients in the Netherlands, go to illumina.com/company/news-center/feature-articles/shining-a-light-on-cancer-of-unknown-primary.html

4. hartwigmedicalfoundation.nl/en/partnerships/hartwig-medical-foundation-australia
5. worldcupawareness.org