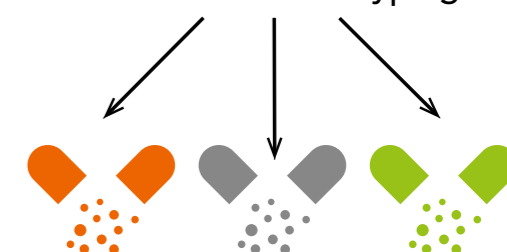




HIV Patients



PANDAA
Resistance Genotyping



Effective ARVs

Overall, PANDAA technology offers a cost-effective, simpler and quicker process compared to other genetic sequencing technologies ”

HIV to others. Furthermore, prescribing ineffective medication does not make economic sense.

Clearly, drug resistance testing plays a critical role in HIV patient care management: if resistance was consistently monitored and identified early, patients could be more readily switched to effective ARVs and have better health outcomes.

ALDATU BIOSCIENCES: A PUBLIC BENEFIT MISSION

The main aim of Aldatu Biosciences (founded by Dr David Raiser and Dr Iain MacLeod in 2014) is to increase access to drug resistance testing through the use of their ground-breaking technology, PANDAA (Pan Degenerate Amplification and Adaptation).

As a result of his experiences working at the Botswana Harvard AIDS Institute and witnessing first-hand the need for better drug resistance testing options, Dr MacLeod was inspired to design PANDAA – a simple, sensitive, and inexpensive way to detect drug-resistant HIV. Aldatu Biosciences' first application of the technology to a specific disease, HIV, led to the development of their lead product PANDAA HIV6. Although developing countries have the highest HIV rates, they often lack the funds and

resources needed to deploy comprehensive HIV management strategies that include drug resistance testing. Local laboratories dedicated to HIV testing are over-burdened and struggle to cope with demand. A simple, affordable solution for HIV drug resistance testing is badly needed in these settings, and PANDAA HIV6 could be the answer.

PANDAA – AN ENABLING TECHNOLOGY

Typically speaking, the number of diagnostic techniques that utilise quantitative real-time polymerase chain reaction (qPCR) molecular probes has been unrivalled in modern genomics. However, with highly polymorphic pathogens, such as HIV, traditional qPCR's greatest strength, its sensitivity and specificity, becomes a weakness. Traditional qPCR cannot tolerate changes in the probe binding sequence, which commonly present at regions near resistance-conferring mutations within the HIV genome. This is referred to as “secondary sequence variation”. As a result, probes designed to detect resistance mutations are unable to bind, resulting in high false negative rates during experimentation – the results show no resistance although resistance is present. So, in other words, HIV's high genomic variability has limited the development of molecular probes for HIV drug resistance genotyping.

The power of PANDAA: enabling resistance testing for better HIV care

Dr Iain MacLeod, along with **Dr David Raiser**, from Aldatu Biosciences, discuss how the use of their innovative PANDAA technology could facilitate drug resistance testing in HIV patients, transforming the quality of patient care, especially in developing countries.

The war against Human Immunodeficiency Virus (HIV) is a global struggle. Worryingly, over 74 countries, particularly South Africa, have experienced an increased rate of new HIV infections between 2005 and 2015.

Despite this grim statistic, more HIV patients than ever are receiving antiretroviral drug (ARV) therapy. These drugs suppress the HIV life cycle, not only ensuring that patients live longer and healthier lives, but also minimising further HIV transmission. By the end of 2015, 18 million of the 38 million people worldwide suffering from HIV had access to ARV treatment.

HIV RESISTANCE AGAINST ARVS

However, HIV is fighting back. Different strains have developed resistance to specific ARV drugs, rendering those drugs ineffective as treatment. Annually, ARV treatment failure occurs in 10% of patients on average, which has severe consequences when allowed to persist: patients continue to suffer from this debilitating disease until alternative medication is administered. Drug resistance testing is necessary to identify effective alternative ARVs, but these tests are simply too expensive for most developing countries. Without resistance testing, patients can remain on ineffective ARVs for up to 18 months, during which time they are also more likely to transmit their



Above: PANDAA testing in action

Right: Drs Iain MacLeod and David Raiser in the Aldatu lab

This is where PANDAA technology comes in. Using proprietary reagent design techniques, PANDAA maintains the superior sensitivity of qPCR without losing any of its specificity. The primers it uses include special design features that strip out secondary sequence variation in the probe binding sequence, while leaving the drug resistance information intact. Not only that, but by harnessing an existing low-cost technology (qPCR), PANDAA provides a technique for HIV drug resistance genotyping that is affordable where it is most needed.

PANDAA has been specifically designed to meet the clinical and economic needs of healthcare settings with limited resources. The technology provides a vital advancement in HIV drug resistance diagnostics that can radically improve HIV patient care delivery and management in lower- and middle-income countries.

ADVANTAGES OF PANDAA OVER CURRENT TECHNOLOGY

Current technology used for drug resistance testing involves collecting long sequences of the HIV genome, using methods such as Sanger or Next Generation Sequencing. However, only around 1% of the sequenced



genome is of clinical importance i.e., provides information on the resistance level of the HIV strain. PANDAA-enabled qPCR allows for a more efficient approach called 'focused genotyping', wherein only those genomic positions that are relevant to a particular therapeutic decision are queried in a given test. For example, PANDAA HIV6 collects information on just six different mutation positions in the HIV genome, and in doing so, it can detect resistance in more than 99% of HIV-infected patients in Africa who are failing their WHO-recommended first line ARV therapy regimen. In essence, focused genotyping allows for simplified and cost-efficient testing product design, which has been employed for PANDAA HIV6.

Current Sanger sequencing-based methods used to detect the presence of drug-resistant variants have a relatively low sensitivity at 80–85%. As such, they are only able to detect variants if they are present within 15–20% or more of the total virus population of a patient. PANDAA, on the

other hand, can detect these variants with 99% sensitivity and in a fraction (roughly 3%) of the time. The technology's 'sample-in/answer-out' format limits the sample handling time and means that less-skilled users can perform the tests – both of which are important considerations within resource-limited environments. PANDAA tests also do not require any new machinery to be purchased, as the technology utilises existing equipment found in centralised testing laboratories. Aldatu's current focus is to offer the tests in a thermostable format. This will mean samples do not have to be kept in cold storage, avoiding the typically high costs involved with cold chain transfer storage and transportation.

FUTURE USES

PANDAA technology is an innovative technology that could potentially be life-saving, especially in developing countries where resources are limited. Currently, Aldatu Biosciences are focusing on targeting HIV patients in Southern Africa who are failing their first-line treatment, especially pregnant women who could transmit HIV to the next generation. Moving forward, Dr MacLeod believes that PANDAA technology could be used to detect drug resistance in other debilitating diseases, including influenza, hepatitis B and MDR-TB. PANDAA technology is a revolutionary step for clinical diagnostics and will undoubtedly transform the lives of many people all over the world.

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Q&A

PANDAA turns existing techniques on their heads. How did you come up with the idea behind the PANDAA technology?

[MacLeod]: PANDAA is the result of a stubborn belief that the technical hurdles preventing qPCR from being used for HIV drug resistance could be overcome. My colleagues and I systematically broke the rules that govern traditional qPCR design, and, with the help of some PCR advancements that have been developed in recent years, figured out a way to harness the strengths of qPCR while mitigating the weaknesses that plagued HIV drug resistance detection. A reviewer of one of our earliest grant applications commented that PANDAA "violates the core principles of primer and probe design" – and we took that as a high compliment!

Why is PANDAA an improvement on existing genetic sequencing techniques?

[Raiser]: The current gold standard technology for HIV drug resistance detection is Sanger sequencing, which is neither quantitative nor particularly sensitive. The protocols require skilled personnel and multiple pieces of equipment, and it can take several days before a result is returned. By enabling qPCR, PANDAA returns quantitative drug resistance results in a sample-in, answer-out format in as few as 90 minutes – and less expensively than Sanger sequencing can.

[MacLeod]: Sanger sequencing also collects large HIV sequences, despite the fact that only a small number of genomic positions are relevant to drug resistance. PANDAA-enabled qPCR allows us to screen the HIV genome for resistance in a targeted way, only collecting the information that will be informative to clinical decision-making.

Could PANDAA be used to test for HIV drug resistance in developed (as well as developing) countries?

[MacLeod]: In short, yes. The speed, sensitivity, test simplicity, and cost

advantages of the PANDAA approach can benefit diagnostic test development for a variety of geographies. In particular, the ability of PANDAA to detect drug resistance earlier and in more patients is especially valuable when testing is performed more routinely, as it is in the US and Europe.

[Raiser]: Furthermore, clinicians in these regions are becoming increasingly interested in so-called "minority variants", which are HIV variants that go undetected with existing tests because they are present at levels below the sensitivity cut-off of Sanger. Early evidence suggests that these minority variants negatively affect patient outcomes, and should be considered when making treatment decisions. A PANDAA-based diagnostic could make detection of these variants possible, and Aldatu is already at work exploring this possibility.

Could PANDAA be used in clinical areas other than drug resistance testing?

[MacLeod]: Yes. PANDAA is a platform technology that can be applied to diagnostics in a number of different disease areas. Several highly polymorphic pathogens, such as influenza, hepatitis B and C, and tuberculosis, present technical challenges similar to those seen in HIV. Wherever secondary sequence variation complicates qPCR diagnostic design, PANDAA can be extremely useful.

Where do you see Aldatu Biosciences' focus in five years' time?

[Raiser]: PANDAA was developed to address a specific problem in HIV drug resistance, and our primary focus in the near-term is to use PANDAA to create diagnostic tools that meet that need. The HIV drug resistance products we are developing now will allow us to demonstrate the utility of the technology in a clinical setting over the next two to three years; after that, we can think about expanding the platform into other disease areas.

Detail

RESEARCH OBJECTIVES

Aldatu Biosciences aim to reduce the cost and increase the speed of drug resistance testing. Their PANDAA technology has been developed to allow clinicians to test patients' existing drug resistance before prescribing an appropriate treatment. At present, their work is focused on HIV-drug resistance – their lead product PANDAA HIV6 could be the key to enabling practitioners to effectively prescribe for patients across the world.

FUNDING

National Institute of Allergy and Infectious Disease (NIAID)
The Charles H. Hood Foundation

COLLABORATORS

Academic: Harvard School of Public Health – PANDAA was invented in collaboration with Christopher Rowley, MD, MPH, in the lab of Max Essex, DVM, PhD; Botswana-Harvard AIDS Institute Partnership (BHP); University of Zimbabwe

Implementation: Clinton Health Access Initiative (CHAI) **Business Support:** Harvard Innovation Labs

BIO

Dr Iain MacLeod holds a PhD in Pathology from the University of Cambridge (2008), and a BSc in Virology from the University of Glasgow. At Aldatu, Iain is Chief Science Officer. Iain maintains an

academic appointment at the Harvard School of Public Health AIDS Initiative. Dr David Raiser is Aldatu's CEO and is head of strategy and business development at Aldatu.

He holds a PhD in Genetics from Harvard Medical School and a BS from the University of Richmond.

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